Morphogenesis of the epidermis of adult abdomen of *Drosophila*

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SUMMARY

Mitotic pattern in the different histoblast nests, and the temporal sequence of fusion and differentiation of these nests and spiracular anlagen resulting in the formation of the different regions of the adult abdomen of *Drosophila melanogaster* were studied by examining whole mount preparations and histological sections of the epidermis from closely timed developmental stages. The relationship between the boundaries of the primary (larval) and secondary (adult) segments was determined by following the points of insertion of the dorsal internal oblique muscles which persist through metamorphosis. These studies indicate that the descendants of the anterior dorsal histoblast nest form the hairy and bristled region of the tergum, while those of the anterior and posterior groups of the posterior dorsal nest give rise to the intersegmental membrane and acrotergite respectively; the ventral histoblast cells give rise to the sternum and pleural region while the spiracular anlage forms the spiracle. These findings confirm and extend the conclusions derived from genetic analyses or after experimental induction of defects, on the lineage of the various histoblast nests.

INTRODUCTION

Histological (Madhavan & Schneiderman, 1977) and genetic (Garcia-Bellido & Merriam, 1971; Guerra, Postelthwait & Schneiderman, 1973; Lawrence, Green & Johnston, 1978) analyses of histoblasts during the entire larval development of *Drosophila* showed that they do not increase in number. Following pupariation the histoblasts undergo mitosis and during adult development they replace the polytene larval cells and form the adult epidermis. Since only cursory accounts of the development of the adult abdomen of *Drosophila* (Robertson, 1936; Roseland & Schneiderman, 1979), or other cyclorrhaphan Diptera (Bautz, 1978; Bhaskaran, 1973; Emmert, 1972) are available, we describe in detail in this report the mitotic pattern seen in different histoblast nests during early stages of adult development and the temporal sequence of the fusion and differentiation of these nests resulting in the formation of the different regions of adult abdominal segment of *Drosophila*. These histological observations provide direct support to the conclusions derived from genetic analyses of the

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lineage of the different histoblast nests and formation of inter- and intrasegmental compartments in the abdomen.

By observing the insertions of the persisting larval muscles and difference in the orientation of the imaginal epidermal cells on either side of the intersegmental line of the primary segments, we found that what was customarily considered to be the anterior region of the adult abdominal segment, developmentally, is formed by the histoblasts of the preceding segment. In the adult, the most posterior of the preceding segment (intersegmental membrane) and the most anterior of the following segment (acrotergite) both differ histologically and developmentally from each other and also from the segment proper. The recognition of these differences provided a new interpretation of pattern duplication of the tergites which is described elsewhere (Madharan & Madharan, 1980).

MATERIALS AND METHODS

_Drosophila_ was maintained on a standard medium containing maize, agar, sugar, water and yeast and at 25 ± 1 °C and 65% humidity. Under these rearing conditions, it takes about 4 days (96–106 h) for completion of larval development after hatching from the egg, whereupon pupariation occurs. Adult development is completed within 4 days following pupariation. Freshly pupariated animals (white puparia) were collected from culture bottles, transferred to Petri dishes lined with moistened filter paper and allowed to develop to the desired stages of development. These animals of known ages were either used for making whole mounts of epidermis or for histological sections.

For making the whole-mount preparations of the epidermis, the pupariating animals were cut longitudinally either dorso-ventrally in the midline (resulting in left and right halves) or laterally (resulting in dorsal and ventral halves). The internal organs from these halves were removed as much as possible with forceps and the cuticle and epidermis were fixed in Kahle's or Carnoy's fixative. Such preparations were made at hourly intervals from animals of 0–8 h and thereafter at 12, 15, 18, 20, 24, 28, 32, 36, 38, 40, 41, 42, 48, 60, 72, 90 and 96 h after puparium formation. These whole mounts were stained with Feulgen's reagent and counterstained with fast green whenever necessary and mounted in Histoclad. Paraffin sections (4–8 μm thick) of whole puparia fixed in Kahle's fixative were cut longitudinally and transversely. Most of these sections were stained with Feulgen's reagent and counterstained with fast green but some were stained with Mallory's triple stain. The sections were made of animals of 0, 4, 8, 10, 12, 18, 24, 32, 38, 48, 72 and 84 h after pupariation and 0, 2, and 4 days after adult emergence to confirm the observations made with whole mounts.

In order to determine the number of cells and the area occupied by each of the histoblast nests at various stages of adult development, an ocular micrometer grid where 1 cm² was divided into 400 small squares was used. Unlike the imaginal discs of _Drosophila_ which have several foldings and the peripodial
Morphogenesis of the epidermis of adult abdomen of *Drosophila*

membrane, the histoblasts appear mostly as a single layer of cells. This facilitated, particularly during the early stages of adult development, counting of the total number of cells and mitotic figures in the different nests. However, during the later stages of development (beginning from 18 h after pupariation), as the number of cells in the nests increased markedly, cell numbers were determined in representative regions of the nest by counting the number of cells in 10 or 40 \( \mu \text{m}^2 \) area. From this and the total area occupied by the nest, the total number of cells present in the whole nest was determined. This arbitrary method is considered adequate for making comparisons in the increment of cell numbers during the growth of the different nests. Although histoblasts are located in the same plane as the larval nuclei during larval and early stages of pupariation, during the later stages of adult development (beyond 18 h after pupariation) there is some superposition of nuclear images at certain regions of the dorsal and ventral histoblast nests due to the slight pseudostratification of their own nuclei as well as due to the presence of other cells like the oenocytes directly underneath the histoblasts, making accurate cell counts harder during these stages.

**RESULTS**

The spiracular anlage

Analyses of whole mounts and sections of various stages of larval abdominal epidermis of *Drosophila* have shown that each hemisegment consists of an anterior dorsal, a posterior dorsal and a ventral histoblast nest amongst the larval epidermal cells (Madhavan & Schneiderman, 1977). In addition to these histoblast nests present in each segment, a pair of spiracular anlagen also appear in the third instar. In the early third instar, the cells at the distal end of the spiracular branch of the transverse tracheal connective on either side of each of the first seven abdominal segments represent the spiracular anlage, which is attached to the epidermis at a level of two to three larval epidermal cells away from the lateral edge of the ventral band of chitinous hooks. The position of the anlage can also be identified by its location posterior to the dorsal insertion of the pleural internal transverse muscle which is present at the anterior lateral intersegmental border of each segment (Fig. 1). In the late third-instar larvae as well as in animals which are 0–2 h after pupariation, the anlage remains relatively inconspicuous and consists of two to three cells arranged in a circlet (Fig. 2), but 2–4 h after pupariation their cell number increases and these structures become increasingly noticeable on the surface of the epidermis.

Recognition of segment boundaries in the larval and adult abdomen

The relationship between the boundaries of the primary (larval) and secondary (adult) segments was recognized by following the points of insertion of some of the larval muscles persisting through metamorphosis. The abdomen of the late embryo and larva of *Drosophila* show primary segmentation similar to that
Fig. 1. Dorsolateral region of the right side of the fourth abdominal segment of a mid-third instar larva (92 h after egg laying) showing the position of the spiracular anlage (SA) at the distal end of the spiracular branch (SB). PITM, pleural internal transverse muscle; TCO, transverse tracheal connective.

Fig. 2. Spiracular anlage (SA) consisting of three to five cells at 2 h after pupariation.

Fig. 3. Longitudinal section of the dorsolateral body wall of a mid-third instar larva showing the insertion sites of dorsal internal oblique muscle (LM) at the primary intersegmental folds (ISF).

Fig. 4. Longitudinal section of a 1-day-old adult showing the persisting larval muscle (LM) and part of the imaginal dorsal longitudinal tergal muscle (IM). Note that the insertion site of the larval muscle is at the junction of the acrotergite and hairy region of the tergite (arrow). Compare this with Fig. 6.
Morphogenesis of the epidermis of adult abdomen of Drosophila described for other insects by Snodgrass (1935). In the larval abdomen most of the longitudinal and oblique muscles are intrasegmental, with the exception of ventral external oblique muscles near the mid-ventral line which are intersegmental, and the intrasegmental muscles are attached to the body wall at the primary intersegmental folds (Fig. 3). These observations have also been recently reported by Szabad, Schüpbach & Wieschaus (1979). Thus, the functional intersegmental lines of the larval body wall coincide with the lines of attachment of its longitudinal and oblique muscle fibers.

During pupariation, the larval cuticle which shows segmentation externally, becomes separated from the body wall and contracts forming the puparial case. Subsequently, the pupa is covered by two membranous coverings, the prepupal and pupal cuticle which do not bear any trace of segmentation in the abdominal area. A few of the larval muscles degenerate following pupariation and most of the remaining ones do so subsequent to pupation. Two pairs of the dorsal internal oblique muscles, although degenerate to some extent, persist all through the remaining period of adult development and histolyse completely only 2–3 days after adult emergence (Figs. 4, 5). Since the insertion of persisting muscles does not vary during molting of the insect (Caveney, 1969), the points of insertion of the dorsal internal oblique muscles are used in the present study to establish the boundaries of the primary segment in the imaginal abdomen which, due to the insertions of the adult muscles, shows secondary segmentation (Fig. 6).

In the secondary segment of the adult, the persisting larval muscle becomes intersegmental since its anterior end is attached at the junction of the anterior smooth region and the hairy region of the tergum (see below) and its posterior end at the corresponding region in the following segment (Fig. 6). Hence, the narrow smooth region of the dorsum of the adult Drosophila lying anterior to the points of larval muscle insertions (primary intersegmental fold) corresponds to the acrotergite of the generalized insect (Snodgrass, 1935).

Based on the morphology of the cuticle, three major regions are discernible on the dorsum of a typical adult abdominal segment like the third or fourth (Fig. 7A–C). The measurements of the widths of these various regions are made in the mid-lateral region of a well-stretched dorsal side of a female adult. The anterior region of the segment, i.e. acrotergite, consists of a narrow area measuring 40–45 μm in width and is hairless, bristleless and generally hidden by the overlapping posterior end of the preceding segment. This region is followed by a wider region which, based on the kinds of cuticular outgrowths and pigmentation, can be subdivided into four regions. The first of these regions measures 55 μm in width and bears about 11 rows of hairs. Following this is the second region which contains about 26 rows of hairs, three to four rows of microchaetae and measures 140 μm in width. The area containing the single row of macrochaetae, two to three rows of hairs and measuring 20 μm in width follows this region. The area containing the last row of microchaetae and macro-
Morphogenesis of the epidermis of adult abdomen of Drosophila

Chaetae is pigmented black. In the more posterior tergites, particularly in males, the pigment band becomes wider and extends further anteriorly. The pigmented area is followed by a 60 μm-wide region containing 10–13 rows of hairs. This is followed by the third major region of the dorsum of the segment which is flexible and membranous and measures 45–50 μm in width. This latter region, intersegmental membrane, is devoid of any cuticular outgrowths and remains folded underneath the posterior hairy region of the tergum during the later half of adult development and most of the adult life, except at eclosion when it becomes visible as a result of stretching of the segment due to the animal's attempt to come out of the puparium. The intersegmental membrane in turn overlaps the smooth anterior region of the following segment. These different regions of the dorsum, except the intersegmental membrane, are sclerotized in varying degrees.

The development of the adult abdominal epidermis is characterized by three kinds of morphogenetic processes occurring in the histoblasts and larval epidermal cells. (1) Marked increase in the number of cells of histoblast nests. (2) Programmed cell death among the larval epidermal cells and the coordinated spreading of the histoblasts replacing the degenerating epidermal cells. (3) Differentiation of the histoblasts resulting in the formation of tendons for muscle attachment and secretion of the cuticle and cuticular outgrowths of the adult. These various events begin soon after pupariation and is completed by the third day of adult development.

Pattern of mitotic activity in the different histoblast nests

Direct counts of cells and mitotic figures were made in the anterior dorsal, posterior dorsal and ventral histoblast nests of the third to fifth abdominal segments.

Figures 5-7

Fig. 5. Cross-section of the fourth abdominal segment of a 1-day-old adult showing the two pairs of persisting dorsal internal oblique muscles (arrows). DV, dorsal blood vessel; S, spiracle.

Fig. 6. A semidiagrammatic representation of section of the abdomen of a 1-day-old adult showing boundaries of the primary and secondary segments. The attachment sites of the persisting larval muscles represent the boundaries of the primary segment, while the junction of the intersegmental membrane and the acrotergite represents those of the secondary segment.

Fig. 7. (A) Scanning EM picture of the dorsal view of a stretched abdomen of a female adult exposing the intersegmental region. (B) An enlarged view of the posterior of the third and anterior of the fourth tergite and the intersegmental region showing the details of cuticular pattern. AHR, anterior hairy region; AT, acrotergite; ISM, intersegmental membrane; MA, macrochaete; MI, microchaete; PHR, posterior hairy region. (C) Longitudinal section through the fourth abdominal segment of a 2-day-old adult male showing the different regions of the tergal cuticle and the insertion point of the imaginal dorsal longitudinal tergal muscle (IM). AT, acrotergite; T, tergite; ISM, intersegmental membrane.
Table 1. *Number of cells in the histoblast nests of the third, fourth and fifth abdominal segments during 0–28 h after pupariation*

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<td>822 ± 31</td>
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</table>

Histoblasts of three to six nests were counted at each stage: ± denotes standard deviation.

* From 20 h onwards the numbers in the anterior dorsal nest represent the total of anterior and posterior dorsal nests.


*Morphogenesis of the epidermis of adult abdomen of Drosophila*

Table 2. *Mitotic index of the histoblasts in the anterior dorsal, posterior dorsal and ventral histoblast nests of the third, fourth and fifth abdominal segments during 0–28 h after pupariation*

<table>
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Histoblasts of three to six nests were counted at each stage.

segments using stained whole-mount preparations made at hourly intervals from the time of pupariation to 8 h thereafter, and also at 12 (pupation), 18, 20, 24 and 28 h after pupariation. These results are recorded in Tables 1 and 2 and Fig. 8A–C. At the outset, these analyses showed that the histoblasts begin to divide earlier than it was reported before (Robertson, 1936; Madhavan & Schneiderman, 1977; Roseland & Schneiderman, 1979).

The general trend in the increase in cell number in the anterior dorsal nests of third to fifth segments appears to be similar. Mitosis begins at 1 (in the third segment) or 1·5 h (in the fourth and fifth segments) and the cells undergo divisions steadily until pupation. During the first division, the plane of division in the histoblasts is mostly parallel to the anteroposterior axis of the puparium (Fig. 9). However, in the subsequent divisions, mitotic figures appear at various angles. The number of cells in the nest doubles by 3, 6, 8·5 and 12 h after pupariation and by 12 h, the nest consists of about 240 cells (Fig. 8A). At 4–7 h of development, when the nest contains several cells, division figures are seen in 5–7 cells which are closely situated to one another indicating that the cells lying in the same neighborhood are in the same stage of cell cycle (Fig. 10).

During the 8 h of development after pupariation, in the anterior dorsal nests of all the three segments, there are three characteristic peaks in the mitotic index occurring at 2, 4 and 7 h respectively. The mitotic index decreases and shows an all time low at 12 h when it is nearly 1 or less than 1. From the trend in the increase in cell number it is evident that there should be a fourth peak of mitosis some time between 8 and 11 h because, in the previous three doublings,
Fig. 8. (A–C). Increase in the cell number (○—○) and mitotic index (△—△) of the anterior dorsal (A), posterior dorsal (B) and ventral (C) histoblast nests during the first 28 h after pupariation. From 20 h onwards the cell numbers in the anterior dorsal nest represent the total of anterior and posterior dorsal nests. The time intervals between arrowheads represent the average cell-doubling time for the various nests.
Morphogenesis of the epidermis of adult abdomen of Drosophila

the peaks in mitotic indices were observed at about 1-1.5 h before the cell number was doubled. During pupation, there is a pause in mitotic activity and it takes about 8.5 h for the next (fifth) cell doubling to take place. By now (20 h) the anterior dorsal nest has begun to fuse with the posterior dorsal nest. It takes about 6 h for the sixth cell doubling to occur and now (26 h) the combined anterior and posterior nests contain about 960, and at 28 h, about 1100 cells. When the cells of the combined anterior and posterior nests underwent divisions, we did not observe any preferential distribution of mitotic figures among them.

In general, the cell doubling time and pattern of mitotic activity in the ventral nest closely resemble those of the anterior dorsal nest (Fig. 8C). In the ventral nest, the first cell to divide is the one which lies immediately dorsal to the polytene cell within the nest (Madhavan & Schneiderman, 1977). Cell division continues among the histoblasts of both the dorsal and ventral nests till about 40 h after pupariation.

In the posterior dorsal nest, mitosis begins at 2 h and three cell doublings take place before pupation (Table 1). By 6 h, the posterior dorsal nest contains a sufficient number of cells and this facilitates, in many segments, the recognition in it of an anterior and posterior group of cells separated by a larval epidermal cell (Fig. 11). In the larvae or in the early hours after pupariation, this grouping of the cells in the posterior dorsal nest was not recognized, due perhaps to the small number of cells present during these stages. The two groups of cells remain distinct till 12 h or, occasionally, until 18 h (Figs. 12 and 13), at which time the intervening larval cells degenerate and the two groups merge. By 20 h, the combined posterior dorsal nest merges with the anterior dorsal nest (Fig. 14).

Spreading of the histoblasts and programmed cell death in the larval epidermis

During metamorphosis of Drosophila, the larval epidermal cells are sequentially replaced by the histoblasts. Since the cells of the histoblast nests during spreading are arranged mostly in a single layer, it is possible to visualize in whole mounts of the abdomen, the pattern of their spreading to areas originally occupied by the larval epidermal cells which undergo programmed cell death. Although cell proliferation in the various histoblast nests occurs quite intensely during the first 8 h after pupariation and cell numbers have increased about six to seven times in the anterior dorsal and ventral nests and four to six times in the posterior dorsal nest, the areas occupied by these nests do not show proportionate increase in size, indicating that the cells of the nests are not spreading out actively. For example, the area occupied by anterior dorsal nest and ventral
Fig. 9. Whole mount of epidermis at 2 h after pupariation showing the region of anterior dorsal histoblast nest (enclosed by dashed line) in which 75% of cells are undergoing their first mitosis. The plane of division is parallel to the antero-posterior axis of the puparium. LEC, larval epidermal cell.

Fig. 10. The anterior dorsal histoblast nest of the fourth abdominal segment at 7 h after pupariation. Note that the dividing cells (enclosed by the dashed line) are in the same neighborhood, indicating that they are in the same stage of cell cycle.

Fig. 11. Posterior dorsal nest at 6 h after pupariation in which an anterior (APDH) and posterior (PPDH) group separated by a larval epidermal cell (LEC) can be recognized.

Fig. 12. Posterior dorsal nest at 12 h after pupariation showing the anterior (APDH) and posterior (PPDH) groups of the posterior dorsal nest still separated by a row of larval epidermal cells (LEC).

Fig. 13. The anterior (ADH) and posterior (PDH) dorsal nests at 18 h after pupariation. Note that there is only a row of larval epidermal cells (LEC) between the spreading anterior dorsal and posterior dorsal nests. The larval epidermal cell (arrow) existing between the two groups of posterior dorsal nest has not yet been replaced.
Morphogenesis of the epidermis of adult abdomen of Drosophila

nest increases from 2200 and 2100 μm² at 4 h to about 3100 and 2500 μm² respectively at 8 h, whereas their cell numbers show an increase of about three times (Table 1). As the number of cells increases, most of them, except those in the periphery, gradually assume columnar shape to accommodate the large number of cells in the limited area. However, as development proceeds, the histoblast cells spread to areas previously occupied by the degenerating larval epidermal cells and merger of different histoblast nests occurs. The details and temporal sequences of these processes are described below.

Fusion of the anterior and posterior dorsal nests

Until pupation, there are two to three rows of larval epidermal cells between the anterior and posterior dorsal nests (Fig. 15), and by 18 h, there is only a single row of larval cells or part of it remaining between them (Fig. 13). Remnants of several larval epidermal nuclei can be seen in the immediate vicinity of these histoblasts. By 18 h, the two groups of the posterior nest have mostly fused, although in some instances the larval epidermal cells present in between the groups have not yet been replaced completely. However, all larval cells disappear completely by 20 h resulting in the merger of the two groups. The fusion of the anterior and posterior dorsal nests begins in anterior segments of the abdomen at 18 h and is well advanced in most of the segments by 20 h. One can see remnants of larval epidermal nuclei in various degrees of degeneration underneath the fusing histoblast nests in the different segments (Fig. 14). During this time, the anterior dorsal and posterior dorsal nests consist of 22–24 and 8–9 vertical rows of cells at their widest regions and 330–370 and 100 cells, respectively. By 24 h, no trace of larval nuclei is seen in between the two nests (Fig. 16). After fusion, the cells of the composite dorsal nest continue to divide until 40 h and direct counting of cells at 48 h of the dorsum of the fourth segment showed about 9000 cells.

Fusion of the ventral nest and spiracular anlage

By 18 h, when the fusion of the anterior and posterior dorsal nests has begun, the ventral nest containing about 250 cells begins to spread towards the spiracular anlage containing about 85 cells arranged in five to seven concentric layers around the future spiracular opening (Fig. 17). Analysis of a series of stages of fusion and measurements of the areas of these spreading nests show that, though both the nests are extending towards each other, the ventral nest cells are spreading more actively (Fig. 18). The cells in the anterolateral corner of the ventral nest spread towards the spiracular anlage replacing two to three rows of larval epidermal cells which are present between the two anlagen. By 24 h, the ventral nests of all the abdominal segments have joined with the spiracular anlage of their corresponding segments (Fig. 19). Even after this merger, the cells belonging to the spiracular anlage can be identified because their nuclei are more compact and intensely stained compared to those of the ventral
Fig. 14. Whole mount of epidermis showing the different stages of fusion of anterior (arrow) and posterior (arrowhead) dorsal nests of second and third abdominal segments at 20 h after pupariation. In the second segment the fusion is almost complete whereas in the third it is in progress. DLN, degenerating larval epidermal nucleus.

Fig. 15. The anterior (ADH) and two groups of posterior dorsal nests (APDH, PPDH) at 8 h after pupariation showing two to three rows of larval epidermal cells between the nests.

Fig. 16. The fused dorsal histoblast nest (arrows) and the spiracular nests (S) of the third and fourth segments at 24 h after pupariation.

Fig. 17. Extension (arrow) of the ventral histoblast nest (VH) towards the spiracular nest (S) at 18 h after pupariation.

Histoblasts which are widely distributed in the vicinity of the anlage. In sections, the cells of the spiracular anlage appear columnar to cuboidal compared to the more squamous type of cells of the histoblast. The ventral nest, after its merger with the spiracular anlage, begins to spread above and below the future spiracular region.
Morphogenesis of the epidermis of adult abdomen of Drosophila

Thus, by 24 h after pupariation, fusion of the anterior dorsal and posterior dorsal nests as well as that of ventral nest and spiracular anlage is complete in all the segments. The fusion of former groups results in the formation of an island of cells in the tergal region and that of the latter in an island of cells in the pleural and sternal regions of the hemisegment.

Fusion of the dorsal nest and the spiracular anlage

Between 24 and 28 h, the anterolateral region of the dorsal nest merges with the spiracular anlage (Fig. 20). The number of cells present in the dorsal nest varies at the time of its fusion with the spiracular anlage in the larger and smaller segments of the developing adult abdomen, indicating that the nests need not have a critical number of cells before merger. For example, the number of cells in the dorsal nests of segments 4 and 5 is about 870 while that in the smaller segment 7 is only about 440 at the time of their fusion with the spiracular anlage of the corresponding segments. By 28 h, when fusion of all the nests is complete in a hemisegment like the fourth, the total area occupied by the dorsal nest is about 18200 µm², that of the spiracular anlage about 1700 µm² and that of the ventral nest about 18000 µm². At this time, the dorsal nest, ventral nest and spiracular anlage contain about 1100, 800, and 185 cells, respectively. Further, there are one or two rows of larval epidermal cells between the dorsal or ventral histoblast nests and four to five rows between the spiracular anlagen of two consecutive hemisegments (Fig. 20).

Fusion of dorsal or ventral nests of neighboring segments

The events leading to the fusion of dorsal or ventral nests of consecutive segments are almost similar in detail and chronology and hence, in the following, we describe only those of the dorsal nests. The larval epidermal cells between the dorsal nests of the first and second segments begin to disappear by about 28 h after pupariation indicating the beginning of merger of the histoblasts of these two segments. One can see a few mitotic figures near the advancing edges of the histoblast nests. Examination of the dorsal and ventral histoblast nests with mitotic figures near the future segmental borders showed that when the dividing cells are three to four rows of cells interior from the very edge of the advancing front, they divide at various angles. However, those closer to the advancing front, i.e. within one or two rows of cells from the front, divide in such a way that the daughter cells are placed either parallel or at a slight angle to the edges of the front (Fig. 21). During this merger, the advancing edge of the posterior of the dorsal nest of the preceding segment and that of the anterior of the following segment spread in such a manner that they equally fill in the space occupied earlier by the larval epidermal cells (Fig. 22). The fusion of dorsal nests of consecutive hemisegments is nearly complete by about 36 h (Fig. 23).
Fig. 18. The ventral histoblast nests (VH) and spiracular nests (S) of the third and fourth left abdominal segments at 20 h after pupariation. Note that the ventral nest cells are spreading (arrows) actively towards the spiracular nest.

Fig. 19. Hemisegments (right side) of the fourth, fifth and part of sixth segment showing the relationship of the dorsal (DH), spiracular (S) and ventral (VH) nests at 24 h after pupariation. The ventral and spiracular nests have fused while there is still a row of larval epidermal cells (LEC) between the dorsal and spiracular nests. Note that there is a dark area in the center of each of the dorsal nests which represents the site of formation of oenocytes (O). This region is less prominent in the ventral nest. The longitudinal split in the dorsal nests was caused during processing.

Fig. 20. Whole mount showing the nature of epidermis at 28 h after pupariation. Note that the dorsal (DH), spiracular (S) and ventral (VH) histoblast nests have fused and the future adult epidermal cells of a hemisegment form a longitudinal island of cells. There are one to two rows of larval epidermal cells between the dorsal and ventral histoblast nests and four to five rows in the interspiracular region of two consecutive segments.

Fig. 21. The dorsal epidermis at the junction of two adjacent segments showing the division plane of the imaginal epidermal cells near the boundary. The cells which are closer to the advancing front of the nests (arrows) divide in such a way that the daughter cells are placed either parallel or at a slight angle to the edges of the front. The dividing cells which are situated three to four rows of cells interior from the boundary (arrowheads) divide at various angles.
Fig. 22. Whole mount showing the merger of the dorsal nests of the third and fourth abdominal hemisegments at 28 h after pupariation. As the last row of larval epidermal cells between these two nests begins to disappear, the imaginal epidermal cells of the consecutive segments equally fill in the space occupied earlier by the larval epidermal cells (arrow).

Fig. 23. Whole mount of the epidermis of the right side of dorsum at 36 h after pupariation showing almost complete fusion of the dorsal nests of second (A2), third (A3) and fourth (A4) hemisegments of right side. There are still two larval epidermal cells (arrow) existing at the fusion point of second and third segments. A transverse band of darkly staining cells representing the oenocytes, underneath the epidermal cells, is seen in the middle region (O).

Fig. 24. Whole mount at 36 h after pupariation showing fusion of the dorsal and ventral histoblast nests at the interspiracular area. S, spiracle; O, oenocyte region.
Morphogenesis of the epidermis of adult abdomen of Drosophila

Fusion of dorsal and ventral nests

We already saw that there are four to five rows of larval epidermal cells between the spiracular anlagen of two consecutive hemisegments. By about 30 h these cells of the anterior hemisegments begin to degenerate and the cells of the dorsal and ventral nests begin to merge and this merger is nearly completed by 36 h (Fig. 24). Merger of the imaginal epidermal cells of the tergal and pleural areas occurs slightly dorsad to the spiracle in the anterior segments. In this process of merging too, the advancing edges of both these regions are equally involved and as a result the line of fusion appears to be almost straight. This is also reflected at the tergo-pleural border of the adult cuticle which bears two types of hairs (Fig. 25). The tergal (as well as sternal) hairs are long and thin with a narrow base and their shafts appear to arise sharply from the general body cuticle (Fig. 26A, B). This is in contrast to the hairs of the pleural area because the cuticle around the bases of the pleural hairs is membranous and thrown into folds. As a result these hairs appear broad-based. The shaft of the pleural hairs appears forked with unequal arms (Fig. 27).

Fusion of the contralateral hemitergites and hemisternites of a segment

At 36 h, the left and right hemitergites of various segments are still separated by three to seven rows of larval epidermal cells on the mid-dorsal side. Similarly, the left and right hemisternites are separated by two to eight rows of larval epidermal cells on the mid-ventral region. However, by about 40 h, the left and right hemitergites are almost completely fused but for a small region consisting of one or two transverse rows of four to six larval epidermal cells at the mid-dorsal junction of consecutive segments (Fig. 28). These larval epidermal cells are at the same level as the posterior insertion of the persisting larval internal oblique muscles located closer to the mid-dorsal line. By 38 h, between the left and right hemisternites of various segments, there is either a complete or partial longitudinal row of larval epidermal cells (Fig. 29). By 40 h, these cells are almost completely replaced by the imaginal epidermal cells. By 41 h, fusion of the adult abdominal epidermis is completed both on the mid-dorsal and the mid-ventral lines.

Figures 25-27

Fig. 25. Scanning EM picture showing the relative positions of the spiracle and the tergo-pleural border line (arrow) in the adult cuticle. PL, pleural region; S, spiracle; T, tergal region.
Fig. 26. (A, B). Scanning EM pictures of the tergal (A) and sternal (B) hairs which are long and narrow and arise abruptly from the general cuticle.
Fig. 27. Scanning EM picture of the pleural hairs. Note that the cuticle around the bases of the hairs is thrown into folds and as a result the hairs appear broad-based, in contrast to those of the tergite and sternite. The shafts of the hairs appear forked in the lateral view.
Morphogenesis of the epidermis of adult abdomen of Drosophila

Density and shape of histoblast cells during their spreading

When the various histoblast nests are beginning to spread during the merger of the individual nests, or after the merger to fill in the rest of the segment, the density of cells is greater in the center than in the periphery of the nests. In histological sections of the nests, this difference in cell density is clearly evident as is the difference in cell shape. The cells in the center of the nest are columnar, whereas those towards the periphery of the nest appear cuboidal. The cells at the very periphery of the nest appear squamous and these spread radially replacing the histolysed larval epidermal cells (Fig. 30).

Orientation of imaginal epidermal cells

In longitudinal sections of animals of 32 h, the orientation of the imaginal epidermal cells can be recognized in the dorsal region. The two imaginal epidermal cells on either side of the larval epidermal cell, which marks the boundary between the two primary segments, appear round. The distal ends of the next four to six imaginal cells belonging to the anterior segment direct posteriorly while those of four to five cells of the posterior segment point anteriorly. The distal ends of nine to ten rows of cells in the middle of the segment point horizontally and there is a smooth transition in the orientation of the distal ends of

Figures 28–31

Fig. 28. Whole mount of the dorsum at 40 h after pupariation. Note that the right and left hemitergites have nearly fused except for a small region at the posterior end of each segment in the mid-dorsal area where there are two to four transversely arranged larval epidermal cells (arrowheads). The degenerating nuclei (arrows) of the recently displaced larval epidermal cells are present in the background in the mid-dorsal area.

Fig. 29. Whole mount of the ventrum at 38 h after pupariation showing one to two rows of larval epidermal cells along the mid-ventral line between the right and left hemisternites. The developing ventral longitudinal (VL) and lateral tergosternal (TS) muscles are seen in the background at the future sternal and pleural regions respectively.

Fig. 30. Longitudinal section of the dorsal body wall at 32 h after pupariation showing the differences in cell shape from the center to the peripheral region of the nest. Note that the cells in the center of the nest are columnar while those towards the periphery gradually become cuboidal to squamous. The two cells immediately anterior and posterior to the existing larval epidermal cell are transversely oriented and hence in section appear spherical. Portions of persisting dorsal internal oblique muscle (LM) and developing imaginal dorsal longitudinal tergal muscle (IM) are also seen.

Fig. 31. Longitudinal section of the dorsal body wall at 32 h after pupariation showing the opposite orientation of the anterior and posterior border cells of the adjoining segments. The larval epidermal cell (LEC) marks the boundary between the primary segments. Note that the distal ends of the cells near the posterior boundary of the preceding segment are posteriorly oriented and those near the anterior boundary of the following segment are anteriorly oriented.
Morphogenesis of the epidermis of adult abdomen of Drosophila

the cells in the intervening regions of the segment (Fig. 31). Due to the difference in the orientation of the imaginal cells at the boundary of consecutive segments, it is possible to recognize the limits of the two segments involved even after the histolysis of the last larval epidermal cell (Fig. 32). These differences in the orientation of epidermal cells are maintained up to 48 h after pupariation and are obliterated by 72 h.

Differentiation of the histoblasts

From 24 h after pupariation, in whole mounts, a darkly staining central region is observed in the dorsal nest (Fig. 19), which represents the site of formation of oenocytes. A similar, but less prominent, darkly staining region is also present in the ventral nest. By 36 h, one can see in the middle region of the tergum a transverse band of darkly staining cells underneath the epidermal cells (Figs. 23, 24). In histological sections, a layer of 8–12 oenocytes is seen between the epidermis and the dorsal longitudinal imaginal muscle (Fig. 33). From these limited observations, we could not confirm the origin of oenocytes from the histoblasts as reported by earlier investigators (Robertson, 1936; Koch, 1945). In animals which are ready to emerge, the oenocytes lie underneath the area occupied by the last row of microchaetae and macrochaetae.

By 32 h, some of the epidermal cells in the posterior region of the dorsum of each segment appear as unicellular internal extensions (Fig. 34) to which developing adult dorsal longitudinal muscles are attached. These are the tendon cells. As development proceeds, these muscles begin to shorten and in doing so, the region where they are inserted to the tendon cells – the posterior of the dorsum – is pulled inwards resulting in a slight infolding which later becomes deep (Figs. 35 and 36). When this infolding is complete, the presumptive intersegmental membrane and acrotergite regions of the segment (as recognized by comparison with the sections of the 72-h-old developing and the emerged adults)

Figures 32–35

Fig. 32. Longitudinal section of the dorsum showing the fusion point of two consecutive primary segments (A₃ and A₄) at 28 h after pupariation. Due to the specific orientation of the cells of the two segments (as in Fig. 31) one can recognize the limits of the primary segment border (arrow). AT, acrotergite area; ISM, intersegmental membrane area; TC, tendon cell.

Fig. 33. Longitudinal section of the tergum at 48 h after pupariation showing a layer of oenocytes (O) between the epidermis and dorsal longitudinal tergal muscle (IM).

Fig. 34. Longitudinal section of the dorsum at 32 h after pupariation showing the insertion of the developing dorsal longitudinal tergal muscle (IM) to the tendon cell (TC). This cell is situated five to six cells anterior to the larval epidermal cell (LEC) which marks the posterior boundary of the primary segment. LM, portion of the persisting larval muscle.

Fig. 35. Longitudinal section of the dorsum at 38 h after pupariation showing the beginning of infolding (arrow) at the intersegmental region due to shortening of the imaginal dorsal longitudinal tergal muscle (IM).
Morphogenesis of the epidermis of adult abdomen of Drosophila

are lying underneath, the tergite region, and the point of insertion of adult muscle is located in the penultimate cell of the intersegmental membrane (Figs. 37, 7C). Following the intersegmental membrane is the acrotergite, behind which is the point of insertion of the persisting larval dorsal internal oblique muscle (Fig. 37). In a well-stretched intersegmental region of the dorso-lateral area of an adult ready to emerge, one can recognize seven to nine and six to seven rows of cells in the intersegmental membrane and acrotergite regions, respectively (Fig. 38).

By 36 h, in both the dorsum and ventrum of the segments, the differentiation of bristle-secreting cells begins, and by 41 h, these cells have differentiated further so that trichogen and tormogen cells are visible. During this time, in the dorsum of the fourth segment which measures about 180 μm in width in its mid-lateral region, the cells in the anterior region (60 μm wide) and posterior region (25 μm wide) do not secrete bristles, while those in the middle region (90 μm wide) secrete bristles as evidenced by the presence of trichogen and tormogen cells in the latter region (Fig. 38). Thus, only about half of the visible area of the dorsum is covered with bristles. Analysis of sections and whole mounts of 48-h-old animals showed that from the presumptive acrotergite to the beginning of microchaetae of the tergum there are about 15–17 rows of cells. The area of both micro- and macrochaetae-secreting cells consists of 26–28 rows of cells. From the posterior edge of the macrochaetae-secreting cells to the edge of the posterior fold of the tergum there are about nine rows of cells. The region of the tergum which is folded inward includes both the posteriormost region of the presumptive tergite and intersegmental membrane and consists of 9–11 rows of cells.

From 41 h onwards, no dividing cells are observed in the abdominal epidermis. By 48 h, the epidermal cells are in the process of secreting the general body cuticle, bristles and hairs (Fig. 39). By 72 h, the hairs and bristles are

Figures 36–39

Fig. 36. Whole mount of the epidermis at 41 h after pupariation showing the deepening intersegmental fold (ISF) between the third and fourth abdominal hemi-tergites due to shortening of the dorsal longitudinal tergal muscle (IM).

Fig. 37. Longitudinal section passing through the intersegmental region of the fourth (A₄) and fifth (A₅) abdominal segments at 72 h after pupariation. By now the in-folding at the intersegmental region is well formed and the dorsal longitudinal tergal muscle (IM) is attached to the penultimate cell of the intersegmental membrane region (ISM) which consists of three to five cells. There are four to five cells between the insertion point of the imaginal and the larval muscles (LM) and they represent the acrotergite region (AT) of the adult fifth segment.

Fig. 38. Whole mount of the epidermis of an adult ready to emerge showing the well-stretched intersegmental region between the A₃ and A₄ segments. The intersegmental membrane (ISM) and acrotergite (AT) contain seven to nine and six to seven rows of cells respectively.
Fig. 39. Whole mount at 41 h after pupariation showing the differentiated bristle-secreting cells (BC) in the middle region of the tergum. ISF, intersegmental fold.

Fig. 40. Longitudinal section of the dorsal body wall at 48 h after pupariation showing the beginning of secretion of adult cuticle (AC). TO, tormogen cell; TRC, trichogen cell; PC, pupal cuticle.
Morphogenesis of the epidermis of adult abdomen of Drosophila

completely formed and by 90 h they become pigmented and appear like those in the newly emerged adult. The pigment band seen in the posterior region of the adult tergite is not visible till eclosion. Direct counting of the hairs and the number of epidermal cells underneath them in the tergum show that each hair-secreting cell produces three to five hairs which are arranged in a transverse row.

DISCUSSION

Cell-doubling time for the various nests

The data in Table 1 and Fig. 8A and C show that from pupariation to pupation the cells of the anterior dorsal and ventral nests have a doubling time of 2–3 h. This agrees quite well with the estimates, deduced from clonal analysis, of 2–7 h for tergite (Garcia-Bellido, 1973) and 3–2 h for sternite (Lawrence, Green & Johnston, 1978). However, until pupation, the cells of the posterior dorsal nest are slower in division and undergo only three cell doublings. After pupation till 26 h, the first and second doubling time for the cells of all the nests is about 8 to 9 h and 6 h, respectively. From the slope of the curve in Fig. 8A we estimate that at 26 h there are about 960 cells in the fused anterior and posterior dorsal nests of a hemitergum. Since cell divisions in the tergum are seen only till 40 h and that at 48 h there are about 4500 cells in the hemitergum, we assume that the dorsal nest cells have undergone after 28 h two more cell doublings before ceasing to divide. Thus in the formation of the adult tergum, the anterior dorsal nest and posterior dorsal nest undergo about eight and seven cell doublings, respectively.

Role of programmed cell death in the larval epidermal cells

During the spreading of the histoblasts, the polytene larval cells degenerate and the histoblasts spread to these areas. This degeneration and replacement of cells occurring in the developing abdomen is so precise that there is no discontinuity in the epidermis at any stage of development. This sequential replacement of larval epidermal cells may serve an additional purpose. During the merger of the dorsal nests of two adjoining segments, or that of the dorsal and ventral nests of a hemisegment, the rows of larval epidermal cells which degenerate last keep the confronting edges of the nests at equal distance from them. When these larval epidermal cells degenerate, the cells of both the advancing edges equally fill in the void. In this way, the larval cells which degenerate last provide the physical barrier and serve as guidelines in establishing intersegmental and intrasegmental boundaries. These boundaries, as visualized by histological methods in the present study, have already been shown to exist by clonal analysis (Guerra et al. 1973 for intersegmental boundary and Lawrence et al. 1978 for intrasegmental boundary between the tergum and pleura).
Identification of boundaries of the primary segments in the secondary segments of the abdomen

During the adult development of *Drosophila*, the primary segmentation seen in the larva is obliterated due to the attachment of the adult dorsal longitudinal muscles to the posterior region of the intersegmental membrane. However, the boundaries of the primary segments in the developing adult abdomen can be recognized by two morphological features: (1) point of insertion of the persisting larval dorsal internal oblique muscles and, (2) the characteristic orientation of the imaginal epidermal cells on either side of the intersegmental line of the primary segments.

In the posterior region of the adult tergum, following the rows of hairs behind the macrochaetae, is a narrow region of infolded, flexible intersegmental membrane which in turn overlaps the smooth, anterior acrotergite region of the following segment giving the characteristic appearance of posterior overlapping to the successive tergal plates. So, conventionally, it has been assumed that the intersegmental membrane forms the segmental boundary and the acrotergite it overlaps, as the beginning of the following segment. However, we saw that the persisting larval muscles, which delineate the primary segments, are attached at the junction of the acrotergite and the following hairy region of the tergite.

Since the limit of the posterior region of the acrotergite can be recognized from the insertion of the persisting larval muscle even at the time of formation of segmental boundaries of the adult, it is evident that the acrotergite is derived from the posterior region of the posterior dorsal nest, and there is no contribution from the anterior region of the dorsal nest of the following segment. So, ontogenetically, the acrotergite of the adult, indeed, belongs to the preceding segment.

Lineage of the different histoblast nests

In the literature there are reports that, in *Drosophila*, the histoblasts besides giving rise to the epidermis of the adult abdomen also form adult muscles, fat body and oenocytes (Robertson, 1936; Koch, 1945; Crossley, 1978; Rizki, 1978). However, the formation of the latter tissues from the histoblasts has not been rigorously demonstrated. In the present study, we did not attempt to determine the various derivatives of the histoblasts and observed only the contribution of histoblasts in the formation of the different regions of the abdominal epidermis. The anterior dorsal nest gives rise to the different regions of the tergum behind the acrotergite. In histological preparations, due to lack of recognizable landmarks, it is difficult to distinguish the boundary between the cells of the posterior region of the anterior dorsal nest and those of the anterior group of the posterior dorsal nest after their merger. However, this boundary can be determined from the results of ablation experiments done by Roseland and Berns (cited in Roseland & Schneiderman, 1979). In their experiments,
since u.v. laser microbeam radiation of most of the posterior dorsal nest did not prevent the formation of hemitergite, it can be concluded that the anterior dorsal nest gives rise to, from anterior to posterior, the following regions of the tergum: the hairy, hairy and bristled, and possibly, the hairy region behind the macrochaetae. From these results we conclude that the posterior dorsal nest gives rise to the intersegmental membrane and acrotergite regions.

Our histological observations on the number of vertical rows of cells of the anterior and posterior nests at the time of their merger and during their subsequent development also support these conclusions regarding the lineage of the anterior dorsal and posterior dorsal nests. There are about four rows of cells in each of the groups of the posterior dorsal nest at the time of their merger as well as the merger of these two groups with the anterior dorsal nest (Figs. 13 and 14). During these stages the anterior dorsal nest contains 22–24 rows of cells. In the adult, the tergite, intersegmental membrane and acrotergite regions contain about 54, 7–9 and 6–7 rows of cells, respectively. When we compare the number of rows of cells present in each of these groups at 20 h with that present in the different regions of the epidermis of the adult segment, it appears that, during adult development, the number of rows has increased about twice. Since there is no evidence of differences in the rate of cell division in any particular area of the combined dorsal nest, and the density of the cells as well as the width in the different regions of the dorsum of the adult segment is nearly similar, we conclude that the anterior dorsal, and anterior and posterior groups of the posterior dorsal nests seems to contribute to the final rows of cells of the tergum, intersegmental membrane and acrotergite, respectively.

The histological details of spreading and fusion of the ventral nests with the spiracular anlagen show that the pleura and sternum are derived from the former nest while the spiracular anlagen gives rise to the spiracle. Results of cautery of ventral nest and spiracular anlagen by Roseland & Schneiderman (1979) support our histological findings. The common origin of the sternum and pleura as deduced from the above studies confirms the observations of Lawrence et al. (1978) who did not find clonal restriction lines within these areas.

**Single or multiple fields in the tergum?**

Since there are three groups of histoblasts isolated from each other by larval cells which form the dorsum of the adult abdomen, we can ask whether these groups function as single or multiple morphogenetic fields. One of the basic properties of a field is that its different regions recognize, through positional information, what is happening in other parts of the field and develop accordingly. As a result of this interaction, the structure is formed.

If the three groups of dorsal histoblasts form a single field, and if they have positional values specified in an antero-posterior sequence as suggested by Roseland & Schneiderman (1979), one would expect that when the anterior dorsal nest is removed completely the posterior dorsal nest should regenerate
the missing dorsal nest. However, they observed that after removal of the anterior dorsal nest, the hemitergite was missing and the area occupied by it now contained only white or nearly transparent cuticle with occasional abnormal trichomes. This result can be explained by assuming that the anterior dorsal nest and posterior dorsal nest function as different fields. Accordingly, when the anterior dorsal nest is removed, then this area could first be filled by cells from the anterior group of the posterior dorsal nest since during normal development the row of larval epidermal cell between it and the anterior dorsal nest degenerate earlier than that located between the anterior dorsal and posterior group of the posterior dorsal nest of the preceding segment. Later in development, when these latter larval epidermal cells degenerate, then these cells from the posterior group of the posterior dorsal nest of the preceding segment could also reach the area. This will result in the formation of an inter-segmental-membrane-like cuticle with or without some cuticular outgrowths, depending on the number of leftover anterior dorsal nest cells, as observed in the actual experiment. Thus, the posterior dorsal nest makes only cells of its kind to fill in the void and cannot regenerate the missing anterior dorsal nest.

Based on the reinterpretation of the results of the microcautery of the posterior dorsal nest (Madhavan & Madhavan, 1980) that if one of the groups on the posterior dorsal nest is missing, then its specific effect on differentiation and polarity of the pattern elements in the tergum is absent, and so the remaining group of the posterior dorsal nest is unable to regenerate the missing one. This suggests that the two groups of the posterior dorsal nest also function as two separate fields.

Within each nest and possibly the surrounding larval epidermal cells we assume that there are positional values for regulation of growth. However, more detailed experimental analyses of the developmental capacities of the different regions of the histoblast nests and larval epidermal cells are needed to assign the sequence of these values within them.

**Compartments in the tergum**

Based on clonal analysis and on the assumption that the posterior dorsal nest gives rise to the bristles of the tergum, Roseland & Schneiderman (1979) suggested that the anterior and posterior dorsal nests do not form compartments in the tergite. Since the posterior dorsal nest gives rise to the intersegmental membrane and acrotergite, neither of which produces any cuticular outgrowths to serve as landmarks for the recognition of clonal boundaries, the presence or absence of compartments in the different dorsal histoblast nests still remains an open question.

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Morphogenesis of the epidermis of adult abdomen of Drosophila

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