Adult deficiencies and duplications of head and thoracic structures resulting from microcautery of blastoderm stage *Drosophila* embryos

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**SUMMARY**

*Drosophila* embryos were damaged by microcautery at the cellular blastoderm stage at the sites of presumptive imaginal cells, identified from fate maps. The resulting adults were analyzed for abnormal structures. Cautery of any of the presumptive imaginal regions can lead to defects in the adult cuticle, though the majority of adults which hatch are morphologically normal. The abnormal adults had one or more discs either deleted, incompletely formed, or misarranged. Several of the structures which were incomplete had duplicated regions. The results suggest that, from the time of their initial formation in the cellular blastoderm, a group of cells determined to be an 'adult' structure possesses a gradient of developmental capacity which is expressed by certain regions duplicating and other cells regenerating. The types of duplications found were similar to those resulting from other experimental treatments of imaginal discs at later stages in development, indicating that the presumptive imaginal disc cells, when they are first established in the cellular blastoderm, have a similar organization to mature imaginal discs.

**INTRODUCTION**

Using microcautery to damage small groups of cells in the blastoderm Bownes & Sang (1974) were able to show that the resulting adult defects were correlated with the initial site of damage. The positions of the presumptive adult disc cells were shown to correspond to those calculated by Hotta & Benzer (1972) using mosaic mapping techniques. One of the interesting results of these experiments was the high frequency of normal adults which hatched after blastoderm treatment. (Possible explanations for the normal adults were discussed by Bownes & Sang (1974).) The reason most likely to account for these normal adults is that presumptive disc cells remaining intact after microcautery compensate for the loss of surrounding damaged cells. This could occur in two ways, either the remaining cells could be reorganized to form a complete presumptive disc again, or the cells at the damaged surface could divide and...
regenerate the deleted regions. If these regulatory mechanisms exist within the presumptive disc cells, we can use the technique of microcautery to answer some questions about the basic organization of the presumptive disc cells when they are first established in the developing embryo.

When an imaginal disc is cut into parts, the parts can be cultured in an adult and then passed through metamorphosis in a larva. Some regions of the disc will regenerate missing parts and other regions will merely duplicate the structures (Schubiger, 1971; Bryant, 1974, 1975). It seems likely that if some of the prospective disc cells in the blastoderm are capable of regenerating missing parts, then complementary regions may duplicate. Any duplications should be recognizable in the resulting structures.

This paper describes in detail the types of duplications and defective structures found in the adults which hatched after blastoderm treatment, and compares them to the kinds of results obtained by other experimental techniques at later developmental stages, e.g. X-rays (Postlethwait & Schneiderman, 1973; Postlethwait, 1975), cutting and culturing discs (Gehring, 1966; Bryant, 1971; Schubiger, 1971; van der Meer & Ouweneel, 1974; Bryant, 1975), and cautery (Santamaria & Garcia-Bellido, 1972). Similar defective adult structures are also observed in temperature-sensitive cell lethal mutants (Russell, 1974; Arking, 1975; Simpson & Schneiderman, 1975), and morphologically abnormal mutants (Sang & Burnet, 1963; Fristrom, 1970; James, personal communication).

MATERIALS AND METHODS

Collection and preparation of eggs

Oregon-R eggs were collected on yeasted agar plates at 25 °C. After aging the eggs for the required time on the collecting plates at 25 °C, they were dechorionated for five minutes in sodium hypochlorite and washed. The dechorionated eggs were placed in 0.9% sodium chloride and eggs which were at the cellular blastoderm stage were selected for treatment.

Microcautery technique

The microcautery techniques used in these experiments were identical to those described by Bownes & Sang (1974). The temperature of the needle was set at 75 °C. Control eggs were manipulated identically to the experimental eggs in all respects except for the microcauterization.

Analysis of defective adults

After microcautery the eggs were placed on agar and incubated at 25 °C for 24 h. The numbers of eggs failing to develop, the number developing abnormally, and the number of hatched larvae were recorded. Yeast paste was then added to the agar plate and the resulting pupae were transferred into vials at 25 °C.
### Table 1. General results of microcautery of head and thoracic regions at blastoderm formation

<table>
<thead>
<tr>
<th></th>
<th>Experiment</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>5,175</td>
<td>1,105</td>
</tr>
<tr>
<td>No development</td>
<td>1,100 (21.2%)</td>
<td>36 (3.9%)</td>
</tr>
<tr>
<td>Abnormal development</td>
<td>2,085 (40.4%)</td>
<td>27 (2.4%)</td>
</tr>
<tr>
<td>Larvae hatched</td>
<td>1,990 (38.4%)</td>
<td>989 (94.3%)</td>
</tr>
<tr>
<td>Pupae hatched</td>
<td>1,170 (22.6%)</td>
<td>907 (82.1%)</td>
</tr>
<tr>
<td>Adults hatched</td>
<td>864 (16.1%)</td>
<td>863 (78.0%)</td>
</tr>
<tr>
<td>Defective adults</td>
<td>116 (2.2%)</td>
<td>5 (0.42%)</td>
</tr>
<tr>
<td>Pupae formed which produce defective adults or pupae (%)</td>
<td>10%</td>
<td>0.6%</td>
</tr>
</tbody>
</table>

![Fate map of the embryo showing the location of presumptive adult cells at the cellular blastoderm stage.](image)

**Fig. 1.** A fate map of the embryo showing the location of presumptive adult cells at the cellular blastoderm stage.
Table 2. *Head and thoracic defects resulting from microcautery at blastoderm formation*

<table>
<thead>
<tr>
<th></th>
<th>Eye palpus</th>
<th>Antenna</th>
<th>Labium</th>
<th>Wing/thorax</th>
<th>Haltere</th>
<th>Legs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole structure absent</td>
<td>10</td>
<td>2</td>
<td>15</td>
<td>12</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Disc duplication and deficiency</td>
<td>1</td>
<td>2</td>
<td>3*</td>
<td>—</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Absent part deficiency</td>
<td>7</td>
<td>3</td>
<td>7</td>
<td>—</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Parts misarranged or abnormally shaped or fused</td>
<td>—</td>
<td>—</td>
<td>11</td>
<td>—</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Uneverted tissue found in the haemocoel</td>
<td>—</td>
<td>—</td>
<td>3</td>
<td>—</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>7</td>
<td>39</td>
<td>12</td>
<td>78</td>
<td></td>
</tr>
</tbody>
</table>

* One wing triplicated.

Adults were checked for morphological defects, and abnormal parts were mounted in ACS mountant (Gurr’s) and analyzed.

RESULTS

Table 1 summarizes all the results of the microcautery experiments compared to the controls. The percentage of abnormal adults and pupae is significantly increased in the experimental group. The only defects in the control animals were abnormal abdomens with fusions and deletions of tergites. Fig. 1 shows a map of the sites of the presumptive disc cells on the blastoderm surface. This map was derived from that of Hotta & Benzer (1972) and was experimentally confirmed by Bownes & Sang (1974). A large number of animals were treated with microcautery for each of the head and thoracic presumptive disc regions. Table 2 classifies the resulting adults with defects in these structures. Either one or more imaginal disc derivatives were totally absent; or one structure was absent and a neighboring structure was abnormal; or a structure was abnormal.

**Figure 2**

Shows examples of normal and defective head structures resulting from microcautery.

*an*, Antenna; *ar*, arista; *e*, eye; *fs*, frontal setae; *fos*, front orbital setae; *mp*, maxillary palpus; *o*, ocellus; *os*, orbital setae; *seg*, segments; *v*, vertex; *vs*, vertical setae.

(A) The normal head of *Drosophila*.
(B–G) Eye antenna defects.
(B) Head with setae (s) missing on the right-hand side.
(C) Head lacking the antenna and a large part of the eye; some facets and the palpus remain.
(D) Only the palpus, segments two and three of the antenna, and a tiny part of the arista remain of this head.
(E) This small remaining fragment of facets and setae failed to evert (*unev.* eye).
(F) The normal antenna.
(G) Antenna with a duplication of the arista beginning to form at point x.
Microcautery of Drosophila embryos
The abnormalities of head and thoracic structures could be classified, as in Table 2, into (a) disc derivatives deleted and (b) duplications (in these cases part of the structure is often absent and the remaining part is duplicated), (c) parts of the structure are absent but there has been no duplication of remaining parts, and (d) the structure is complete, but misarranged; (e) uneverted disc situated either in the thorax or in the abdomen.

These abnormalities will be discussed in relation to the structures which were defective rather than the area treated, as in several cases more than one disc is damaged by treatment of a particular region.

**Head defects**

The head of an adult *Drosophila* is derived from six discs. One eye, one antenna, and half of the palpus arise from one disc and half of the mouthparts from the labial disc. Fig. 2A shows the normal head. When microcautery was performed on presumptive labial or antennal, eye and palpus discs, 27 head defects were found. This low number is probably due to the importance of the cells surrounding the presumptive disc cells for larval head and mouthhook formation. Microcautery of this region frequently results in embryonic death due to abnormal larval head formation and any damage to presumptive disc cells would not therefore be detected.

(a) *Antennal, eye, and palpus defects*

Several flies showed a deletion of the imaginal derivatives of this disc. One eye, one antenna, and half of the palpus arise from one disc and half of the mouthparts.

Flies with deletions of parts of the disc presented very variable expressions of damage, ranging from cases in which just a palpus or a region of the bristles on the head were lacking to flies lacking the antenna and part of the eye, and flies with just a small remaining patch of eye facets and some cuticle and bristles. Some examples of these defects can be seen in Figs. 2B–E. Fig. 2B shows a head lacking some of the vertical and orbital setae, the ocellar seta, postvertical seta, and all of the frontal and frontorbital setae. The fly in Fig. 2C lacks a large number of head elements. The head has some facets, the vibrissae, the postorbital setae, the vertical setae, and two of the three orbital setae. The gena, parocciput, and part of the occiput are present. The whole of the antenna is absent along with the ptilinum, frons, vertex, ocellus, intraocellar and provertical setae. There are no frontorbital or frontal setae. The area deleted is from the mid-region of the eye antenna disc (Gateff & Schneiderman, 1974) leaving intact the eye region near the stalk and the palpus. There are some problems as to how this fly might have formed with regions which are separated in the third instar disc both being present in the adult. One might expect that the palpus would become detached when the adjoining cells are missing. Another
Fig. 3. (A) Normal mouthparts: \(lp\), labial palpus; \(p\), pseudotracheae; \(br\), bristle region. (B) The pseudotracheae of the labial palpus are duplicated and fused to the remains of the 1st leg. (C) The pseudotracheae are almost deleted and the setae of the labial palpus are duplicated.

(b) Labial defects

Fig. 3A shows the normal mouthparts of an adult Drosophila. After microcautery of the presumptive labial region the mouthparts were often split at the line of bilateral symmetry, only one of each of the paired structures being present. Two duplications were observed. In one case the duplicated region had fused to the first leg and was consequently joined to the thorax. Fig. 3B shows this duplication and part of the other labial disc derivative which was normal. The dense patch is derived from the leg and thorax. The labium and other labial
Degenerated tissue

4 claws

Lacks groups of sensillae

D
disc derivatives were not present. In another fly (Fig. 3C) one labial palpus was perfect, but the second was misplaced and not joined to its partner. It lacked pseudotracheae and some of the large bristles of the labial palpus, but the region with smaller bristles and hairs was duplicated. In another case the labial palpus was absent on both sides, but the maxillary lobe and labium were intact. Sometimes the labial palpus alone was absent. There are no good fate maps of the labial disc, or any details of which regions normally duplicate, so these results cannot be compared to experiments at late stages. Misarrangements were observed where the two discs had not fused correctly, but all parts were present.

**Leg defects**

The morphology of the male and female prothoracic (1st) legs has been described by Schubiger (1968). There are slight differences in the bristle patterns of the pro, meso and metathoracic leg (Hannah-Alava, 1958). Fig. 4A shows a normal first leg. Schubiger (1968) also made an anlage plan for the 1st leg; it is assumed that it is similar for all three legs.

The most frequent result of microcautery in the presumptive leg region on the blastoderm was the deletion of one or more neighboring legs. Deletion or damage to legs on both the right and left side of the body were not observed. A number of flies were found with obviously misshapen legs, but were not lacking any pattern elements. All the other flies showed partial deletion of structures, and some of these also had duplicated regions. Postlethwait & Schneiderman (1973) studied the effects of X-ray induced cell death on late embryos and early larvae and found duplications of certain leg structures. There are some differences between the types of duplicated legs which they
Fig. 5. (A–H) The legs shown in Figs. 4(A–H) respectively, showing the regions duplicated and deleted superimposed on Schubiger's anlage plan. □, Deleted structures; ☐, structures present once; ☒, duplicated structures. In (D) more parts of the leg were duplicated and present in one copy but since they did not occupy regions containing sensilla or bristle markers they cannot be placed on the fate map. C, coxa; T, trochanter; f, femur; ti, tibia; ta 1, tarsal segment 1; ta 2–5, tarsal segments 2–5; Cl, claw.

observed and those resulting from microcautery. When a leg resulting from X-irradiation showed a duplication it was always complete and showed a total duplication of distal regions and a number of medial structures missing. The size of the duplicated area, but not the type of pattern varied. Essentially three types of duplications were found after microcautery: (1) medial parts were missing; distal parts were completely duplicated (Fig. 4B, C); (2) medial parts missing, some distal parts duplicated and some slightly more proximal regions present only once (Fig. 4D); (3) distal regions of the leg were present once and slightly more proximal leg segment regions duplicated. One everted leg and two uneverted parts of legs, found in the haemocoel (Fig. 4E) fell into this category.

The regions deleted; present in one copy, or duplicated are superimposed on Schubiger's anlage plan (Fig. 5). The observations of type 3 duplications where proximal parts are duplicated and distal parts are present in one copy is very unusual and has not been observed after X-ray induced cell death in either legs (Postlethwait & Schneiderman, 1973) or wings and halteres (Postlethwait,
Microcautery of Drosophila embryos

1975). This could be due to a difference in the techniques used to cause cell death or due to the difference in age of the presumptive imaginal disc cells at the time of treatment.

No fusions of prothoracic legs resulted from microcautery, though this result was frequent after X-ray treatment of older embryos. This is probably because the presumptive left and right 1st leg cells are quite distant on the blastoderm whereas at later stages they are in close proximity.

Although the exact type of duplication found is somewhat different from those found in other experiments the regions which did duplicate were always consistent with Schubiger's (1971) regeneration and duplication experiments. Structures in the upper medial quarter which is capable of regenerating the whole leg were only duplicated in one example (Fig. 5B) and even in this leg the bulk of the duplicated region was not in this quarter.

A large number of flies hatched with parts of the leg deleted. Sometimes these legs failed to evert and were present, unattached, inside the fly. In all examples of everted legs with missing parts the upper medial quarter of the disc was present. Deleted regions almost always included the claws and some of the tarsi (Fig. 4F, G), although sometimes certain other pattern elements were missing, e.g. Fig. 4H. The remaining regions of the leg in these cases arise from cells which would be expected to regenerate the structures normally present in the deleted regions.

Implants usually lacked regions close to the stalk. They often consisted of regions which may be predicted to duplicate (Fig. 4E), and in one example the apical bristle had indeed duplicated, and in another case the claws and parts of the tibia and femur were present and parts of the trochanter and coxa were duplicated. The reason for the failure to evert may well be due to abnormalities of the adult at the point of attachment of the disc.

It can be seen in Fig. 5A–H that in general both the duplications and deletions were consistent with Schubiger's findings on the 3rd instar 1st leg discs. Two remaining questions however are why the deleted regions are not regenerated in cases where the upper medial quarter of the disc is intact and why, in other cases, is the process of duplication incomplete.

Wing defects

The morphology of the wing and thorax has been described in detail by Bryant (1975), who has also mapped the wing disc of the third-instar larva and observed its patterns of duplication and regeneration. Fig. 6A shows the details of a normal wing and 6H of the normal thorax and Fig. 7A shows part of Bryant's fate map.

As with treatment of presumptive leg regions, the most frequent result was the deletion of all the derivatives of this disc. Only three flies were observed with duplicated structures. One had duplicated thoracic parts including the scutellum; no wing was observed. Another fly showing a duplication was very
(A) Normal wing and thorax.
(B–J) Examples of abnormal wings resulting from microcautery.
(C) The defective thorax was rotated 90° as seen in the whole fly. (C2) Fusion of haltere to wing and duplicated structures can be seen at the point of the wing hinge.
(D) A small part of the posterior wing compartment is deleted.
(E) The anterior and posterior wing blade is deleted.

unusual. The wing had fused to the haltere at the position of the alar lobe. Near to the line of fusion wing structures are duplicated (Fig. 6C1, C2). Only a small region of the wing was deleted (Fig. 7C shows this on Bryant’s anlage plan) and this type of loss would not be predicted to result in duplication. Bryant found that this part corresponding to the remaining wing structures was
Figure 6

(F) Part of the wing blade can be seen as an uneverted tissue, the rest had everted.
(G) Mis-shapen wing.
(H) Normal thorax.
(I) Thorax rotated 90°.
(J₁) Thorax rotated 180° partial wing blade is next to the other half thorax. Whole fly. (J₂) Mounted thorax shows that only the distal part of the wing blade is deleted.
always regenerated. Furthermore, the duplication has occurred in a direction towards the high point of the proposed gradient of developmental capacity in the wing rather than in the normal direction down the gradient. One possible explanation is that the wing and haltere discs have fused and their gradients have interacted. This possibility will be discussed.

One fly had a wing blade containing a triplication (Fig. 6B). This would be expected to be a very rare event from microcautery as the needle is large compared to the number of presumptive disc cells and triplications arise when a small region of cells within a gradient is destroyed. This result is important in that it gives direct evidence for regeneration occurring after microcautery. Complete regeneration from a disc fragment is usually undetected. Triplications can result after the induction of cell death by X-rays in young larvae (Postlethwait, 1975), and after cutting discs *in situ* (Bryant, 1971). The difference in the frequency of occurrence of triplications in these results and the microcautery results would be due to the techniques rather than the age of treatment. Since X-rays may kill cells at random, one might expect them to delete central positions of the gradient as easily as the high or low points of the gradient.

Deletions of parts of the wing were also observed. Deletions of everted structures were restricted to the wing blade, sometimes parts of the posterior wing compartment (Garcia-Bellido, Ripoll & Morata, 1973) were deleted and sometimes parts of both the anterior and posterior compartments were deleted. Deleted regions did not follow compartment borders in all cases but this fact does not provide any evidence either in support of or against the existence of compartments, since the processes of regeneration and duplication are not restricted by these boundaries (Bryant, 1974). Some of the wings with deleted parts are shown in Figs 6D–F. In Fig. 6D just part of the posterior compartment is deleted. In Fig. 6E the whole anterior and posterior wing blade has been deleted. Part of the wing was everted in the fly shown in Fig. 6F but the remainder was present as an implant. Microcautery alone may not be directly responsible for this defect: some regeneration may have occurred subsequenitly.

Many flies hatched with obviously mis-shapen wings (Fig. 6G). Often the dorsal and ventral wing surfaces failed to fuse, producing a wing filled with haemolymph. Several flies had very abnormal thoracic arrangements. In some cases the structures were defective and in others they were perfect. In Fig. 6I the thorax is perfect but rotated 90° from normal. Other flies had the thorax in approximately the correct location but the two halves of the thorax failed to fuse at the midline. The most extreme example, Fig. 6J₁ and J₂, had half of the thorax rotated 180° from normal such that the wing everted between the two thoracic halves. In this example most of the wing blade was deleted. How this type of misarrangement occurs is unclear, but it may be the result of scar tissue causing a rotation of presumptive disc cells, or it may result from abnormal attachment to surrounding disc derivatives in the adult. Some large uneverted pieces of wing and thorax tissue were also found in the abdomens of
flies apparently lacking the whole disc. Two flies lacked thoracic structures near the stalk, another lacked part of the wing blade. No duplications were observed in these implants.

In general, the results are consistent with experiments in which 3rd instar wing discs have been cut and cultured, and with experiments in which first instar larvae have been treated with X-rays. Some abnormal wings are shown superimposed on the anlage plan in Fig. 7B–C. The deleted regions were all positioned such that the missing structures could have been regenerated. Very few duplications were found. After X-ray treatment Postlethwait (1975) found that duplications were the largest class of defects in wings. No flies were observed after X-rays or microcautery with parts of the wing duplicated and other parts present in one copy, as was observed in the legs resulting from microcautery. However, since so few duplications resulted from microcautery we cannot be sure that this result would not be possible.
Haltere defects

Only total deletions of haltere disc derivatives were observed. This may be because of the small number of presumptive haltere cells at the time of microcautery.

DISCUSSION

Chan & Gehring (1971) dissociated blastoderm cells and were able to show, by culturing cells derived from anterior and posterior parts in adults and subsequently passing them through metamorphosis in a larva, that adult cells are determined at this stage. Since anterior cells produced head and thoracic derivatives, and posterior cells produced thoracic and abdominal derivatives there must also be some anterior/posterior polarity of adult determination. Illmensee & Mahowald (1974) confirmed this determination of presumptive adult cells in the blastoderm. They obtained labial derivatives in the adult haemocoel when anterior blastoderm cells were transplanted to posterior regions of host eggs.

Microcautery destroys a region on the surface of the egg, which at the blastoderm stage probably results in the death of the cells in the damaged area. The types of adult structures resulting from this damage suggest that the technique is equivalent to surgical removal of part of a disc. The occurrence of duplicated structures suggests that the presumptive disc cells, which are set aside from the surrounding larval cells of the blastoderm and subsequently give rise to the adult cuticle, are already organized such that certain regions are capable of duplication. It is presumed that certain regions are also capable of regeneration, leading to normal adult structures. Since the duplicated regions in the leg disc resulting from microcautery are similar to those with the areas shown to duplicate at later stages (Schubiger, 1971) we can conclude that there is probably a gradient of developmental capacity already established at the time of blastoderm formation within the groups of cells determined to become 'adult' structures. Herth & Sander (1973) have shown by ligating Drosophila eggs into anterior and posterior halves at various times in early development that, after ligature at nuclear multiplication stage, only the first and last larval segments differentiate; ligaturing a little later results in two or three larval segments differentiating in each part and finally ligature at blastoderm formation allows differentiation of all the larval segments. This shows that contact between the anterior and posterior of the egg is no longer required after blastoderm formation in order to establish larval segmentation. Larval segmentation is therefore fixed at a similar time in development to adult cell determination. Until this time, however, there are presumably cortical interactions in the egg leading to the establishment of differently determined cells once the totipotent nuclei (Zalokar, 1971; Illmensee, 1972; Okada, Kleinman & Schneiderman, 1974) migrate to the egg surface and the cellular blastoderm is formed. The gradients within the presumptive adult discs may therefore merely reflect gradients within
Fig. 8. Possible explanations for the results of microcautery in terms of Bryant's model of developmental capacity. (a–d) Structures of one disc. (x–z) Structures of a second disc. (A) Normal fly; (B) deletion; (C) duplication; (D) incomplete duplication; (E) fusion accompanied by duplication. It is assumed that the cells at the damaged surface respond as if they are at level x and make structure y, but differentiate as structure b as they maintained their original determination. (F) Triplication.
larval segments or gradients within whole eggs. As yet we have no ideas of the molecular nature or organization of cortical interactions, although preliminary studies with u.v. irradiation of early Drosophila embryos (Bownes & Kalthoff, 1974) suggest that proteins may be involved.

When a disc is cut in half and allowed to grow, either in situ (Bryant, 1971) or by culturing the fragment for a number of days in an adult abdomen (Gehring, 1966; Schubiger, 1971; Bryant, 1974; van der Meer & Ouweneel, 1974), the cells at the cut surface proliferate and when metamorphosis occurs they give rise to new structures. The structures produced depend upon the region of disc which was cultured. Bryant proposed that there is a gradient of developmental capacity within a disc and the dividing cells at the cut surface can only produce structures at lower levels in the gradient. This idea describes the fact that certain regions of the disc regenerate missing parts while others duplicate the remaining structures (Fig. 8). The area which is the high point of the gradient and leads to regeneration of missing structures is not the same for all discs, but all the discs studied show properties which can be described by a gradient of developmental capacity. The results obtained by microcautery will be discussed in relation to Bryant's theory and compared to the results obtained after X-irradiation of older embryos and young larvae.

X-irradiation induces cell death (Postlethwait & Schneiderman, 1973; Postlethwait, 1975). These authors have studied the defective leg, wings, and halteres resulting from X-irradiation of young larvae and late embryonic stages. The types of defects which were observed after microcautery are analogous with X-ray treatment of slightly older disc cells. In addition to duplications, deletions of certain structures were a consequence of both microcautery and X-rays. Deletions were explained by Postlethwait and Schneiderman as being the result of incomplete regeneration. After microcautery this could also be the case, since in all cases the deleted region should have regenerated, according to the prediction.

No examples of incomplete duplication were observed after X-ray damage to discs, but they were observed in the legs after microcautery at blastoderm. Sometimes structures were present in only one copy, while other structures were duplicated. One might predict that, if cells are proliferating at the damaged surface, structures added on by duplication might occur in a specific direction. Either structures next to the cut surface would arise first or structures from a low point of the gradient and furthest from the cut edge would arise first. In the leg disc we are not sure exactly where the low point of the gradient of developmental capacity is, but the high point is in the upper medial quarter (Schubiger, 1971). The results from the microcautery do not, however, help to answer this question, as they do not fall into a consistent pattern. Sometimes distal parts of the leg claws are duplicated and more proximal regions are present in one copy and in other cases the reverse occurs. With respect to the third instar disc there is also no consistent pattern. Sometimes duplicated regions are towards
the upper part of the disc and regions present in one copy occupy lower parts of the disc and in other cases the reverse occurs. One might have expected that the same regions of the disc would always be duplicated first and hence patterns of incomplete duplication would be similar. Establishing the direction in which structures are added on during the growth of cells at the cut surface will help us to establish if a series of inductive steps, e.g. A → B → C → D accounts for regeneration and duplication or if cells at the cut surface produce prospective structures furthest from the damaged region first, and then fill in the intermediate regions.

X-ray-induced cell death causes triplications. Only one case of this resulted from microcautery and this occurred in the wing disc. Triplication can be explained by a regeneration and a duplication (Fig. 8). One would expect this to be a rare event after microcautery because this technique damages large localized regions. One would therefore rarely expect to affect a small central part of a disc. On the other hand, X-rays may kill small groups of cells and they might be expected to lead to small centrally located lesions more frequently. The one example of a triplication does, however, give us direct evidence that presumptive disc cells can regenerate after damage at blastoderm stage.

Postlethwait & Schneiderman found fusions of prothoracic legs after X-ray treatment and explained this by suggesting that the two first leg discs have a common gradient. Fused thoracic legs were not found after microcautery, but they would not be expected since the location of the presumptive right and left first leg cells are quite distant on the blastoderm and only later do they come into close contact. Two cases of fusion between other discs were observed. In one case fusion occurred between the first leg and proboscis and in the other between the wing and the haltere. In both cases a duplication was observed in one of the structures. In the wing this was a very unusual duplication which occurred up the gradient of developmental capacity in the wing disc. In the case of the labial duplication we cannot conclude anything since we do not know where the high and low points are in this disc. The duplicated wing fused with the haltere can be explained in two ways. The duplication could have arisen by a transdetermination of haltere structures to wing structures or, more probably, by the wing cells at the cut damaged surface responding to the haltere gradient. This is shown diagrammatically in Fig. 8. Such fusions between discs, if they could be induced at a sufficient frequency, could provide a great deal of information about the similarities of pattern and determination between discs and about the general nature of developmental gradients.

All of the above discussion has been with reference to the parts of the adult which develop during larval life in pockets of cells, the imaginal discs which are in contact with relatively few larval cells. The abdominal histoblasts do not develop in this fashion, they lie in close proximity to surrounding larval cells and do not divide until puparium formation (Garcia-Bellido & Merriam, 1971; Guerra, Postlethwait & Schneiderman, 1973). It has also been shown by cautery
of the larval segments at later stages in development (Santamaria & Garcia-Bellido, 1972) that the larval cells play an important role in histoblast development. It is not possible at blastoderm formation to precisely damage specific abdominal segments by cautery, though it is possible at later stages. In other insects there seems to be a gradient in the whole segment with the high and low points at the anterior and posterior margins respectively (Lawrence, 1973). The histoblasts may be an integral part of this gradient, the larval cells may provide a substrate for the proliferation of histoblasts in later development and may even provide signals for later pattern formation. The histoblast cells of the presumptive tergite are separated into dorsal anterior and posterior nests (C. Roseland, personal communication) and ventral nests of cells lead to the sternites.

The types of abdominal defects resulting from microcautery at blastoderm formation (Bownes, unpublished observation) were similar to those found in genetic stocks and after experimental manipulations of later developmental stages (Sobels, 1952; Lobbecke, 1958; Santa-Maria & Garcia-Bellido, 1972). Duplications, complete and partial deletions of tergites and sternites were observed and in this respect results of cautery of presumptive abdominal regions were consistent with results from other presumptive disc areas. However, many interactions also occur between the remaining histoblast cells after cautery. For example, hemitergites fuse abnormally, or can be located in the wrong position. The results of these experiments will be presented in a separate paper.

The results of the microcautery experiments presented in this paper indicate that the behaviour of imaginal discs at the time of the initial determination in the blastoderm is similar to their behaviour at later stages of development. They show that, when the disc may consist of as few as 2–40 cells, these cells behave as a population in which some cells occupy a high point and some a low point in the gradient of developmental capacity.

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REFERENCES


Microcautery of Drosophila embryos


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