Embryonic defects in \textit{Drosophila} eggs after partial u.v. irradiation at different wavelengths

By MARY BOWNES$^1$ AND KLAUS KALTHOFF$^2$

From the School of Biological Sciences, University of Sussex and the Biologisches Institut I (Zoologie) der Universität, Freiburg

SUMMARY

\textit{Drosophila} eggs at nuclear multiplication or blastoderm stages were regionally irradiated with monochromatic u.v. Twenty-four hours after irradiation, the results were classified as normal larvae, undifferentiated eggs, or defective embryos; the latter were subdivided into embryos with anterior or posterior defects, or embryos without anterior and posterior specificity. The irradiation of anterior (posterior) egg regions and the occurrence of anterior (posterior) defects were strongly correlated. These correlations were found using eggs of both stages. The size of the irradiated area did not obviously influence the types of defect.

Dose-response curves were established irradiating anterior quarters of eggs at 245, 265, 285, or 305 nm wavelength. The frequency of embryonic defects increased with increasing u.v. doses, whereas the dosages applied did not increase the frequency of undifferentiated eggs over the control level. In eggs during nuclear multiplication 285 nm radiation was most effective in producing embryonic defects. After blastoderm formation, the efficiency of irradiation was generally increased but similar at all wavelengths employed. The induction of embryonic defects was photoreversible after u.v.-irradiation at the blastoderm stage. The data reported also include transmittance spectra of chorion and egg membrane preparations from \textit{Drosophila} eggs.

INTRODUCTION

Spatial pattern formation is a basic problem in developmental biology. For an analysis of the underlying processes, experimental techniques which allow us to modify the formation of a spatial pattern might be considered especially useful. U.v. irradiation induces aberrant body segment patterns in eggs of chironomid midges, as described by Yajima (1964), Kalthoff & Sander (1968), and Kalthoff (1971a, b; 1973). In particular, the aberrant pattern 'double abdomen' is caused by irradiation of anterior pole regions of the egg of \textit{Smittia}. The embryo then develops a second abdomen, in mirror image symmetry to the first, in place of the normal head, thorax and anterior abdominal segments. Similarly, the mutant bicaudal (bi) of \textit{Drosophila melanogaster} produces an embryonic abnormality that closely resembles the u.v.-induced double abdomen of \textit{Smittia} (Bull, 1966).

Experiments interfering with epigenetic processes in \textit{Drosophila} eggs, e.g.

$^1$ Author's address: Center for Pathobiology, University of California, Irvine, California 92664, U.S.A.

$^2$ Author's address: Biologisches Institut I (Zoologie) der Universität, Katharinenstr. 20, D 7800 Freiburg, Germany.
u.v. microbeam irradiation (Hathaway & Selman, 1961) or microcautery (Bownes & Sang, unpublished), so far have produced only defective embryos, but no drastically aberrant patterns like double abdomens. The experiments described in this paper were carried out in an attempt to produce a phenocopy of the bicaudal mutation of Drosophila using the techniques developed for u.v. induction of double abdomens in Smittia.

As we failed to obtain this specific result in the course of our experiments, we have described and classified the embryonic defects obtained after irradiation of different egg regions, since comparable data in this field are still scanty and partly conflicting. We have also tried to obtain some indication of the molecular processes involved in the generation of these embryonic defects, by observing which wavelengths produce them most frequently. This led us to record transmittance spectra of chorion and egg membrane preparations from Drosophila eggs which might be useful for further photobiological experiments on these eggs.

MATERIALS AND METHODS

Origin and preparation of eggs. All eggs were of Oregon K stock. Eggs were collected at 25 °C on agar plates coated with a paste of fresh yeast and sugar. After the first hour of laying, eggs were collected at 30 min intervals and used immediately or left to incubate for a further 45 min. The eggs were washed from the agar with 0.9% sodium chloride, then dechorionated with 3% sodium hypochlorite for 5 min. This procedure removes the chorion, rendering the remaining membranes (probably vitelline membrane, wax layer, and inner membrane, after King (1970)) perfectly transparent. The eggs were then washed with water and put in a mild detergent to prevent the eggs from clumping together and from floating on the surface of the solutions during experimental procedures. From these batches of eggs, the following stages in development were selected for experimental use:

Stage ‘NM’ was during nuclear multiplication; eggs were irradiated 60 ± 15 min after deposition. At this stage, the nuclei are shielded from incident radiation by periplasm and yolk material.

Stage ‘Syn Bl’ was after the migration of cleavage nuclei into the periplasm to form a syncytial blastoderm but before the formation of a cellular blastoderm. Only one experiment was done with eggs at this stage.

Stage ‘Bl’ was after the formation of a cellular blastoderm but before the beginning of morphogenetic movements; eggs were irradiated 150 ± 15 min after deposition. At this stage, the nuclei in the blastoderm cells are no longer shielded by yolk material.

Generation and measurement of u.v. radiation. Far-u.v. radiation was obtained from an apparatus including a xenon arc (Osram XBO 450 W) and a grating monochromator (Farrand foci, f/3·5). The bandwidth used was 5 nm, and the scattered energy was less than 2%. Dose rates were measured by a compensated
thermocouple (Hilger & Watts FT 12) and a microvoltmeter (Keithley 150 B) calibrated against a u.v.-standard. Local deviations of dose rate within the radiation beam leaving the exit slit of the monochromator were kept below \(\pm 5\%\). For the experiments involving photoreversion, we used blue light of 433 nm wavelength produced by another xenon arc and an interference filter.

**Irradiation procedure.** Eggs were irradiated with far-u.v. through the quartz bottom of a small vessel placed above the exit slit of the monochromator. Eggs were submerged in detergent solution and lined up in a groove between two glass pieces with cut edges (Fig. 1). One of these pieces could be adjusted to accommodate eggs in various positions. Usually, eggs were lined up with their long axes perpendicular to both the direction of the incident radiation and the walls of the groove. In this position, those egg regions intended to be shielded were covered by a razor blade stuck to the bottom of the vessel from below (Fig. 1). Thus parts of the egg referred to as anterior fourth, posterior eighth, etc., with respect to the long egg axis could be irradiated. In order to irradiate pole caps eggs were placed in the groove with their long axis parallel to the incident radiation. The pole region facing the exit slit of the monochromator was irradiated while other egg regions were shielded by the egg contents. This procedure is referred to as irradiating the anterior or posterior pole cap, respectively. The correct position of the eggs during irradiation was monitored using a stereo microscope. All irradiations were carried out at room temperature (about 24 °C). After irradiation, eggs were rinsed with tap water, placed on agar in plastic Petri dishes, and incubated at 25 °C in the dark.

**Scoring.** After approximately 24 h the irradiated eggs were classified into:
1. Normal larvae.
2. Undifferentiated eggs which had completely failed to continue development after irradiation.
3. Defective embryos. The defective embryos were further classified according to the structures affected, as described in the following paragraph. Eggs were mounted in 0.9% sodium chloride on a slide, and covered by a coverslip (supported by pieces of coverslip to prevent the
eggs from bursting) which flattened the eggs somewhat, making the internal organs more easily recognizable. Photographs were taken with a Leitz Ortholux microscope with an orthomat camera attachment.

Classification of embryonic abnormalities. Our experiments resulted in a large variety of embryonic defects which will be described in some detail below. For quantitative evaluations of our data, we have classified the defects very broadly as follows:

Class I. The embryos included in this class show only anterior defects. Mouthparts or other head structures may be abnormal or absent, the gut may be extruded at the anterior end of the embryo. Anterior abdominal segments may be lacking, too, but the posterior end of the abdomen including the spiracles was formed by all embryos in this class.

Class II. Embryos without definite anterior or posterior specificity have been pooled in this class. They show yolk patches and contracting masses of gut tissue; some of them have formed bristle rows on the surface.

Class III. This class comprises all embryos with clearly posterior defects. The gut may be extruded at the posterior end of the embryo, abdominal segments may be formed partially or replaced by a mass of yolk and gut. All embryos in this class show head structures and at least some indication of mouthpart formation.

RESULTS

Transmittance of the chorion

U.v. and visible radiation are considerably reflected, scattered, and absorbed by the chorion of Drosophila eggs. These losses are reduced by our dechorionation procedure. To assess the optical effects of dechorionation, we measured the transmittance of vitelline membrane and wax layer, and complete chorions with inner membranes after preparing them mechanically and embedding in glycerol between quartz slides and coverslips. The chorions with inner membranes were prepared as double layers in order to minimize alterations due to mechanical stress. The transmittance was recorded by a microspectrophotometer (Zeiss UMSP 1) using an objective with a numerical aperture of 0-4. Figure 2 shows the average of three measurements each; the maximum deviations from the averaged curves were ± 1% for the endochorion and ± 5% for both complete chorion, and chorion with inner membranes.

The transmittance of all preparations was wavelength dependent and essentially decreased towards shorter wavelengths, probably due to scattering. A slightly decreased transmittance around 280 nm might be ascribed to absorption by protein components. At all wavelengths, the transmittance of the inner membranes was much higher than that of the chorion. The transmittance of the chorion with inner membranes, as drawn in Fig. 2, is probably still overestimated for the following reasons: the embedding medium might have leaked into spaces between the layers of the chorion, thus lowering losses due to
reflexion. Moreover, the transmittance of the chorion with inner membranes has been calculated by taking the square root from the transmittance recorded for double layers of chorions. Actually, the transmittance of the double layers should have been increased by multiple reflexion and backward scattering. This is also indicated by the fact that the transmittance of the chorion with inner membranes was higher than would be expected from combining the transmittances of inner membranes and chorion.

Our data disagree with a result of Goldman & Setlow (1956). Using a Beckman spectrophotometer these authors found a transmission of 85% for the chorion of Drosophila eggs, which was constant over all wavelengths from 230 to 313 nm. In our opinion this result is unlikely to be true, for both physical and biological reasons. According to our results, the transmittance of the chorion is rather low and wavelength dependent, and there are difficulties in measuring it precisely, whereas the transmittance of the inner membranes is much higher and easier to measure. Therefore, in contrast to most previous investigators, we decided to remove the chorion from the eggs before irradiation. This procedure, together with the submersion of the eggs in detergent solution, caused the high mortality rate of preblastoderm eggs in our experiments. On the other hand, the 'dechorionation' facilitated the sorting out of retained or irregularly developed eggs before irradiation as well as the scoring of the results after development.
Table 1. Results of dorsal and ventral u.v. irradiation of anterior quarters of Drosophila embryos

<table>
<thead>
<tr>
<th></th>
<th>Total eggs treated</th>
<th>Undifferentiated eggs</th>
<th>Normal larvae</th>
<th>Defective embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal</td>
<td>57</td>
<td>34</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>Ventral</td>
<td>59</td>
<td>36</td>
<td>3</td>
<td>20</td>
</tr>
</tbody>
</table>

Dorsal and ventral irradiation of the anterior fourth of the egg

In the experiments to be described in the following section, we had to irradiate a defined egg region in a large quantity of eggs. This is done most easily if the eggs are lined up perpendicularly to the incident radiation as indicated in Fig. 1. Since irradiating the eggs dorsally or ventrally might cause different frequencies, or types of abnormalities, we checked this first. The anterior fourths of eggs at stage NM were irradiated at 285 nm wavelength. As can be seen from Table 1 there was no significant difference in the frequencies of defective embryos after irradiation of different sides of the egg. Within the limited number of eggs examined, there were also no major differences in the types of embryonic defects found. Therefore, in all subsequent experiments of this kind, eggs were lined up without respect to their dorsoventral orientation.

Wavelength dependence of u.v. induction of embryonic defects

In order to test the influence of u.v. dose and wavelength, dose-response curves were established at 245, 265, 285, and 305 nm. In these experiments, the anterior fourths of eggs were irradiated at stages NM or Bl. Dose rates were adjusted to 0.1 micro-einstein per square centimetre per minute at each wavelength (1 einstein = 6 x 10^23 quanta). The u.v. doses that passed the inner membranes of the eggs were calculated from the incident dose rate, the duration of irradiation, and the transmittance of the endochorion as shown in Fig. 2. The frequencies of defective embryos were plotted against the u.v. doses thus computed (Fig. 3). About 9% of these embryos were of class I showing clearly anterior defects.

The frequency of defective embryos is plotted as a percentage of developing eggs (i.e. as 100% minus frequency of normal larvae), because the frequency of undifferentiated eggs shows no correlation with the u.v. dose applied, except for one extraordinary result described below. The frequency of undifferentiated eggs was about 40% with eggs during nuclear multiplication. This high mortality rate was probably caused by the dechorionation procedure and the submersion of the eggs in detergent solution. In the experiments with eggs at the blastoderm stage, those eggs that failed to develop were easily sorted out before irradiation, so the frequency of undifferentiated eggs was about 7% in these experiments. The plots against u.v. dose zero represent the frequencies of defective embryos in control eggs which received the same treatment as did the irradiated eggs but
U.V.-irradiation of Drosophila eggs

Fig. 3. Dose-response curves for u.v. induction of embryonic defects in Drosophila eggs obtained at different wavelengths. The anterior fourths of eggs were irradiated during nuclear multiplication (left panel) or after blastoderm formation (right panel). The incident dose rate was adjusted to 0.1 micro-einstein per square centimetre per minute at all wavelengths (1 einstein = 6 x 10^28 quanta). The u.v. doses passing the inner membranes of the eggs were calculated from the incident dose rate, the duration of irradiation, and the transmittance of the inner membranes as shown in Fig. 2. The u.v. doses thus computed are plotted against the frequency of defective embryos which is expressed as percentage of developing eggs. About 90% of the defective embryos were of class I showing clearly anterior defects. Number of eggs per point, 1000.

Increasing u.v. doses produced increasing amounts of embryonic defects. The dose-response curves obtained with eggs during nuclear multiplication are more or less sigmoid (Fig. 3, left panel). This type of dose-response curve which is characteristic of multiple hit effects, was also found for the u.v. induction of the aberrant body segment pattern 'double abdomen' in the egg of the chironomid midge Smittia (Kalthoff, 1971b). The dose-response curves obtained after irradiation of Drosophila eggs at blastoderm stage did not show sigmoid shapes but might do so after addition of plots at lower u.v. doses (Fig. 3, right panel).

During nuclear multiplication 285 nm radiation produced embryonic defects most effectively (Fig. 3, left panel). This result is in agreement with an action spectrum for the u.v. induction of double abdomens in Smittia eggs, which has been worked out in more detail under similar experimental conditions (Kalthoff, 1973). A maximum in the efficiency of irradiation around 260 nm, as found by...
Goldmann & Setlow (1956) in an action spectrum for photoinactivation of early normal development of Drosophila by irradiating whole eggs, could not be observed after the partial irradiation employed in our experiments. Observing the types of anterior defects occurring after irradiation at different wavelengths in some more detail, we found that the maximum efficiency of 285 nm radiation in eggs during cleavage was accompanied by an overproportional increase in the frequency of embryos with gut extrusions at the anterior end (Fig. 4H–J). The pronounced efficiency of 285 nm radiation was no longer found after blastoderm formation. At this stage, 245, 265 and 285 nm radiation displayed rather similar efficiencies in producing embryonic defects. With both stages 305 nm radiation was least effective. Generally, irradiations at blastoderm stage were more effective than corresponding irradiations during nuclear multiplication. This was also found by Goldman & Setlow (1956) and by Ghelelovitch (1966), who irradiated whole Drosophila eggs, and in corresponding observations after irradiation of whole Smittia eggs (Kalthoff, unpublished). In contrast, Hathaway & Selman (1961) found that Drosophila eggs were more easily damaged by microbeam irradiations during nuclear multiplication than after blastoderm formation.

Table 2. Influence of illumination with blue light after u.v. irradiation: Anterior fourths or pole caps of eggs at blastoderm stage were u.v. irradiated at 265 or 285 nm wavelength. Subsequently, half of the eggs were illuminated with blue light (433 nm) at a dose rate of about 200 ergs.mm$^{-2}$.sec$^{-1}$ for 30 min (+), while the other half was incubated in the dark immediately after (u.v.) irradiation (–).

<table>
<thead>
<tr>
<th>Irradiated egg area</th>
<th>Anterior pole cap</th>
<th>Anterior fourth</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.v. dose (ergs.mm$^{-2}$)</td>
<td>1500</td>
<td>1500</td>
</tr>
<tr>
<td>U.v. wavelength (nm)</td>
<td>265</td>
<td>285</td>
</tr>
<tr>
<td>Number of single experiments</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Blue light after u.v.</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Total number of eggs</td>
<td>141</td>
<td>149</td>
</tr>
<tr>
<td>Number of normal larvae</td>
<td>54</td>
<td>88</td>
</tr>
<tr>
<td>Number of defective embryos</td>
<td>86</td>
<td>54</td>
</tr>
<tr>
<td>Number of undiffer. eggs</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>% normal larvae</td>
<td>38</td>
<td>59</td>
</tr>
<tr>
<td>Difference % normal larvae</td>
<td>21</td>
<td>38</td>
</tr>
</tbody>
</table>

Experiments involving photoreversion

U.v. irradiation is an unspecific treatment at the molecular level because many types of biologically important molecules absorb u.v. radiation. However, when accompanied by photoreversal in a living system, u.v. effects may be considered more specific. Photoreversal, which is usually referred to as photoreactivation after u.v. inactivation, is defined as partial reversal of far-u.v. effects by subsequent treatment with near u.v. or visible radiation. According to present know-
U.V.-irradiation of Drosophila eggs

U.V.-irradiation is observed in living systems only if nucleic acids are involved as effective targets of u.v. While u.v. is thought to produce pyrimidine dimers in nucleic acids, photoreversal is considered as a light-dependent enzymic process splitting these dimers (Setlow, 1966; Levin & Jordan, 1973; Trosko & Wilder, 1973). Therefore, we looked for photoreversal of the u.v. effects observed in our experiments.

In the experiments involving photoreversion, eggs remained in position in the vessel after u.v. irradiation, and were then immediately irradiated with blue light. Control eggs taken from the same batch were incubated in the dark after u.v. irradiation. The results of these experiments have been compiled in Table 2: illumination with blue light after partial u.v. irradiation of eggs at blastoderm stage caused a significant increase in the proportion of normal larvae. The difference in the percentages of normal larvae, obtained with and without photoreversal, were 21 or 38% after u.v. irradiation at 265 nm or 285 nm, respectively. Preliminary experiments have indicated that little if any photoreversal occurred after u.v. irradiation of eggs at nuclear multiplication or syncytial blastoderm stages. This has also been observed by Ghelelovitch (1966).

Descriptions of defects resulting from irradiations of different areas of the egg

The experiments described in the following section were designed to obtain a survey of the embryonic defects resulting from irradiation of different egg areas at the two stages used. Eggs during nuclear multiplication were irradiated at 285 nm, which is the most effective wavelength for the production of anterior defects at this stage. Eggs in Bl stage were irradiated at 265 nm; at this wavelength the nucleic acids in the chromatin of blastoderm cells should be damaged as specifically as possible. The time of irradiation was varied in inverse proportion to the length of the area irradiated, so that all eggs absorbed similar amounts of energy. The results of these experiments are compiled in Table 3 and some examples illustrating the range of defects within the classes are shown in Fig. 4 D–Q. For comparison Fig. 4 A–C shows normal embryos from dorsal, ventral and lateral views at the time of hatching.

Irradiation of anterior quarters, eighths, halves and anterior pole caps led to defects classified as I or II, with similar ranges of defects within the classes. Figure 4 D–J shows some of the varying degrees of abnormality in the anterior embryo. Figure 4 D and E show embryos with no heads or mouthparts, but with all of the abdominal segments present, and spread to the anterior of the egg. This type of defect, which might be considered to be most specifically anterior, occurred only after irradiation of the anterior pole cap at stages NM or Syn Bl. Figure 4 F and G show examples of abnormal head involution. Figure 4 H–J shows examples where the anterior of the egg contains extruded yolk, but the posterior has some segmentation and the spiracles are present. Embryos similar to this were found with just a few abdominal segments abnormally formed or with a range of levels of organization up to those with all posterior segments and a
Table 3. The effect of u.v. irradiation on various regions of the egg at nuclear multiplication (NM), syncitial blastoderm (Syn Bl) and blastoderm (Bl) stages

<table>
<thead>
<tr>
<th>Stage</th>
<th>Areas</th>
<th>Number treated</th>
<th>Normal larvae</th>
<th>Undifferentiated eggs</th>
<th>Embryos defective</th>
<th>Distribution of defects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Class I</td>
</tr>
<tr>
<td>NM</td>
<td>Anterior eighth</td>
<td>81</td>
<td>24</td>
<td>30</td>
<td>27</td>
<td>23</td>
</tr>
<tr>
<td>NM</td>
<td>Anterior quarter</td>
<td>133</td>
<td>9</td>
<td>58</td>
<td>66</td>
<td>60</td>
</tr>
<tr>
<td>NM</td>
<td>Anterior half</td>
<td>121</td>
<td>1</td>
<td>58</td>
<td>62</td>
<td>56</td>
</tr>
<tr>
<td>NM</td>
<td>Posterior eighth</td>
<td>76</td>
<td>11</td>
<td>30</td>
<td>35</td>
<td>4</td>
</tr>
<tr>
<td>NM</td>
<td>Posterior quarter</td>
<td>108</td>
<td>3</td>
<td>34</td>
<td>71</td>
<td>1</td>
</tr>
<tr>
<td>NM</td>
<td>Posterior half</td>
<td>53</td>
<td>1</td>
<td>18</td>
<td>34</td>
<td>1</td>
</tr>
<tr>
<td>NM</td>
<td>Middle quarter</td>
<td>76</td>
<td>3</td>
<td>35</td>
<td>38</td>
<td>23</td>
</tr>
<tr>
<td>NM</td>
<td>Anterior pole</td>
<td>186</td>
<td>3</td>
<td>99</td>
<td>95</td>
<td>82</td>
</tr>
<tr>
<td>NM</td>
<td>Posterior pole</td>
<td>91</td>
<td>1</td>
<td>27</td>
<td>63</td>
<td>1</td>
</tr>
<tr>
<td>Syn Bl</td>
<td>Anterior pole</td>
<td>47</td>
<td>1</td>
<td>24</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>Bl</td>
<td>Anterior eighth</td>
<td>53</td>
<td>18</td>
<td>23</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Bl</td>
<td>Anterior quarter</td>
<td>94</td>
<td>21</td>
<td>12</td>
<td>61</td>
<td>58</td>
</tr>
<tr>
<td>Bl</td>
<td>Anterior half</td>
<td>41</td>
<td>1</td>
<td>7</td>
<td>33</td>
<td>31</td>
</tr>
<tr>
<td>Bl</td>
<td>Posterior eighth</td>
<td>54</td>
<td>22</td>
<td>19</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Bl</td>
<td>Posterior quarter</td>
<td>74</td>
<td>18</td>
<td>13</td>
<td>43</td>
<td>2</td>
</tr>
<tr>
<td>Bl</td>
<td>Posterior half</td>
<td>31</td>
<td>1</td>
<td>2</td>
<td>28</td>
<td>4</td>
</tr>
<tr>
<td>Bl</td>
<td>Middle quarter</td>
<td>45</td>
<td>—</td>
<td>5</td>
<td>40</td>
<td>28</td>
</tr>
<tr>
<td>Bl</td>
<td>Anterior pole</td>
<td>589</td>
<td>46</td>
<td>51</td>
<td>492</td>
<td>382</td>
</tr>
<tr>
<td>Bl</td>
<td>Posterior pole</td>
<td>48</td>
<td>—</td>
<td>1</td>
<td>47</td>
<td>7</td>
</tr>
</tbody>
</table>

**Figure 4**

Fig. 4. A sample of embryonic defects resulting from u.v. irradiation of different regions of the egg.

A, B and C are normal embryos at hatching stage.

D and E show embryos with just an abdomen, the segments spreading to the anterior of the egg. These result from irradiation of anterior pole caps at NM and Syn Bl only.

F and G are examples of abnormal head formation and H, I, and J show embryos with gut extruded at the anterior, with varying numbers of abdominal segments. These are found after irradiation of anterior eighths, quarters, halves and pole caps at NM and Bl. D–J represent class I.

K shows an embryo with a gut mass at the posterior and yolk at the anterior, L has a contracting gut mass only, and H a posterior yolk mass and gut at the anterior. All these are unspecific class I defects resulting from irradiation of all egg regions at NM and Bl.

N shows an embryo with abnormal head and mouthparts at the anterior, and a contracting gut mass at the posterior. O, P and Q have abnormal head and mouthpart formation, with varying numbers of abdominal segments; the gut is extruded at the posterior. These class III defects result from irradiation of posterior eighths, quarters, halves and posterior pole caps at NM and Bl.
well-formed tracheal system and possibly some thoracic segments (4H and I). Figure 4J shows an egg with the gut extruded anteriorly with the posterior well segmented, but with the segments curled around the posterior, and the spiracles situated dorsally. Figure 4K–M show the unspecific class II abnormalities. The embryo shown in Fig. 4K has a large, dark yolk patch at the anterior of the egg,
Fig. 4. For legend see page 338.
Fig. 4. For legend see page 338.
and the posterior is a contracting gut mass; such embryos sometimes have a few abnormally arranged bristles on the surface. Figure 41 represents a contracting gut mass, and Fig. 4M shows a posterior yolk patch with an anterior gut mass.

Irradiations of posterior eighths, quarters, halves and posterior pole caps produced class III embryos with abnormal posterior organization, as seen in Fig. 4N–Q, and the unspecific class II defects described above.

Figure 4N demonstrates a type of posterior defect where the head has formed abnormally, but with mouthparts present, and the rest of the embryo a mass of yolk and gut. Figure 4O–Q shows embryos with the head and mouthparts perfectly formed, and increasing numbers of segments also formed; the gut is extruded at the posterior in all cases. Occasionally damage of anterior structures was found, such as in Fig. 4H–J described previously. This is difficult to explain; possibly these are secondary defects occurring after abnormal head involution which in turn might be caused by various failures in germ band formation. However, those defects regarded as most specifically anterior, i.e. embryos with just abnormal head or mouthparts were never found as a result of posterior irradiation. Irradiation of middle quarters of the egg led to defects shown in Fig. 4H–M; these are the least specific anterior abnormalities found in class I and the unspecific types of class II. No posterior defects were found after irradiation of the middle of the egg.

Irradiations at nuclear multiplication and blastoderm stages led to the production of similar abnormalities, with the possible exception of irradiating the anterior pole cap. After treatment embryos were found with no head or mouthparts (Fig. 4D and E). These embryos occurred after irradiation at NM and SynBl, but none were found after irradiation of the cellular blastoderm.

DISCUSSION

This study was undertaken to test the effects of partial u.v. irradiation on the early development of Drosophila eggs and to compare these effects with the results after similar experiments using Smittia eggs, and with the effects resulting from other experimental manipulations on Drosophila eggs. The early embryogeneses of higher Dipterens are very similar. Moreover, eggs from these species display common features with respect to aberrant development after experimental manipulations. After transverse fragmentation, both the anterior and posterior parts produce partial germ bands with normal polarity, the anterior fragment including head structures but not posterior structures, and the posterior fragment including at least the last abdominal segment but no anterior structures. Some intermediate segments are usually missing from both fragments; the number of missing segments decreases with the age of the egg at fragmentation (Herth & Sander, 1973).

By partial u.v. irradiation the body segment pattern of Smittia can be drastically altered. The targets responsible for the u.v. induction of double abdomens
U.V.-irradiation of Drosophila eggs

in Smittia are extranuclear and they are obviously prelocalized in oogenesis (Kalthoff, 1971b). The occurrence of the maternally inherited mutation 'bicaudal' demonstrates that at some stage during Drosophila oogenesis the polarity of the anterior half of the oocyte can be reversed. The double abdomen type of abnormality occurs spontaneously or can also be experimentally induced in the eggs of other insect species (Price, 1958; Sander, 1961; Yajima, 1964; Schnetter, 1965).

However, the experiments described in this paper show that, by the irradiation techniques so effective in Smittia eggs, the polarity of the anterior of the Drosophila egg can no longer be reversed one hour after oviposition. It seems that the egg is determined at least with respect to its anterior/posterior axis at this time, as anterior irradiations lead to anterior defects and posterior irradiations lead to posterior defects. This was also found by W. Herth (Diploma thesis, Freiburg, 1970) after partial u.v. irradiation of Drosophila eggs using u.v. from a germicidal lamp. Our experiments do not substantiate the idea that the egg is completely mosaic, as irradiations of eighths, quarters and halves give similar deficiencies and irradiation of middle quarters produces anterior defects. One reason for this could be that the initial damage caused in small or large areas affects the subsequent morphogenetic movements in some way, giving similar final abnormalities. To check this the development of these abnormalities after irradiation must be followed. By such observations it should also become clear if those anterior defects occurring after irradiation of posterior or middle egg regions are caused indirectly, e.g. by interference with normal head involution. A correlation between the u.v. irradiated egg area and the type of the resulting embryonic defects was also found by Hathaway & Selman (1961), who irradiated areas of 60 μm diameter in Drosophila eggs at the same stages used here. As with our experiments the results were similar after irradiation at these two stages, except that they found a higher sensitivity of the eggs to u.v. damage during nuclear multiplication rather than after blastoderm formation. Nöthiger & Strub (1972) irradiated lateral and slightly dorsal areas measuring 69 x 27 μm in Drosophila eggs aged 2 through 20 min. They could not find a correlation between the site of irradiation and the location of the defect in the adult fly. However, they note that the apparatus employed in their experiments produced a halo of u.v. of different wavelengths around the target area.

Experimental manipulations other than regional u.v. irradiation have also been used to demonstrate correlations between the site of a damaged egg region and the type of abnormality that results. Microcautery (Bownes & Sang, unpublished) damages smaller areas of the egg than the irradiations used in the experiments described here and consequently most of the resulting embryos have much smaller defects. Many embryos show small defects in several systems, such as mouthparts, tracheals, gut, segmentation or head involution, but some are found corresponding to the defects shown in Fig. 4H–J and Fig. 4P–Q, where one half of the egg has developed well and the other is just a mass of gut.
or undifferentiated tissue. These types of defects arise from microcautery of areas in the anterior three-quarters of the egg or areas in the posterior half of the egg, respectively. There seems to be an overlap in the third quarter of the egg, as either anterior or posterior deficiencies occur. There is no distinct difference in the pattern of abnormalities thus produced in eggs aged 1 or 3 h. Physical removal of blastoderm cells by pricking the embryo (Bownes & Sang, unpublished) also leads to embryonic abnormalities consistent with the results of our u.v. irradiations. Anterior pricking leads to anterior defects like the ones shown in Fig. 4F–L; posterior pricking causes defects similar to those shown in Fig. 4L–Q. Mid-lateral removal of cells leads most frequently to anterior defects, which is consistent with our results from irradiations of middle quarters of the embryo.

The wavelength dependence of the u.v. induction of embryonic defects in Drosophila as observed in our experiments does not allow us to identify the types of target molecules, since too few wavelengths have been tested. The pronounced efficiency of irradiation at 285 nm (Fig. 3, left panel) might be taken as a hint on the involvement of a protein in the production of anterior defects at nuclear multiplication stage. These targets are probably extranuclear since the nuclei are heavily shielded by yolk material at this stage. After blastoderm formation, irradiations at 245, 265 and 285 nm were of similar efficiency, which was higher than in corresponding experiments with eggs during nuclear multiplication (Fig. 3). These differences can be interpreted on the assumption that molecules not exposed at the earlier stage, e.g. nucleic acids in the chromatin of the blastoderm cells, act as additional targets at the later stage. However, our results can be interpreted as well by assuming that (1) embryonic defects not distinguished in our scoring procedure are produced by u.v. damage to different types of molecules, that (2) the ability of the embryo to repair or compensate for the u.v. damage to these molecules soon enough decreases with later stages, and that (3) the decrease in the capacity for repair or compensation occurs with different kinetics for different types of molecules.

The results of the experiments involving photoreversion also do not allow definite conclusions about the molecular mechanisms effective in the u.v. induction of the embryonic defects observed. The photoreversibility of defects occurring after u.v. irradiation at blastoderm stage might indicate that nucleic acids are involved as effective target molecules at this stage. However, this conclusion is complicated rather than confirmed by the fact that photoreversion after u.v. irradiation at 265 nm was less effective than after u.v. irradiation at 285 nm. Corresponding results obtained with Smittia eggs have been discussed by Kalthoff (1973).

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