Experimental manipulations of early *Drosophila* embryos

II. Adult and embryonic defects resulting from the removal of blastoderm cells by pricking

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**SUMMARY**

Cells were removed from three regions (anterior, mid-lateral and posterior) of *Drosophila* eggs at blastoderm formation, by pricking. Embryonic defects were generally correlated with the site of damage, as is also found with microcautery and with u.v. irradiation. But many structures can develop autonomously, independently of surrounding damage. Most hatching adults were normal, suggesting that there may be mechanisms which permit compensation for cell loss. Malformed or missing adult structures are those expected from the fate map obtained from studies of genetic mosaics.

**INTRODUCTION**

It has been assumed for some time that the *Drosophila* egg is mosaic at least at, if not prior to, blastoderm formation (Wigglesworth, 1939). If this is true, each cell should form a specific part of the embryo or adult, and if cells are damaged or removed the relevant parts should be missing. Howland & Child (1935) reported some correlation between the site of pricking blastoderm eggs with particular defective structures found in adults. The age of their eggs was rather variable, and as very few adult defects were found their evidence cannot be used as proof of determination at this stage. Howland & Sonnenblick (1936), on the other hand, found some evidence of regulation in nuclear multiplication stage *Drosophila* embryos after removal of material by pricking. The implication was that determination was established in the uniform cellular blastoderm, but not before. The subject has now been pursued using this simple technique.

The experiments described in this paper were designed to test the idea of mosaic determination in blastoderm eggs of *Drosophila* with respect to both larval and adult organization. The important difference between this technique and u.v. irradiation (Hathaway & Selman, 1961; Nöthiger & Strub, 1972;
Bownes & Kalthoff, 1974) or microcautery (Bownes & Sang, 1974), which have been used to damage regions of the egg, is that cells are physically removed from the blastoderm. Damaged molecules may be repaired by mechanisms within the egg after u.v. irradiation (Setlow, 1966) or possibly after microcautery; but if cells are actually removed and normal larvae and adults are produced, then cells in the surrounding area must be capable of compensating for the absent cells, and regulation of some type is occurring.

Pricking experiments can show that determination for adult structures has occurred if damage to areas known from somatic cross-over studies to contain presumptive adult cells (Hotta & Benzer, 1972) consistently produce defects in the expected adult structures. However, if defects are not found it does not prove that the areas were not determined at this time. Schubiger (1971) has shown that the discs of 3rd instar larvae are capable of regenerating the missing parts when they are cut into portions, so long as a specific area of the disc is left intact. It seems likely that this could also be true at earlier stages, so the chances of damaging or removing all the cells necessary to produce an adult with defective cuticular structures may be quite small. Another possibility is that a small group of cells may be determined to be an adult disc, but at this stage removal of any part of them does not result in a defective adult, due to compensation for the loss by surrounding cells. It is probable, too, that many surrounding larval cells will be removed, the changes of embryonic or larval death will be increased, and adults may rarely emerge. But larval defects can then be identified.

Three areas of the embryo were pricked – the anterior, posterior and mid-lateral areas shown in Fig. 1. From the data, resulting embryonic defects could be compared with those found after partial u.v. irradiation of Drosophila embryos at blastoderm formation (Bownes & Kalthoff, 1974), and the expected adult defects could be predicted from Fig. 2.

If the egg is fully determined at blastoderm formation, some larval cells should always be removed and all treated eggs should produce defective embryos or larvae, even if these cannot always be identified. As the number of disc cells is in all cases less than the number of cells removed (Nöthiger, 1972), adult defects should be found, provided the larvae survive. The probability of producing adults with abnormal heads should be high after pricking the anterior region; mid-region damage should cause thoracic deficiencies, and posterior treatments would be expected to cause abdominal defects.

**MATERIALS AND METHODS**

*Origin and preparation of eggs*

All eggs were of Oregon K stock and were collected on agar plates coated with a paste of yeast and sugar. Egg collections were made every 30 min (after the first hour of laying, in order to exclude any partially developed retained eggs). Eggs were then left to develop for a further 45 min, before being washed from the
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**Experimental procedure**

Eggs of the correct developmental stage were placed on a piece of black filter paper, as this makes the eggs easily visible for orientation and also dries them. If the surface of the egg is damp, pricking causes the egg to collapse completely as the contents drain out by capillary action. However, with slight drying the globule of cells remains on the surface of the egg (Fig. 3). The eggs were pricked with a fine tungsten needle mounted in a glass holder. The needle was made the required shape by electrolytically removing some of the tungsten at the tip in sodium hydroxide; suitable needles were chosen by trial. The pricked eggs were then transferred on the filter paper to small dishes, and covered in liquid paraffin. The dishes were placed in damp sealed boxes at 25 °C for 24 h.

**Scoring**

The eggs were classified into (1) normal larvae, (2) undifferentiated eggs which failed to continue development after pricking and (3) defective embryos.

agar with 0.9% sodium chloride, then dechorionated with 3% sodium hypochlorite for 5 min. They were washed in isotonic sodium chloride, and eggs at the cellular blastoderm stage of development were selected, using a dissecting microscope.

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**Fig. 1** Location of regions where cells were removed for these experiments.

**Fig. 2** Location of presumptive adult disc cells. This figure is based on the map produced by Hotta & Benzer (1972) using genetic mosaic studies. The egg is 0.42 mm long.
Fig. 3. Typical appearance of an egg after pricking at blastoderm stage, with a small group of cells on the surface.

Analysis of defective embryos

Defective embryos were mounted in paraffin oil on a cavity slide, and covered by a coverslip to prevent the eggs from collapsing further. These eggs were looked at under higher magnification using Nomarski optics and classified according to the damaged structures. Photographs taken under these conditions are very unsatisfactory due to the collapsed vitelline membrane and the paraffin oil, but detail within the eggs could be seen by refocusing the microscope.

Classification of embryonic defects

Class I. These embryos show only anterior defects. Mouthparts or other head structures may be abnormal or absent, and the gut may be extruded at the anterior of the embryo. Anterior abdominal segments may also be lacking, 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 have formed irregular 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 partially formed or replaced by a mass of yolk and gut. All embryos in this class show head structures, and at least some indication of mouthparts.
Table 1. General results after removal of blastoderm cells from Drosophila eggs

<table>
<thead>
<tr>
<th>Region damaged by pricking</th>
<th>Anterior (%)</th>
<th>Middle (%)</th>
<th>Posterior (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs failing to continue development</td>
<td>50.6</td>
<td>64.4</td>
<td>57.1</td>
</tr>
<tr>
<td>Larvae hatched</td>
<td>18.0</td>
<td>12.5</td>
<td>23.4</td>
</tr>
<tr>
<td>Abnormal embryos</td>
<td>31.4</td>
<td>23.0</td>
<td>19.7</td>
</tr>
<tr>
<td>Larvae dying</td>
<td>15.0</td>
<td>10.0</td>
<td>15.1</td>
</tr>
<tr>
<td>Pupae failing to hatch</td>
<td>—</td>
<td>0.3</td>
<td>—</td>
</tr>
<tr>
<td>Normal adults hatched</td>
<td>2.9</td>
<td>2.0</td>
<td>7.1</td>
</tr>
<tr>
<td>Abnormal adults hatched</td>
<td>—</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Total number of eggs treated</td>
<td>472</td>
<td>312</td>
<td>264</td>
</tr>
</tbody>
</table>

Analysis of adults

The hatched larvae were put into vials containing yeasted Lewis medium and kept at 25 °C to continue development. Adults were scored for abnormalities in external morphology, and any pupae failing to hatch were dissected and abnormalities noted. Adults were kept at least 5 days to ensure that they did not die early due to deficiencies in the soft internal parts of the gut; their fertility was checked, and they were then dissected to note any abnormalities of the ovary or testis.

RESULTS

Table 1 shows that many eggs treated in each of the three regions failed to continue development. These eggs usually collapsed because too many cells were removed from the embryo. Some defective embryos which failed to hatch and some larvae of apparently normal morphology also resulted from treatment of each area. Many of these larvae died, but in all cases some adult flies were found.

Embryonic data

The occurrence of apparently normal adults and larvae from eggs damaged at three different areas shows that embryonic cells are capable of limited regulation, and that surrounding cells may be able to compensate for the loss of nearby embryonic cells.

Table 2 shows the distribution of embryonic abnormalities resulting from damaging different regions of the egg. The classification scheme is the same as that used for the results of irradiating different regions of the blastoderm egg with u.v. by Bownes & Kalthoff (1974). It can be seen from Table 2 that the distribution of embryonic defects obtained was very similar to that found after u.v. irradiation of these areas. Anterior pricking produced anterior defects, and
Table 2. Classification of defective embryos after removal of cells by pricking and u.v. irradiation

A. Pricked eggs

<table>
<thead>
<tr>
<th>Area damaged by pricking</th>
<th>Total number defective embryos</th>
<th>Distribution into classes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Anterior</td>
<td>148</td>
<td>53·5</td>
</tr>
<tr>
<td>Middle</td>
<td>72</td>
<td>43·0</td>
</tr>
<tr>
<td>Posterior</td>
<td>51</td>
<td>4·0</td>
</tr>
</tbody>
</table>

B. Irradiated eggs (data of Bownes & Kalthoff, 1974)

<table>
<thead>
<tr>
<th>Area damaged by u.v. irradiation</th>
<th>Total number defective embryos</th>
<th>Distribution into classes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Anterior quarter</td>
<td>61</td>
<td>95·1</td>
</tr>
<tr>
<td>Middle quarter</td>
<td>43</td>
<td>70·0</td>
</tr>
<tr>
<td>Posterior quarter</td>
<td>40</td>
<td>4·7</td>
</tr>
</tbody>
</table>

posterior damage produced posterior defects. At all sites a significantly greater number of the more damaged, less specific Class II embryos were found after pricking experiments than after u.v. irradiation. As with irradiation of a central band of the egg, pricking in the mid-lateral region led to essentially anterior abnormalities (there was no significant difference between the distribution of abnormalities after pricking at the anterior or the mid-region, at the 5 % confidence level using a $\chi^2$ test). The variation of defects within the classes is the same as that described and illustrated with photographs by Bownes & Kalthoff (1974). These results corroborate the u.v. data, and suggest that there is a general anterior/posterior organization present in the egg at blastoderm formation, but that regulation within these areas is still possible.

Apart from the general variation of embryonic defects within the classification used, observation of specific eggs can help our understanding of the organization of the egg at this stage in development.

Figure 4 shows how eggs with extremely collapsed membranes, which have obviously lost a large number of cells, are able to develop to advanced stages of embryogenesis. This egg, which was pricked in a mid-lateral region, has developed all the abdominal segments. It has fully formed spiracles, and Malpighian tubules are present at the posterior. The head has failed to form at the anterior. Figure 5 shows an embryo, pricked at the posterior, with a normal head and all the abdominal segments formed in the anterior half of the egg, with gut extruded at the posterior. Tracheal tissue has formed in this area. The embryo in Fig. 6 was pricked at the anterior and shows that, conversely, an embryo formed in the
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Fig. 4. The vitelline membrane of this embryo has collapsed considerably due to loss of cells and yolk, yet some larval differentiation has occurred, including abdominal segmentation, and formation of spiracles and Malpighian tubules. A, Anterior.

Fig. 5. An embryo resulting from posterior pricking. The head and mouthparts (m) are normal and parts of all eight abdominal segments are present in the anterior half of the egg. The posterior contains a yolk mass (y), with trachea (t) above. A, Anterior.

Posterior two-thirds of the egg has all the abdominal segments and some abnormal thoracic and head formation; the rest consists of a mass of undifferentiated tissue.

The two types of embryo illustrated in Figs. 5 and 6 suggest that reorganization of the embryo is possible after blastoderm cells have been removed by pricking, as most of the normal structures of the embryo are formed, occupying a smaller area than usual; much undifferentiated tissue is present, which must normally have a function in embryonic development.

One unique embryo (Fig. 7) resulted from pricking the mid-region of the egg. Instead of destroying basically anterior structures, as is usually seen, this embryo is damaged in the middle. The embryo shows segmentation that is well developed along the dorsal edge. It has a complete cephalo-pharyngeal complex situated
obliquely at the anterior of the embryo, and has spiracles with tracheal tracts at the posterior. Ventrally, and to the extreme anterior and posterior of the embryo, the egg contains a mass of undifferentiated tissue. It seems in this case that the egg has reacted in a more determined fashion, suggesting that the mid-region of the anterior–posterior axis had become fixed. This occurred only once, but may suggest that fixation of the mid-region occurs at a time close to formation of the blastoderm.

Figures 8 and 9 show that once certain structures have become determined they seem to be able to differentiate amongst a mass of undifferentiated tissue; they do not need to be in their correct position, nor do they need the presence of organs normally closely associated with them in development. Figure 8 shows detail from an egg containing nothing but some dorsally situated spiracles, and Fig. 9 shows a few segments radiating from a central point over a mass of undifferentiated tissue. Thus at least some determined cells may differentiate whatever their locus.

**Adult defects**

Three of the four adult defects found were correlated with the sites of damage indicated in Fig. 1. No abnormalities were found in flies damaged in the anterior region. In one pupa which failed to hatch, the wing, thorax and one leg were all defective; this embryo had been pricked in a mid-lateral region where the map indicated that these cells are located. Another embryo damaged in this region had a smaller defect in the meta-thoracic leg. One of the eggs pricked in the posterior region produced an adult with an abnormally shaped abdomen. Another adult which hatched had no abnormalities in external morphology, but
Fig. 7. An exceptional embryo resulting from mid-region pricking. The anterior has well formed mouthparts (m) and the posterior contains spiracles (s). Segments are formed along one side of the embryo, yet are broken on the other side where yolk has escaped. Yolk (y) occupies one side of the embryo and part of the anterior and posterior regions of the egg. A, Anterior.

died only one day after hatching, and probably had an abnormality in the internal structures. Abnormalities occurring with this low frequency cannot prove that these areas of the egg are determined at this stage in development, but the occurrence of several normal adults from each area, when the probability of damaging or removing the presumptive disc cells is very high, suggests that if the cells are determined, there is some regulative capacity within the determined area.
Fig. 8. An embryo in which spiracles have formed in the absence of other differentiated tissues. D, Dorsal.

Fig. 9. An embryo in which rows of segmental bristles (b) radiate out from one point, in the absence of other differentiated tissue. P, Posterior.
Perhaps the most surprising result of these experiments is that most of the adults formed after pricking in all of the three regions are apparently normal, contrary to expectation. Howland & Child (1935), Howland & Sonnenblick (1936), Ilmensee (1972) and Nöthiger & Strub (1972) all similarly reported a high proportion of hatched normal adults after damaging various regions of the egg. There could be several reasons why defects are not formed. (1) Not all structures need be present and complete to form a viable larva or adult (e.g. paired organs or large organs like fat body, etc.). Defects of this class could arise from hemi-lateral pricking, and would not necessarily be detected. However, this should be an exceptional class with respect to adult external morphology, and is unlikely to be a general explanation of the high adult survival, if only because all regions of the egg have been damaged in the experiments noted above and all still give normal adults. (2) Cells may not be determined at the time of treatment, and a more or less normal pattern of determination may be established subsequently. Chan & Gehring's (1971) experiment would argue against this since it shows that adult disc cells are determined in the blastoderm, and that this determination can survive disaggregation of anterior (or posterior) halves of blastoderm eggs, their reaggregation, and transplantation and growth in adults. Bownes & Sang (1974) have shown that this determination is specific for each disc. (3) There may be cellular regulation, as is found in the regeneration of missing parts of larval discs (Schubiger, 1971), and this seems the most likely explanation of these results.

Another interesting observation is the rare occurrence of head defects after experimental damage. Only one was found by Bownes & Sang (1974) after microcautery. Nöthiger & Strub (1972) found 9 head defects, but 37 thoracic and 116 abdominal defects. And in a most comprehensive survey, using u.v. irradiation of entire dorsal or ventral surfaces of eggs at various stages of development, Levin (1971) found 18 head defects, but 110 defects of thorax and scutellum, 245 wing defects, 175 leg defects and 625 abdominal defects. Unfortunately, Levin's dosages of u.v. vary with embryonic age (to give a constant mortality), so it is difficult to do more than note this phenomenon and its indication of differential sensitivity of different discs.

The types of embryonic defects found after pricking correlate well with experimental damage by u.v. irradiation (Bownes & Kalthoff, 1974) and by microcautery (Bownes & Sang, 1974), indicating the extent of determination in comparable areas. As we have noted, small areas frequently show a high degree of autonomy, and develop independently of the damaged systems around them. This might be expected from the cell culture studies of Shields & Sang (1970; and unpublished observations) where the cells from disaggregated 4–6 h embryos form the majority of cell and tissue types in vitro. That is, the embryonic cells show a high degree of determinacy at this stage and proceed to their final
differentiated state; they would similarly be expected to do so in the damaged embryo.

We would attach some weight to the positive results, where damage to adult structures is precisely that which would be expected from genetic mapping experiments (Hotta & Benzer, 1972). Like the mapping experiments, these results do not tell us exactly when cells become determined to be adult structures. Our earlier results (Bownes & Sang, 1974) suggest that this is not at pre-blastoderm stages, which implies that the egg is not mosaic, in the sense that determinants of differentiation are already patterned in some final form in the egg cortex. Subsequently, determination is a progressive and developmental process which rapidly seems to reach a state which defines at least larval structures, and the allocation of characteristics of particular discs to other cells. These disc cells multiply in the larva, and their progeny also go through a series of determinative steps which settle their fate within a particular structure (e.g. leg) so as to give it its final form (see Nöthiger, 1972). Thus, determination is a sequential, epigenetic phenomenon which we define experimentally and somewhat arbitrarily. It does not follow that cortical structure and content are not of primary importance for this process: our experiments tell us nothing about this. What they do imply is that determination of adult disc cells is established during a very short space of time after blastoderm formation, and that there may be some regulation if the egg is damaged then. This would argue that the distinction between mosaic and regulative eggs is one of degree rather than of kind.

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REFERENCES


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