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Further analysis of the effect of ultra-violet irradiation on the formation of the germ line in *Xenopus laevis*

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SUMMARY

Ultra-violet (u.v.) irradiation of the vegetal pole of newly fertilized eggs has three documented effects: reduction of primordial germ cells (PGCs), cytological damage to the vegetal hemisphere and disruption of the normal mechanism by which the vegetal yolk mass induces the formation of the dorsal axis of the embryo.

In this study, we find that 90° rotation of the egg for various periods after irradiation rescues the dorsal axial structures but does not restore the number of PGCs found in the dorsal mesentery of the gut; neither is there any correlation between reduced numbers of PGCs and disruption of cleavage at the vegetal pole. We therefore conclude that the effect on the germ line is separate from the other two phenomena.

Secondly, 90° rotation of non-irradiated eggs was found to significantly reduce germ cell numbers migrating in the dorsal mesentery of the gut.

INTRODUCTION

Since Bounoure (1937*a*,*b*) first discovered that irradiation of the vegetal pole of newly fertilized *Rana temporaria* eggs with u.v. light caused a reduction in the number of primordial germ cells (PGCs), much work has been directed at investigating this finding (see Smith & Williams, 1979 for a review). Several anuran amphibian species have been used (principally *Rana pipiens* and *Xenopus laevis*) and in all cases, irradiation of the vegetal pole during early development causes a significant reduction in PGCs (Smith, 1966; Tanabe & Kotani, 1974; Züst & Dixon, 1975).

How this reduction is brought about has been a subject of much contention. Two main views emerge: the first follows the original viewpoint of Bounoure that there is a specific germ-cell determinant in the vegetal pole cytoplasm that is sensitive to u.v. irradiation and which can be destroyed, rendering the animal

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sterile. The second is that u.v. has a generalized effect on the vegetal pole cytoplasm and the resulting disruption of cleavage is sufficient to cause a reduction in the number of PGCs that colonize the gonad.

It has been demonstrated that u.v. has a direct effect on the formation of dorsal axial structures in *Rana* and *Xenopus* (e.g. Grant, 1969; Grant & Wacaster, 1972; Malacinski, Benford & Chung, 1975). This effect is seen only when irradiation is carried out using relatively high doses of irradiation during the first two thirds of the first cell cycle, and therefore was not seen in the original germ-cell ablation experiments. Scharf & Gerhart (1980) have demonstrated rescue of dorsal structures by orientation of irradiated embryos at 90° to their original axis. How this rescues dorsal axial structures is unknown, though it is thought that a period of oblique orientation may cause a shift in cytoplasm relative to the egg cortex similar to that which would normally occur in early development, but which is inhibited in u.v. irradiated embryos. Since rotation of the egg clearly has far-reaching effects on egg organization, it would be interesting to see firstly, whether rotation can correct another effect of irradiation (i.e. germ-cell reduction) and secondly to study the effect of rotation alone on PGC number in the ensuing embryos.

There are few reports in the literature concerning delays or disruption of cleavage at the vegetal pole subsequent to u.v. irradiation. Grant & Youndahl (1974) using *Rana pipiens* and irradiating 45 min to 1 h postfertilization with 100 000 ergs/mm^2 found delays in cleavage in 60 % of embryos. Malacinski, Brothers & Chung (1977) irradiating *X. laevis* embryos up to 1 h postfertilization with 18 000 ergs/mm^2 (erg = 10^{-7} J) found some degree of delayed cleavage. Beal & Dixon (1975) and Züst & Dixon (1975) irradiating *X. laevis* embryos between the beginning of first cleavage and the end of second cleavage with a variety of u.v. doses (mostly in excess of 11 000 ergs/mm^2) found that cleavage-furrow formation was impaired and in some cases absent in the vegetal pole region. Absence of cleavage gave rise to a syncytium that either resolved or resulted in aborted development at gastrulation. This data presents a very incomplete picture. Careful series of experiments detailing the sensitivities of embryos to u.v. effects on dorsal structures and germ-cell reductions have been carried out; however, a similar study of the u.v. effect on cleavage is lacking in amphibia. It might be expected that using high doses of irradiation (100 000 ergs/mm^2 as Grant & Youndahl, 1974) or irradiation coincident with active cleavage processes (Beal & Dixon, 1975; Züst & Dixon, 1975) might potentiate the detrimental effect on cleavage. Since the latter time coincides with the polymerization of the cytoskeleton at the vegetal pole as the first cleavage furrow moves through it, then one might expect that a comparatively low dose of u.v. irradiation would affect cleavage as well as the germ line. It becomes very important therefore to see if the two effects are coincident or causally linked. We have chosen an irradiation time well before the formation of the first cleavage furrow, in order to separate as far as possible, the effects of u.v. on the two phenomena.

The other important aspect of this type of experiment concerns the irradiation conditions. In only one reported study has a spectral analysis been carried out on the effects of u.v. light on the germ line. This study (Smith, 1966) showed that light at 254 nm had a maximum effect on germ-cell reduction with no discernible cytolytic damage, whereas light at 230 nm had a cytolytic effect. We felt it particularly important in this study to carefully characterize the spectral emission of our lamp.

In this study, therefore, we have irradiated fertilized *X. laevis* eggs within the first half of the first cell cycle with various doses of u.v. and analysed three effects on the same batches of embryos. We are able to confirm several previous findings. In addition to this we show that u.v.-induced dorsal axial reduction is rescued by rotation of the eggs, whereas germ cell number is not rescued in the same embryos. Secondly, we show that the number of PGCs migrating in the gut mesentery at stage 45 can be reduced to zero without detectable effect on either the timing or the pattern of the first two cleavage furrows or the number of blastomeres at stage 8 of development (Nieuwkoop & Faber, 1956). Lastly, we show that rotation alone of fertilized *X. laevis* eggs causes a consistent small reduction in the number of PGCs during their subsequent migration to the gonad.

MATERIALS AND METHODS

Oocytes were obtained from *Xenopus laevis* females primed with 1000 i.u. of HCG (Profasi, Serono Lab Ltd.) by gently squeezing them to induce laying. Oocytes were collected as they were laid and immediately fertilized with a suspension of *Xenopus laevis* testes in sterile normal Gurdon's modified Barth's saline (N-GMB) (Hamburger, 1960; Elsdale, Gurdon & Fischburg, 1960). After 5 min in sperm solution excess N/10-GMB was added and eggs left for a further 5 min before dejellying in 2 % cysteine-HCl in distilled water brought to pH 7·8 using 1 M-sodium hydroxide. Eggs were then washed repeatedly in N/10-GMB and sorted; fertilized eggs were divided into groups for irradiation. Only batches with less than 10 % pigment abnormalities were used. Groups contained between 50 and 100 embryos depending on the size of the clutch laid.

Once the embryos had rotated within their vitelline membrane, irradiations were carried out by placing them in a minimal amount of N/10-GMB on a quartz glass slide 15 cm above the u.v. lamp and exposing them to radiation for various lengths of time. All irradiations were carried out within the period 25–35 min postfertilization (with first cleavage at 75 min). The u.v. source used was a Mineralight (Ultra-violet Products, Ltd.). A spectral analysis of the lamp was carried out using a fluorescence spectrometer as supplied by Applied Photophysics. Radiation from the u.v. lamp was introduced into the high irradiance monochromater (blazed at 500 nm) and using the Mullard DUVP photomultiplier tube, the spectral emission was assessed between 200–500 nm. This simply

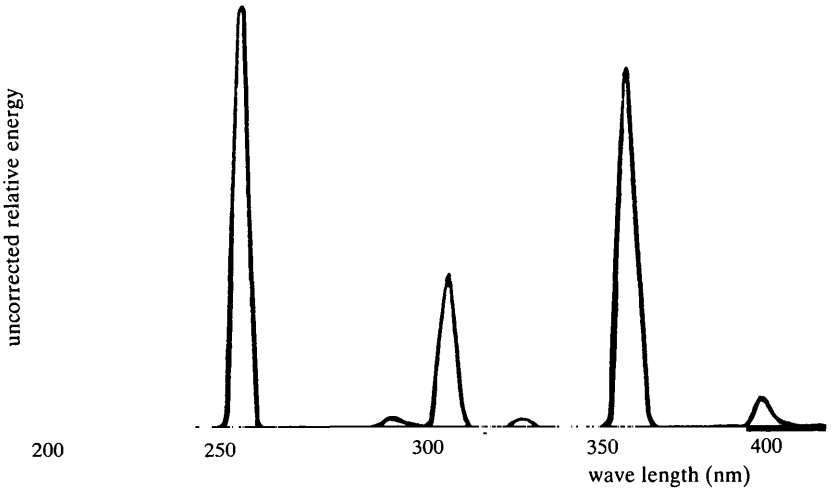


Fig. 1. Spectral analysis of u.v. lamp emission measured with a fluorescence spectrometer. The spectrum shows the wavelengths of u.v. emitted but has not been corrected to account for varying efficiency of the N-window tube in detecting u.v. of different wavelengths, i.e. heights of peaks do not indicate relative energies. See Table 1 for corrected data.

identifies electromagnetic emission peaks (see Fig. 1). To assess relative energy outputs at different wave lengths, reference was made to a representative graph of N-window efficiency i.e. a correction curve for the Mullard DUVP photomultiplier tube supplied by the manufacturer. While not perfectly accurate, these measurements do give a reasonable assessment of the spectral characteristics at the time the lamp was used for these experiments.

Absolute energy emitted at 254 nm was measured using a Latarget meter, readings being taken at 15 cm via a filter and the quartz-glass slide. From this measurement, relative energies at other wave lengths were converted to absolute energies shown in Table 1. It can be seen from the table that emission at 254 nm accounts for 56 % of the total emission of the lamp and that there is no emission at 230 nm.

Immediately after irradiation, batches of irradiated embryos and non-irradiated controls were divided into groups to be rotated and those to be left as non-rotated controls.

In order to rotate the eggs to 90° from their normal orientation, the vitelline space was dehydrated using 10 % Ficoll 400 (Sigma) in N-GMB (after Kirschner, Gerhart, Hara & Ubbels, 1979). Embryos were then oriented so that the equator of the egg was uppermost. This was facilitated by the very distinct black–yellow, animal–vegetal pole cortical pigment gradient in *Xenopus*. Eggs were held in this position under Ficoll in troughs made in plates of 2 % agarose in N-GMB. Eggs were 'derotated' i.e. placed back into N/10-GMB after various lengths of time

Table 1. *Absolute energy at each emission peak in spectral analysis of u.v. lamp*

Wavelength u.v. (nm)	absolute energy* ergs/mm^2/s	% of total energy at each maximum
254	21·8	56
296	0·5	1
312	5·4	14
333	0·4	1
365	9·8	25
403	0·9	2

*measured at 15 cm from lamp through filter and quartz glass slide

Relative energies emitted at each wavelength were obtained from the spectral analysis by reference to a representative graph of N-window efficiency. These were converted to absolute energies shown in Table 1 by measuring energy emission at 254 nm and deriving energy emitted at other wavelengths from their corrected relative energies (see materials and methods for details).

whereupon they assumed their original orientation, with the darkly pigmented animal hemisphere uppermost. Routinely, derotation was carried out after first cleavage, that is at about 75 min postfertilization. A second series of experiments investigated the effect of varying the duration of rotation.

The frequency of different cleavage patterns was assessed by counting the embryos in each group whilst observing the vegetal poles via a Zeiss inverted microscope over a period of 5 min during the 4-cell stage. Photographs of stage-8 (Nieuwkoop & Faber, 1956) vegetal hemispheres were taken using the inverted microscope. From these photographs, the number of blastomeres visible in the vegetal hemisphere of ten embryos from each irradiation group were counted. Photographs of controls and experimentals were taken alternately over a period of 10 min. Dorsal abnormalities were assessed at stage 40–42 of development, using a classification system devised by Malacinski *et al.* (1975).

In order to assess the number of PGCs present in the mesentery during the migratory phase (stages 43–45) tadpoles were dissected as described by Heasman, Mohun & Wylie (1977) and placed into disaggregating saline containing 0·1 % EDTA. The PGCs round up out of the mesentery and can be dissected free and counted under a dissecting microscope. In each experiment, ten embryos from each treatment group were assessed in this way.

RESULTS

U.v. irradiation reduces germ cell numbers

Fig. 2 shows the results of increasing doses of u.v. irradiation of the eggs on the number of PGCs present in the dorsal mesentery of the gut at stage 43–45 of *Xenopus* development (Nieuwkoop & Faber, 1956). As can be seen, u.v.

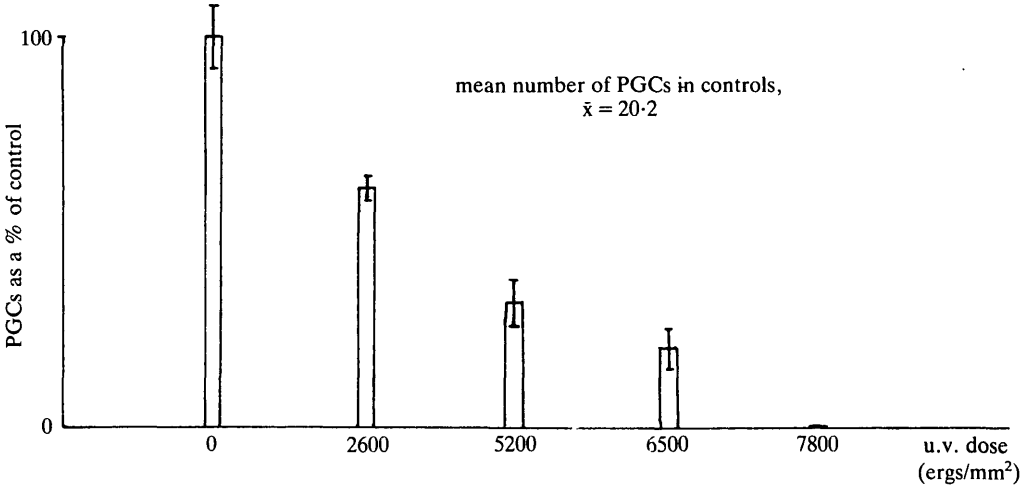


Fig. 2. The effect of u.v. radiation, prior to first cleavage, on the germ line in *Xenopus laevis*. The histogram shows the number of PGCs (mean from 10 embryos) expressed as a % of control, from a representative experiment. PGCs were assayed at stage 43–45. Bars represent standard error.

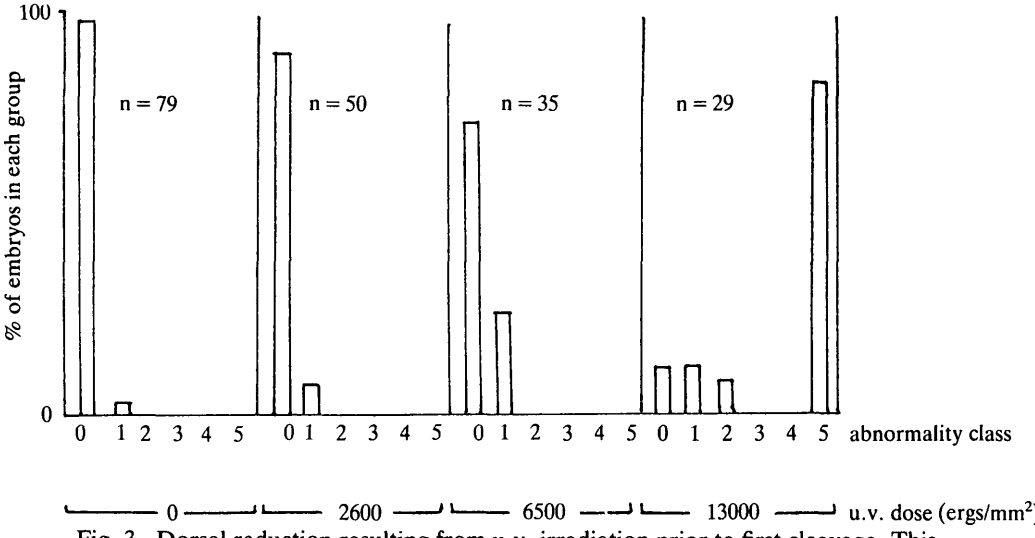


Fig. 3. Dorsal reduction resulting from u.v. irradiation prior to first cleavage. This shows the % of embryos in each abnormality class (Malacinski *et al.* 1975) after different doses of u.v. irradiation. n = the number of embryos scored in each group.

causes a significant reduction in PGCs even at low doses of about 2600 ergs/mm^2. There is a progressive reduction in PGCs as u.v. dose increases. Complete absence of PGCs from the gut mesentery at stages 43–45 is attained with a dose of 7800 ergs/mm^2.

U.v. irradiation causes abnormalities of dorsal axial structures

Fig. 3 shows the effect of irradiation on the dorsal axial structures. In the

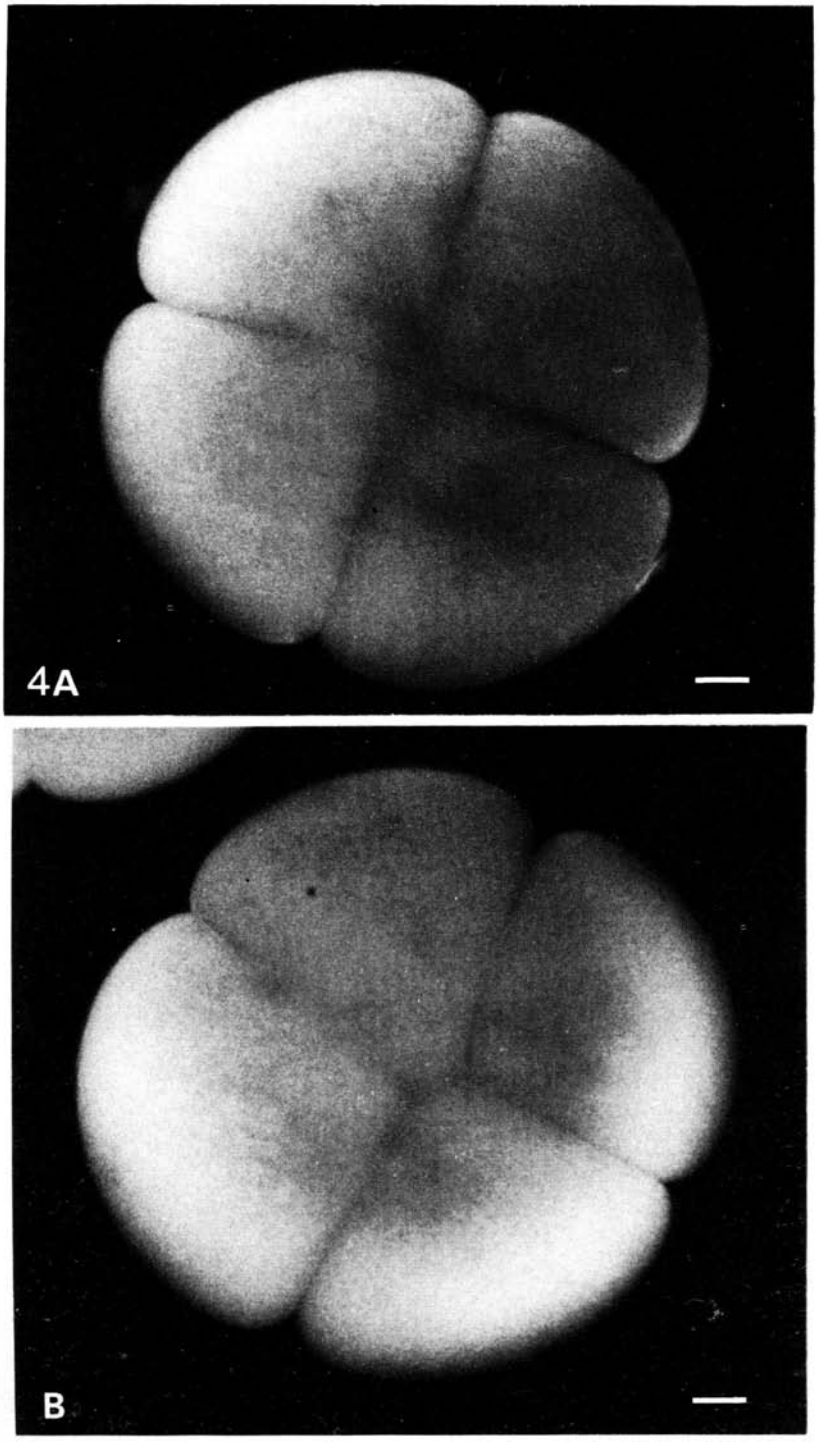


Fig. 4. Two representative photomicrographs of cleavage patterns at the vegetal pole of 4-cell embryos. (A) shows furrows meeting at 90°, (B) shows the other extreme of an 'offset' pattern of cleavage. (Bar = 0·1 mm).

control group and low-irradiation groups, over 90 % of the embryos are normal i.e. fall in the zero abnormality group, the remainder being only slightly abnormal. In the higher doses of irradiation more abnormals are seen and by 1300 ergs/mm^2 about 80 % of the embryos are in class 5, the severely abnormal group.

Effect of u.v. irradiation on cleavage rate and pattern

In Fig. 4, representative photographs of the vegetal poles of 4-cell embryos are shown. These represent the two extremes of cleavage pattern at the vegetal pole. Embryos were observed with patterns ranging from a 90° intersection between cleavage planes (Fig. 4A) through to the 'offset' pattern seen in Fig. 4B. These patterns occurred in about equal proportions in irradiated and non-irradiated groups (Table 2) counts being carried out over a period of less than 10 min. The pattern of the first two cleavage furrows and the time of their appearance is thus not altered by u.v. irradiation in the dose range used.

Table 3 shows the number of blastomeres in vegetal hemispheres of irradiated and non-irradiated blastulae (stage 8, Nieuwkoop & Faber, 1956). The data indicate that, by stage 8, both the irradiated and the non-irradiated embryos have

Table 2. *Incidence of cleavage patterns in irradiated and non-irradiated embryos at the 4-cell stage*

	n	⊕	⊗
non-irradiated	257 (100%)	130 (50·6%)	127 (49·4%)
irradiated	247 (100%)	129 (52·2%)	118 (47·8%)

The diagrams represent the cleavage furrows visible on the vegetal hemisphere of the four cell embryo.

Table 3. *Vegetal pole blastomere count*

	embryo number											
dose of u.v. (ergs/mm^2)	1	2	3	4	5	6	7	8	9	10	$\bar{x}$	σ
0	34	25	40	28	30	32	30	28	32	28	30·7	4·16
2600	30	31	29	30	37	30	28	28	26	30	29·9	2·88
5200	33	31	34	32	26	38	26	26	34	28	30·8	4·16
6500	27	25	36	33	40	36	31	33	28	40	32·9	5·22
7800	35	31	33	32	34	36	34	36	28	36	33·5	2·59

(ten embryos analysed per irradiation group)
overall $\bar{x} = 31{\cdot}6$; overall $\sigma = 4{\cdot}01$

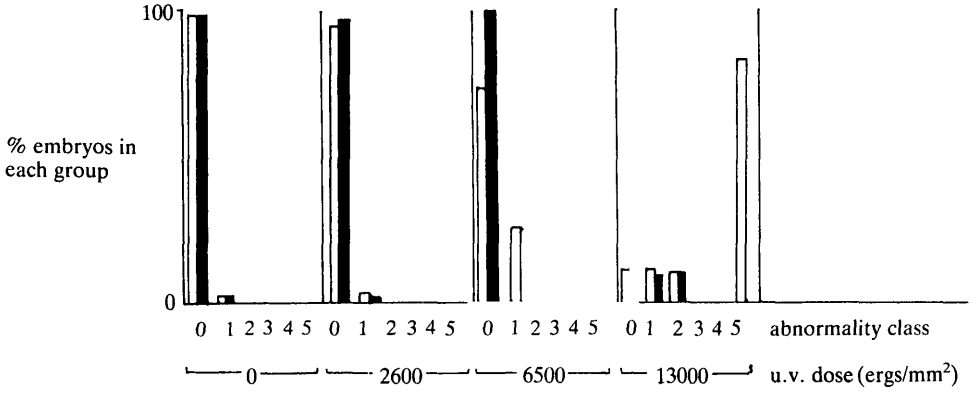
Using a one-way analysis of variance, the F ratio was 1·57 arising with a probability of 0·2 so that the null hypothesis that all observations come from the same population has high significance.

similar numbers of blastomeres in the vegetal hemisphere. If irradiation does delay cleavage, this effect has been overcome by stage 8.

Any alteration in cleavage planes would be most readily observed during the earliest cleavage divisions when cleavage planes are most regular. Any maintained alteration in cleavage rate would be more obvious later in cleavage when blastomere numbers are larger. The data in Table 3 and Fig. 4 indicate that there is no alteration in cleavage planes or maintained change in cleavage rate in embryos irradiated with up to 7800 ergs/mm^2.

Rotation rescues dorsal structures in irradiated embryos

In Fig. 5, the effect of rotation following irradiation on the formation of the dorsal structures can be seen. Rotation enables the irradiated embryos to develop apparently normal dorsal structures. This is shown in the group irradiated with 13 000 ergs/mm^2, a dose which normally causes a high incidence of aneural tadpoles to develop (shown by the open bars). However, rotated embryos were restored to normal (solid bars).



number of embryos in each irradiation group:

	0	260	6500	1300	(u.v. in ergs/mm^2)
non-rotated	79	50	35	35	
rotated	40	32	18	40	

Fig. 5. The effect of rotation on dorsal abnormalities in embryos irradiated prior to first cleavage. The histogram shows the % of embryos in each abnormality class after irradiation (open bars) and after rotation (solid bars); the number of embryos in each irradiation group is shown in the table.

Rotation does not rescue PGCs in irradiated embryos

In Fig. 6, the effect of rotation on the number of PGCs present in the mesentery of stage-43 to -45 embryos is shown. Rotation did not reverse the reduction of PGCs in the gut mesentery caused by u.v. light. With intermediate doses of u.v., there was sometimes a slight increase in PGC number on rotation (see Fig. 6), however, sometimes a slight decrease was observed. These results were not statistically significant.

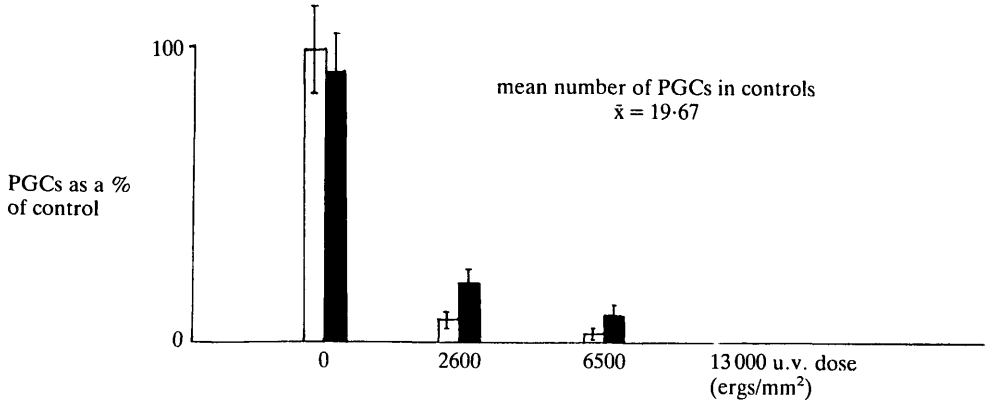
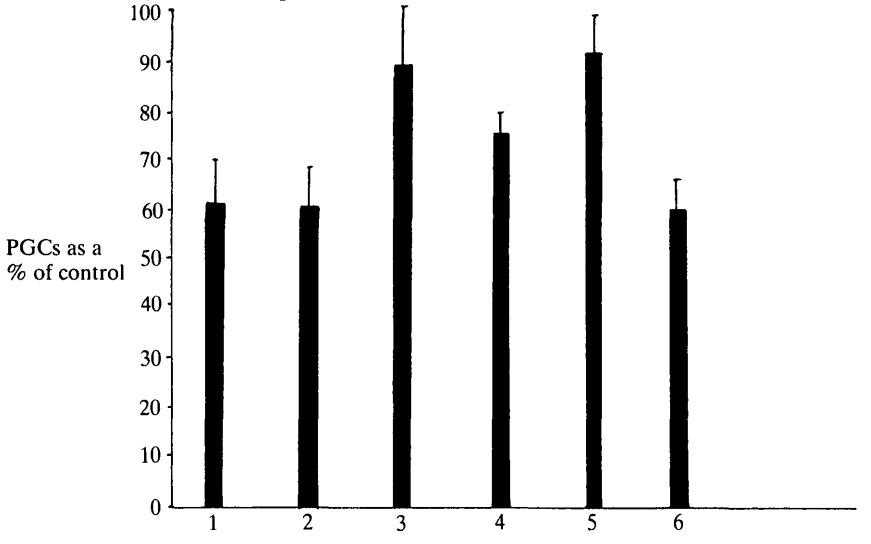


Fig. 6. The effect of rotation on the germ line in embryos irradiated prior to first cleavage. The histogram shows the number of PGCs (mean of 10 embryos) expressed as a % of controls from a representative experiment. Solid bars represent results in rotated embryos. Lines represent standard error.



RESULTS OF SIX SEPARATE EXPERIMENTS
mean number of PGCs in each control:

1	2	3	4	5	6
27·1	24·3	19·1	15·6	19·7	13·5

Fig. 7. Reduction in germ cells after rotation prior to first cleavage. The histogram shows the effect of rotation on the PGCs of embryos not exposed to irradiation; each bar represents a separate experiment and expresses the number of PGCs in rotated embryos as a % of the PGCs seen in controls from the same experiment. The table shows the mean PGCs in the controls for each experiment. In each case 10 embryos were assayed from rotated and non-rotated groups. Lines represent standard errors.

Rotation alone reduces the number of PGCs present in the non-irradiated embryos

Rotation alone reduced the number of PGCs seen in the embryos that were not exposed to u.v. irradiation. Fig. 7 shows six such results; in each case, embryos have been left in the rotated position for 30–60 min and then returned to N/10-GMB whereupon they assume their original orientation i.e. animal hemisphere uppermost. They were then allowed to develop to stage 43–45 at which time their PGCs were assayed as before and the numbers compared with those in non-rotated siblings from the same batch.

There is a reduction in PGCs in the non-irradiated, rotated embryos varying between 10 % and 40 %. We never found an increase in PGCs nor did we see the same number of PGCs in non-rotated and rotated embryos. Using a non-parametric matched pair randomization test we found that the mean reduction in PGCs seen in batches of embryos would have arisen by chance with a frequency of considerably less than 0·01 so that the reductions seen, although small in each case, together are highly significant statistically.

Effect of varying the duration of rotation on PGC numbers

Fig. 8 shows the results of a series of timed rotations of different durations. From our preliminary results, it would seem that rotation reduces the number of

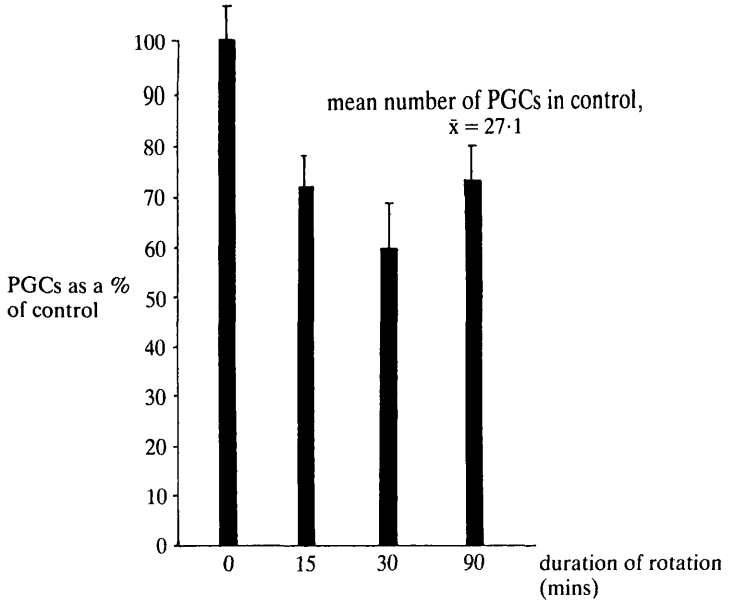


Fig. 8. Reduction of PGCs found in embryos rotated prior to first cleavage and left in the rotated position for various lengths of time. This shows the effect of varying the duration of rotation on the PGCs of embryos not exposed to u.v. irradiation. In each case 10 embryos were assayed for PGCs at stage 43–45. Lines represent standard error.

germ cells seen in the mesentery at stages 43–45 by a similar amount, regardless of the duration of rotation.

In embryos rotated for 90 min, the position of the dorsoventral axis relative to the original animal–vegetal axis was markedly changed due to the embryos not 'derotating' i.e. they remained in a position at 90° to their original orientation despite rehydration of the vitelline space (based on the evidence of their pigment distribution). This resulted in lightly pigmented cortex originally situated in the vegetal hemisphere becoming integrated into small, animal-hemisphere-like blastomeres situated in the upper half of the embryo. Similarly, darkly pigmented cortex formed large vegetal-hemisphere-like blastomeres. These embryos still had PGCs in about the same numbers as rotated embryos in which the axis was not changed (see Fig. 8).

DISCUSSION

From our results, we conclude that the effect of u.v. on the germ line is separate from any generalized effect on the vegetal pole leading to visible alterations in cleavage, and from the effect on the formation of dorsal axial structures.

In each case, the dose response to u.v. is different; the dose of u.v. needed to cause sterility is much lower than that required to significantly reduce dorsal structures, disruption of the pattern or overall rate of cleavage not being seen at the doses used. The observed effect of u.v. radiation on the germ line is a reduction in the number of PGCs present in the dorsal mesentery of the gut during the migratory phase of germ-cell development. Whether this reflects a permanent reduction in PGCs or a delay in their migration is not clear. In Smith's experiments (1966), analysis of the effect of u.v. was carried out at slightly later stages (stage 25 of *Rana* development) and he found that the gonadal ridges formed in irradiated embryos but did not contain gonocytes. Other workers (Züst & Dixon, 1975) and subsequent work by Smith (Smith & Williams, 1979) have shown that in some cases, gonadal ridges do become colonized but at later stages and with reduced numbers of PGCs. Currently, we are analysing tadpoles of later stages to assess the effect seen with our lamp i.e. using predominantly 254 nm radiation.

There is no evidence for disruption of vegetal pole cleavage at the doses used based on analysis of pattern and number of blastomeres. We would therefore conclude that a generalized effect on the vegetal cytoplasm, resulting in a disruption of cleavage, cannot be the cause of the other two effects.

Other workers have observed generalized effects on the vegetal pole but discrepancies in timing of irradiations and the wave lengths and doses of u.v. used make the comparison of results from different laboratories difficult, a situation further complicated, in most cases by the lack of information given concerning the spectral analysis of the u.v. lamp used.

The response curve to u.v. in the three effects is also different; the germ-cell

reduction follows an exponential curve tending to zero as the u.v. dose increases. The dorsal reduction results appear to reflect a far more complex mechanism as shown by the discontinuous nature of the distribution. To a certain extent this may be a function of the artificial groupings imposed upon the abnormalities. However, even when high numbers of vegetalized embryos were produced, within the same irradiation group there were still a large number of normal and only mildly affected embryos.

In an attempt to circumvent the problems of the decay parameters of u.v. lamps with age and use, we have made a careful study of the type and intensity of u.v. irradiation emitted by the lamp used in this study (see Fig. 1 and Table 1) and have found that the majority of the energy emitted is of 254 nm wave length. This wave length, according to Smith (1966), causes minimal cytolytic damage and has maximal sterilizing efficiency i.e. decreases PGC numbers arriving in the mesentery during the migratory phase. Smith (1966) also showed that 230 nm wavelength u.v. radiation is associated with cytoplasmic damage leading to abnormal cleavage and delayed development. The lamp used in this study was demonstrated to have no emission at 230 nm. Our results coincided with Smith's findings in that u.v. irradiation at 254 nm reduced germ cells in the mesentery but had no discernable generalized effect.

The dorsal structures of the irradiated embryo are rescued by rotation by an, as yet, unclear mechanism but possibly by mimicking the normal mechanism by which the initial asymmetry is established (Scharf & Gerhart, 1980).

Rotation does not restore the PGCs to irradiated embryos, this supports the suggestion that u.v. light acts directly on a cortically located, u.v.-sensitive factor – possibly germinal granules. There is considerable evidence to support this view. Firstly as the penetration of u.v. radiation is relatively low (about 10 μm through the egg cytoplasm (Grant & Wacaster, 1972)) it is likely that the sensitive factor is located at or near the surface of the vegetal pole. Secondly, the sensitivity of the egg to 'sterilization' varies, being most sensitive at the time when germ plasm is seen to be closest to the surface (just prior to cleavage) and progressively less sensitive as the eggs divide and the germ plasm is redistributed to a juxtanuclear position (Tanabe & Kotani, 1974). Lastly, centrifugation, which would be expected to move dense particles such as germ plasm towards the centre of the egg, also results in decreased sensitivity to sterilization by u.v. (Tanabe & Kotani, 1974).

In addition to this, in 1966, Smith performed rescue experiments and showed that u.v.-induced sterility in amphibians could be rectified by injecting subcortical cytoplasm from the vegetal hemisphere into the irradiated embryo, subcortical cytoplasm from the animal hemisphere having no effect. Wakahara (1977) extended these studies by injecting fractions of non-irradiated vegetal hemisphere cytoplasm prepared by centrifugation and found that one fraction increased PGCs by 30 % of control numbers. Electron microscopic studies of this fraction showed that it contained membranous structures, mitochondria and

aggregates of electron-dense material resembling 'nuage' purported to be the electron microscopic equivalent of germ plasm. Another study by Ikenishi, Kotani & Tanabe in 1974, demonstrated that u.v. irradiation resulting in the absence of PGCs also resulted in fragmentation of germinal granules and their associated mitochondria in the germ plasm of cleaving *Xenopus* eggs.

How rotation of non-irradiated embryos reduces the number of PGCs at stage 43 is an interesting question. One possibility corresponds with the idea of a small founder clone of about four cells containing germ plasm that will divide to give rise to germ cells (Whitington & Dixon, 1975; Ijiri & Egami, 1976). A slight displacement of germ plasm caused by rotation could reduce the size of the founder clone by one cell so that there will be a reduced number of germ cells in the embryo at stage 43. This could be accomplished by displacement so that only three cells receive germ plasm or so that only three cells receive enough germ plasm to achieve expression of the germ-cell phenotype. This could account for the similar reductions in germ cells seen in embryos after both short and long periods of rotation. Currently, we are analysing rotated and non-rotated embryos to compare the number of vegetal hemisphere blastomeres containing germinal granules.

The observation that prolonged periods of rotation of embryos results in a change in pigment distribution between blastomeres has been reported earlier by Scharf & Gerhart (1980) who demonstrated that in rotated eggs at the 4-cell stage there are two light and two dark blastomeres, rather than the normal situation of four almost equally pigmented blastomeres (the presence of the grey crescent on the prospective dorsal side of the normal embryo does mean that the pigment density over two blastomeres is slightly less on one side).

In conclusion, our experiments strongly suggest that the effects of u.v. on the germ line, dorsal axial structures and cleavage at the vegetal pole are independent of each other. Our irradiations have been carried out at an earlier stage than in many other studies, thus avoiding the possible complication of u.v.-sensitive cleavage-furrow formation, and with a carefully characterized light source. In addition to this, we have shown that rotation alone causes a reduction of PGCs found in the mesentery of stage-43 tadpoles.

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