

Cell Interactions in the Developing Epidermis of the Leech *Helobdella triserialis*

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In embryonic development of the leech *Helobdella triserialis*, each of the four paired ectodermal teloblasts contributes some progeny to a characteristic dorsal or ventral territory of the epidermis. To ascertain the relative roles of cell lineage and cell interactions in generating the highly regular epidermal distribution pattern of the various ectodermal cell lines, a series of experiments was carried out in which the ablation of particular teloblasts was combined with the intracellular injection of cell lineage tracers. The results showed that, after the ablation of an OP proteloblast, or of an O, P, or Q teloblast, the epidermal progeny of the remaining ipsilateral and contralateral teloblasts spread into the territory normally occupied by the epidermal progeny of the ablated teloblast. In this spreading process, cells may cross the ventral midline but not the dorsal midline. The spread of epidermal progeny of one teloblast in response to ablation of another teloblast is contrasted with the failure of the neuronal progeny of one teloblast to replace any missing neural tissue. It appears, therefore, that all epidermal cell lines are of equal developmental potential, regardless of their teloblast of origin, with the eventual location of any epidermal cell in the body wall being governed by interactions between cells within the developing epidermis.

INTRODUCTION

The intracellular injection of cell lineage tracers into the early embryo of the leech *Helobdella triserialis* has shown that each of its identifiable blastomeres regularly gives rise to a specific portion of the developing animal. In particular, each of the bilaterally paired N, O, P, and Q ectodermal teloblasts undergoes a series of iterated, highly unequal cleavages that generates a bandlet of several dozen primary blast cells. Each of these bandlets contributes to a specific portion of the neural and epidermal tissues of each of the 32 metameric body segments (Weisblat *et al.*, 1978, 1980a,b, 1983). Thus, many of the identifiable neurons in the leech can be assigned to one of four bilaterally paired "neuronal kinship groups" with respect to teloblast of origin (Blair, 1983; Blair and Stuart, 1982; Stuart *et al.*, in preparation; Kramer and Weisblat, in preparation). As for the epidermis, its dorsal aspect is derived from the ipsilateral Q teloblast; its ventral aspect is derived mainly from the ipsilateral O and P teloblasts, with the progeny of both O and P teloblasts forming interdigitating fingers; and a small patch of medioventral epidermis in each segment is derived from the N teloblast (Weisblat *et al.*, 1983). The distribution of the epidermal progeny of these teloblasts is in accord with the relative position of their

blast cell bandlets within the nascent germinal plate from which nervous system and epidermis develop: the N-derived bandlet lies next to the ventral midline; the Q-derived bandlet lies at the margin of the plate (and hence in the future dorsal territory of the nascent body wall), and the O- and P-derived bandlets lie in between (and hence in the future ventral territory of the body wall).

To ascertain the relative roles played by cell lineage and cell interactions in generating the stereotyped developmental distribution pattern of various ectodermal cell lines, the use of lineage tracers has been combined with specific ablation of teloblasts or teloblast precursors (Weisblat *et al.*, 1980a; Blair, 1982; Blair and Weisblat, 1982). These studies provided no evidence for the existence of any regulative cell interactions in the developing leech nervous system, since ablation always results in the loss of a particular neuronal kinship group, though cell interactions were found to have some role in consigning a particular neuron to its eventual position. However, no deficits were evident in the epidermis of such neurally deficient, teloblast-ablated specimens. Hence it appeared possible that regulative cell interactions in the developing leech epidermis compensate for the ablation-induced loss of specific epidermal precursor cells.

In the work presented here, the effects of ablation of specific teloblasts on epidermal development were ascertained, to see if regulative interactions help govern

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ectodermal cell fate in the epidermis. As will be shown, the changes in epidermal cell fate following ablation of an O, P, or Q teloblast indicate that cell interactions are of paramount importance in the development of the normal epidermis.

METHODS AND MATERIALS

The embryos of *Helobdella triserialis* used in these experiments were obtained from the breeding colony maintained in this laboratory (Weisblat *et al.*, 1978). The embryos were cultured at 25°C in HL saline (4.8 mM NaCl, 1.2 mM KCl, 2.0 mM MgCl₂, 8.0 mM CaCl₂, and 1.0 mM Tris-HCl, pH 6.6), which was changed daily. After blastomere ablation, the HL saline was supplemented with 4 mg garomycin, 10,000 units penicillin, 10,000 µg streptomycin, and in some experiments, 5 mg tetracycline/100 ml. For lineage tracing, blastomeres of early embryos were injected with solutions of horseradish peroxidase (HRP) or rhodamine-conjugated dodecapeptide (RDP), as previously described (Weisblat *et al.*, 1978, 1980a,b). The HRP solution contained the dye fast green (about 1 g/100 ml), to allow visual monitoring of the progress of the injection (Parnas and Bowling, 1977). Blastomeres were ablated by injection with a 1% solution of DNase I (Sigma type III) in 0.15 N NaCl (Blair, 1982). In some cases, the vital dye malachite green was added to the DNase solution (about 0.5 g/100 ml), to allow visual monitoring, and thus better control of the amount and location of solution injected (Blair, 1983). At the desired later stages of development, HRP-injected embryos were fixed in glutaraldehyde and stained for HRP, and RDP injected embryos were fixed in formaldehyde and counterstained with the DNA-specific fluorescent stain Hoechst 33258. The survival rate of injected embryos ranged from 0 to 86% in these experiments, with 30–50% being typical. For sectioning, observation and photography, the embryos were treated as described elsewhere (Weisblat *et al.*, 1978, 1980a,b, 1983). The cell designations and the system of developmental staging of glossiphoniid leech embryogenesis used here are those of Fernandez (1980) and of Weisblat *et al.* (1980b).

RESULTS

Contralateral Epidermal Spreading

To investigate the effect of ablation of one or more teloblasts upon the epidermal distribution pattern of the progeny of the remaining teloblasts, several experiments were carried out. In the first of these, horseradish peroxidase (HRP) was injected into the left OPQ blastomere (precursor of the left ectodermal teloblasts O, P, and Q) in a series of stage 6a *Helobdella* embryos. In some of these embryos the right OP proteloblast (pre-

cursor of the right teloblasts O and P) was ablated at stage 6b. Development was then allowed to proceed to stage 10, at which time the distribution of the tracer-labeled progeny of the left OPQ blastomere was examined in the epidermis, as well as in the CNS, of normal and operated embryos. In normal embryos the entire epidermis of the left side was labeled, and no labeled epidermal cells were present to the right of the dorsal or ventral midlines. Similarly, in the CNS, labeled neurons were present only in the left half of the segmental ganglia (Fig. 1). This finding is in accord with the results of previous cell lineage tracer experiments, which have shown that in normal leech embryogenesis each teloblast contributes its progeny only to the ipsilateral body half (Stent *et al.*, 1982; Weisblat *et al.*, 1983). In the epidermis of all 12 operated embryos, by contrast, labeled epidermal cells were present also to the right of the ventral midline, extending in a continuous sheet from the left (unoperated) side of the germinal plate, across the ventral midline into the ventral portion of the right (operated) germinal plate (Fig. 1). Hence it may be concluded that following ablation of the right OP proteloblast, the epidermal progeny of the labeled left OPQ blastomere spread from the unoperated side to the operated side, filling in some of the ablation-induced deficit in epidermal precursor cells. No corresponding spread of labeled cells was found across the dorsal midline to the operated side, even though the two margins of the expanding germinal plate had already begun to fuse at the dorsal midline to close the leech body wall. In accord with earlier findings (Blair and Weisblat, 1982), no spread of labeled neurons was found across the midline of the segmental ganglia of OP-ablated embryos.

In the second experiment, HRP tracer was injected into the left OPQ blastomere of a series of stage 6a embryos, and in some of these embryos the right Q teloblast was ablated at stage 6b or 6c. As in the first type of experiment, development was then allowed to proceed to stage 10, at which time the distribution of the tracer-labeled progeny of the left OPQ blastomere was examined in the epidermis and CNS of normal and operated embryos. The normal embryos were identical to those described above. In the epidermis of the 9 operated embryos, labeled progeny of the OPQ blastomere spread across the ventral midline to the operated side, just as in the first type of experiment, though here the spreading of OPQ progeny was rather sporadic, and extended only a short distance into the contralateral epidermis (Fig. 1). As in the first experiment, no spreading of cells was observed across the dorsal midline of the epidermis (Fig. 1), nor was there any crossing of labeled neurons to the operated side of the segmental ganglia. Thus, in both experiments, after ablation of an OP proteloblast or Q teloblast, cells of contralateral origin

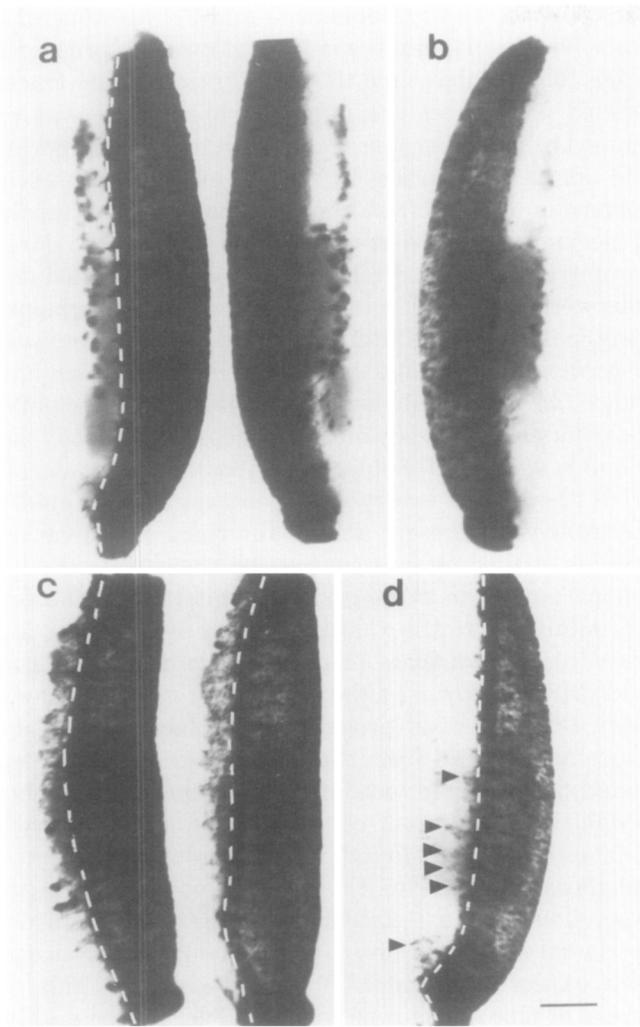


FIG. 1. Whole-mounted late stage 10 *Helobdella* embryos in which the left OPQ blastomere was injected with HRP tracer at stage 6a. Because of the high contrast, the lateral edge on the unlabeled side of the embryo is not apparent in most of these photographs. The ventral midline in these embryos is indicated by a dashed line. The dark spots in the unlabeled side of the embryo (a, b, and d) represent botryoidal tissue which contains an endogenous peroxidase activity (Fischer *et al.*, 1976). Upon examination of these embryos under the stereodissecting microscope, these cells are readily distinguished from labeled epidermal cells because they lie well below the surface of the embryo. The gut is visible as a shadow on both sides of the embryo (a and b). (a) Ventral (left) and dorsal (right) views of a normal (unoperated) embryo. The entire left epidermis is labeled and no labeled cells have crossed the ventral or dorsal midline. (b) Dorsal view of an embryo in which the right Q teloblast was ablated at stage 6b. No labeled cells have crossed the dorsal midline. Similar results are obtained when the right OP proteloblast is ablated. (c) Ventral view of two embryos in which the right OP proteloblast was ablated at stage 6b. The labeled epidermal progeny of the left OPQ blastomere have spread extensively into the right (apparent left) side across the ventral midline. (d) Ventral view of an embryo in which the right Q teloblast was ablated at stage 6b. The labeled epidermal progeny of the left OPQ blastomere have spread sporadically across the ventral midline, as indicated by the arrows. Anterior is up; scale bar, 100 μ m.

spread across the ventral midline within the epidermis, but not the CNS, to the operated side.

Bandlet Switching

Among the OP- or Q-ablated embryos of both experiments, a small minority of specimens was obtained in which, in the rear part of the embryo, labeled cells were present in noncontiguous zones of both left and right epidermis and on both sides of the segmental ganglia. These specimens are likely to represent cases of "switching" of the labeled blast cell bandlets from the nonoperated to the operated side at the point of origin of the germinal bands, as has been previously observed to occur in about a third of the embryos in which an OPQ blastomere was ablated (Blair and Weisblat, 1982). To confirm that ablation of an OP proteloblast or a Q teloblast does, in fact, induce the switching of a blast cell bandlet into a deficient germinal band, the left NOPQ blastomere (precursor of the left N, O, P, and Q teloblasts) was injected with rhodamine-peptide tracer (RDP) at stage 5 in a series of embryos. The right OP blastomere or Q teloblast was ablated at stage 6b, and the distribution of RDP tracer was examined by fluorescence microscopy at late stage 8. At this stage, the posterior ends of the right and left germinal bands have not yet coalesced along the ventral midline to form the germinal plate. Hence cell bandlet switching from the nonoperated (left) to the operated (right) side would be detected by the appearance of an RDP-labeled bandlet in the right germinal band. Switching was found to occur in the posterior zone of the germinal bands in 5 out of 25 Q-ablated specimens, and in 1 out of 15 OP-ablated specimens, in accord with the frequency with which bilateral, noncontiguous labeled epidermal cells were observed in more advanced embryos. Thus, ablation of an OP proteloblast or a Q teloblast does lead to blast cell bandlet switching, albeit at a somewhat lower frequency than does ablation of an OPQ blastomere.

Ipsilateral Epidermal Spreading

In the first section, it was demonstrated that upon ablation of an OP proteloblast or Q teloblast, some of the progeny of teloblasts on the *nonoperated* side spread across the ventral midline and give rise to epidermal tissue on the operated side. Three further experiments were now carried out to examine whether upon ablation of an OP proteloblast, or of an O, P, or Q teloblast, the progeny of the remaining teloblasts on the *operated* side similarly spread into abnormal territories of the ipsilateral epidermis. In the first of these experiments, the right Q teloblast was injected with HRP in a series of stage 6b embryos, and in some of these embryos the right OP proteloblast was ablated at the same time.

The distribution of tracer-labeled cells was then examined at stage 10. It was found that in the epidermis of 18 operated specimens, the labeled progeny of the Q teloblast had spread considerably beyond their normal territory. Whereas in normal embryos Q-teloblast derived epidermal cells were present only in the dorsal skin, in OP-ablated specimens, such cells were present also in a large portion of the ipsilateral ventral skin, in some places having spread almost as far as the ventral midline (Figs. 2a, b). Hence, the progeny of the ipsilateral Q teloblast fill the lateral component of the epidermal deficiency created by ablation of the OP proteloblast. The medial component of this deficiency is evidently filled by spreading of epidermal precursors from the nonoperated side across the ventral midline, as demonstrated in the first section.

In a second experiment, the right OP proteloblast was injected with HRP at stage 6b; the right Q teloblast was ablated at stage 7; and the distribution of tracer-labeled cells was examined at stage 10. In the epidermis of 20 such specimens, the labeled progeny cells of the OP proteloblast were found to have spread from their normal, ventral territory into dorsal territory, all the way to the dorsal midline (or, in the rear portions of the embryos, where the leech body wall had not yet closed, all the way to the lateral margin of the still expanding germinal plate) (Figs. 2c, d). Thus, the progeny of the ipsilateral OP proteloblast appear to fill the entire deficiency created in the dorsal epidermis by ablation of the Q teloblast. But the abnormal dorsal spread of the progeny of the OP proteloblast sometimes appears to leave a gap in the medioventral component of the ipsilateral epidermis which they normally occupy (see Fig. 2d). This medioventral gap is evidently filled by spreading of contralateral epidermal precursors, as demonstrated in the first section.

Finally, in a third experiment of this series, either the O teloblast or its sister teloblast P was injected as usual with HRP and fast green dye in 34 stage 6c embryos. At stage 7, the injected teloblast could still be identified by its green color, at which time the uninjected ipsilateral P or O teloblast was ablated. Upon examination of the distribution of HRP tracer at stage 10, the embryos were found to contain an anterior zone in which the label formed a pattern corresponding to that produced normally by the progeny of an O or P teloblast. This normal zone was evidently derived from progeny of primary blast cells born prior to the ablation. However, posterior to this normal zone was an abnormal zone in which labeled epidermal cells had spread from their normal positions characteristic of either the typical O or the typical P teloblast progeny, into positions characteristic of progeny of the ablated sister teloblast. Thus, in the absence of epidermal precursor cells normally

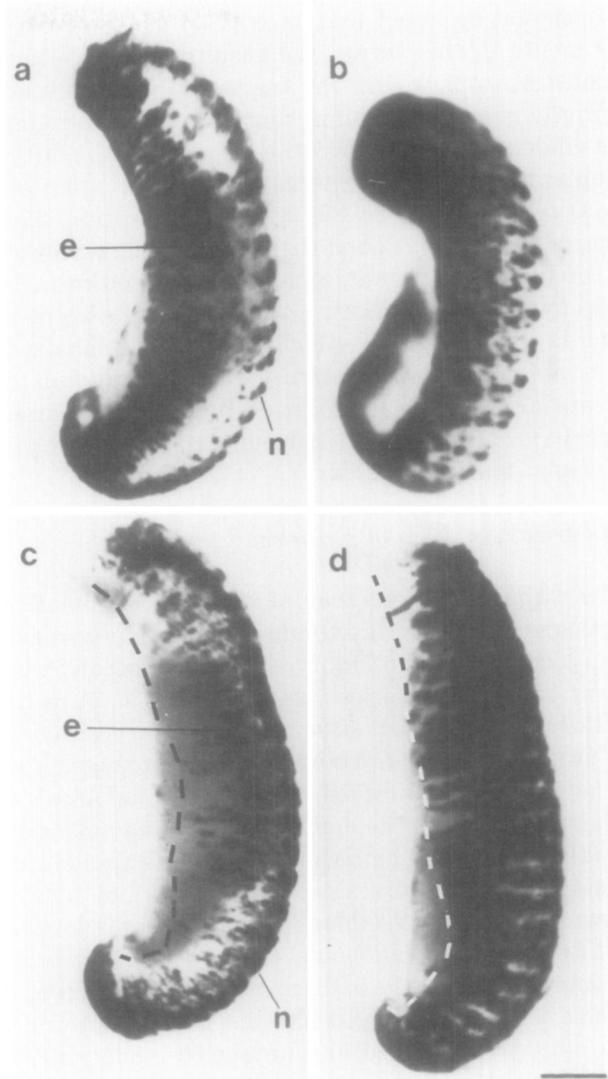


FIG. 2. (a) and (b) Oblique ventral views of whole-mounted stage 10 embryos (dorsum to the left) in which the right Q teloblast was injected with HRP at stage 6b. (a) Normal (unoperated) embryo. The labeled epidermis (e) lies dorsally. The label does not extend into ventral body wall and does not reach the (labeled) nerve cord (n) on the ventral midline. (b) Embryo in which the right OP proteloblast was ablated at stage 6b. Although this operated embryo is slightly younger than the normal embryo shown in (a), here the labeled epidermis extends almost to the ventral midline, especially at the anterior end. (c) and (d) Lateral views of whole-mounted stage 10 embryos in which the right OP proteloblast was injected with HRP at stage 7. Dorsum to the left. (c) Normal (unoperated) embryo. The labeled epidermis occupies a largely ventral territory. (The greyish area covering the entire midbody section of this specimen represents diffuse label in the embryonic gut derived from the digested labeled teloblasts.) (d) Embryo in which the right Q teloblast was ablated at stage 6c. The labeled epidermis extends into the dorsal territory all the way to the margin of the still expanding germinal plate. Lateral margin of the germinal plate indicated by dashed line in (c) and (d). Anterior is up; scale bar = 100 μ m.

derived either from the O or the P teloblast, the epidermal precursor cells of the remaining ipsilateral P or O teloblast spread to occupy the ablation-induced gap.

It should be noted that in both of the first two experiments in this series, the resulting distribution of labeled neurons within the segmental ganglia was essentially normal. Hence, in contrast to the situation in the epidermis, in the CNS the progeny of the OP proteloblast do not fill in the gap created by ablation of the Q teloblast, nor do the Q teloblast progeny fill the gap created by ablation of the OP blastomere. However, in the third experiment, abnormal distributions of the progeny of the O teloblast were found in the segmental ganglia of specimens in which its sister P teloblast had been ablated. This ablation-induced change in fate ("transfating") of O teloblast-derived neuronal precursor cells will be described and analyzed in another paper (Weisblat and Blair, 1984).

Numerical Analysis of Epidermal Spreading

To estimate the number of cells per segment that participate in the ablation-induced epidermal spreading, the epidermis of two embryos from the second experiment in this last series was examined in 3- μ m-thick sections. As described above, in these embryos the right OP proteloblast was injected with HRP at stage 6b, and, in half of these embryos, the right Q teloblast was ablated at stage 7. The embryos were fixed and stained for HRP at stage 10, embedded in Epon, sectioned transversely, and counterstained with toluidine blue. As shown previously (Weisblat *et al.*, 1980a), individual cell profiles containing HRP label can be recognized in such sections by their yellowish color, as distinct from unlabeled cells, which appear blue due to the counterstain. The number of labeled and unlabeled epidermal cells present was estimated by counting the large nucleoli visible in labeled and unlabeled cell profiles (cf. Fig. 3). These cells typically contain only one nucleolus. Since most nucleoli appear in only one 3- μ m-thick section, and the length of a midbody segment of a stage 10 embryo is about 45 μ m, the number of epidermal cells per segment is approximately equal to the total number of epidermal nucleoli counted in 15 successive serial sections.

Four classes of epidermal cells were distinguished in these sections, according to their location and content of label. Class OPi: labeled cells; class Qi: unlabeled cells, ipsilateral and dorsal to the labeled cells within the germinal plate; class MVi: unlabeled cells ipsilateral and medioventral to the labeled cells; and class Ec: unlabeled cells in the entire epidermal perimeter contralateral to the labeled cells. The results of this experiment are presented in Table 1. These data show that the midbody epidermis of a normal stage 10 *Helobdella* embryo contains, on the average, about 250 cells per segment. On the labeled side of the embryo, about 60 of these

cells belong to class OPi; they form the ventral segmental epidermis and, being labeled, are obviously derived from the labeled OP proteloblast. About 50 cells belong to class Qi; they form the dorsal segmental epidermis and are presumably derived from the Q teloblast ipsilateral to the labeled OP blastomere. And about 10 cells belong to class MVi; they form a longitudinal array of medioventral patches of epidermis, and are presumably derived from the unlabeled N teloblast ipsilateral to the labeled OP proteloblast. On the unlabeled side of the embryo, approximately 130 epidermal cells are scored as class Ec; they form the entire segmental epidermis of that other side and are derived from the four unlabeled teloblasts contralateral to the labeled OP proteloblast. Presumably their relative numerical subdivision into OP-, Q-, and N-derived cells corresponds to that seen on the labeled side.

The midbody epidermis of the operated embryo was seen to contain, on the average, only about 210 cells per segment, although in view of the relatively high standard deviation in cell counts, the observed decrease of about 40 epidermal cells per segment in the operated as compared to the normal specimen lies at the border of significance. On the labeled side of the operated embryos, about 70 cells belong to class OPi, but here class OPi also occupied the dorsal epidermal territory normally populated by class Qi. The scatter in cell counts places the increase of about 10 class OPi cells per segment, in the operated as compared to the normal specimen, below the level of statistical significance in that the values from normal and operated embryos are within one standard deviation. In accord with the observed spread of class OPi cells into dorsal territory, only about 5 cells per segment were scored here in class Qi. This decrease of about 45 cells per segment compared to the normal specimens is definitely significant. In principle, in these embryos class Qi should contain no cells at all, since its Q teloblast progenitor was ablated. The residual 5 cells per segment scored here as belonging to class Qi may be derived from the micromere cap, or may represent a posterior migration by epidermal progeny of Q blast cells produced prior to ablation. About 20 cells per segment were scored as belonging to class MVi, a statistically significant increase of about 10 cells per segment compared to the normal embryo. And about 120 cells per segment were scored as belonging to class Ec, with the decrease of about 10 cells per segment in the operated as compared to the normal embryo being barely significant statistically.

The data of Table 1 suggest (but, because of the marginal statistical significance of some of the observed numerical differences, do not rigorously prove) that the filling in of ablation-induced epidermal deficiencies does not result from an increase in the number of epidermal

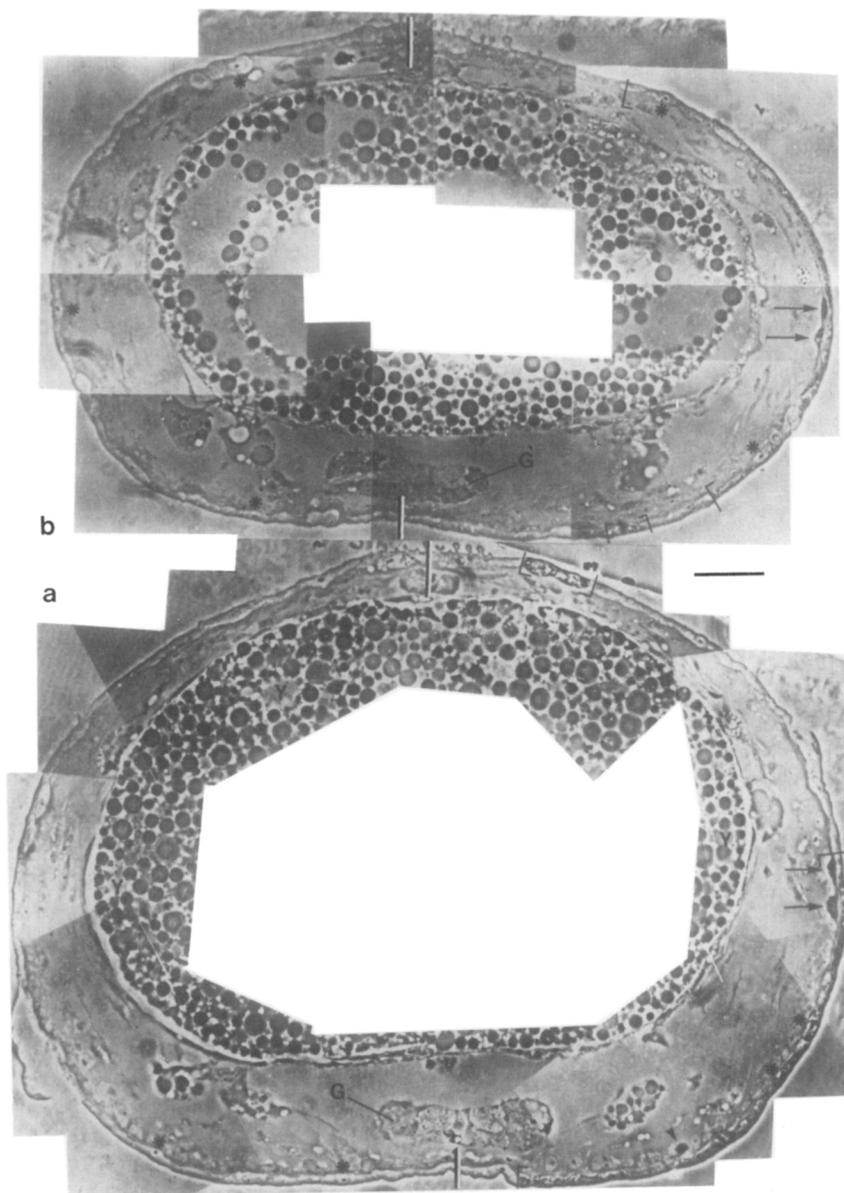


FIG. 3. Transverse sections (counterstained with toluidine blue) through stage 10 embryos, in which the right OP proteloblast was injected with HRP at stage 6b. HRP stain, which appears black in the whole-mounted embryo, appears yellow-brown with black flecks against the toluidine-blue background in these sections, and is thus difficult to see in black and white photographs. Thus, HRP-labeled epidermis is indicated here as lying between brackets, and labeled (OP-derived) peripheral neurons are indicated by arrows. Some of the epidermal nucleoli in stained and unstained epidermis are indicated by asterisks. (a) Normal (unoperated) embryo. Labeled cells lie mostly in the ventral body wall. Additional stained cells are seen in the body wall just next to the future dorsal midline. These OP-derived cells are beyond the margin of the germinal plate in the micromere cap region of the embryo (see Weisblat *et al.*, 1983). (b) Embryo in which the Q teloblast was ablated at stage 7. The labeled epidermis is shifted dorsolaterally but the labeled (OP-derived) peripheral neurons still lie midway between the dorsal and ventral midlines. Abbreviations: G, ganglion; Y, yolk platelets from the remnants of teloblasts in the gut. Dorsal and ventral midlines are indicated by black and white lines. Dorsal is up. Scale bar 25 μ m.

cells per segment produced by the remaining teloblasts. Rather, it would appear that in the operated embryo each teloblast has produced its normal complement of epidermal progeny, which are merely spread over a greater area of the epidermis. Thus, ablation of the Q teloblast would eliminate approximately 40 cells per segment that normally populate the ipsilateral dorsal

epidermis. Their place would be taken by some of the 60 to 70 cells per segment derived from the O and P teloblasts that normally populate only the ipsilateral ventral epidermis. The dorsal spread of the O- and P-derived cells would, in turn, generate a deficit in the ipsilateral medioventral epidermis, which is filled by about 10 epidermal cells per segment of contralateral

TABLE 1
AVERAGE NUMBER OF EPIDERMAL NUCLEOLI PER SEGMENT IN STAGE
10 EMBRYOS OF *Helobdella triserialis*^a

Epidermal cell classes	Nucleoli/segment	
	Normal embryos	Operated (Q-ablated) embryos
O _P i	62 ± 4	68 ± 18
Q _i	47 ± 7	5 ± 5
Mi _V	8 ± 3	18 ± 3
Ec	132 ± 6	120 ± 10
Total	249 ± 16	211 ± 22

^a In both normal and operated embryos, the right OP proteloblast was injected with HRP at stage 7. In operated embryos, the right Q teloblast was ablated at stage 6c. Nucleoli of epidermal cells were counted in 60 serial, 3- μ m-thick sections made from midbody segments of both a normal and an operated stage 10 embryo. The counts of 15 consecutive sections were summed and taken to be equal to the total number of nucleoli per segment in epidermal cells of a given class. These numbers were averaged over the four segments counted in both embryos, and their standard deviation was calculated. Because of flaws in parts of some sections, not all classes of epidermal cells could be scored in every section.

origin that spread across the ventral midline. It should be noted, however, that these (tentative) conclusions pertain only to epidermal development up to stage 10. Since there occurs an extensive proliferation of epidermal cells in postembryonic development and growth (unpublished observations), it is possible that the numerical deficit of operated embryos vanishes at later stages.

Invariant Position of Peripheral Neurons

In addition to the epidermal cells, on which our attention has so far focused, HRP-stained cell bodies and processes of OP-derived peripheral neurons can be identified in the body wall of sectioned embryos. The cell bodies of some of these neurons lie between epidermal and mesodermal cell layers near the future lateral edge of the animal, midway between ventral and dorsal midlines. Accordingly, on the labeled side of the normal embryos these peripheral neurons lie near the border between class O_Pi (i.e., OP-derived, labeled) and class Q_i (i.e., Q-derived, nonlabeled) epidermal cells (Fig. 3a). On the labeled side of the Q-ablated embryos, where the class O_Pi epidermal cells have spread beyond the future lateral edge to the dorsal midline, the peripheral neurons are nevertheless still located at about their normal position, midway between ventral and dorsal midlines (Fig. 3b). Hence following ablation of the Q teloblast, the positioning of these peripheral neurons is not affected by the extensive dorsal spread of their OP-derived epidermal sister cells. That is to say, the

developmental cue that assigns these neurons to their particular peripheral position cannot be the interface between Q-teloblast and OP-proteloblast derived epidermal cells.

DISCUSSION

The experiments presented here show that upon ablation of one or more teloblasts, the epidermal cells derived from the remaining teloblasts take on abnormal positions in the embryonic body wall, occupying territories of the epidermis where ablation has created a deficiency in the normal cell complement. All epidermal cell lines, therefore, seem to be of equal developmental potential regardless of their teloblast of origin, with the eventual location of any cell in the body wall being governed by the relative position of its blast cell bandlet of origin in the germinal plate and by intercellular interactions within the developing epidermis. This conclusion is further supported by the finding that in ablations of O, P, or Q blastomeres, the resultant epidermal spreading entails the movement of cells into completely nonhomologous positions. In particular, cells that would normally lie in the dorsal body wall are found in the ventral body wall, or vice versa.

These findings indicate that the types of cell interactions which lead to the stereotyped distribution of ectodermal cells in the leech differ in epidermal and neural tissues. In contrast to the ablation-induced spreading of epidermal precursor cells into deficient skin territories, neuronal precursor cells do not move to nonhomologous locations to replace neurons that happen to be missing due to teloblast ablation (Blair, 1983; Blair and Weisblat, 1982) [except for the special case of "transfating" (Weisblat and Blair, 1984)]. Although neuronal precursor cells may cross the midline upon ablation of an N teloblast, such crossover appears merely to redistribute neurons of determinate fate into their homologous contralateral positions in the CNS and their biochemical traits are unchanged (Blair and Weisblat, 1982; Blair, 1983). It is unlikely that the more determinate fate of neuronal precursor cells is attributable merely to their being located in the CNS, since as shown here and elsewhere (Blair, 1983), peripheral neurons also remain in their normal position in conditions under which epidermal precursor cells from the same teloblast lineage spread into abnormal skin territories.

Thus the developing leech epidermis is analogous to a viscous fluid layer formed by pouring a fixed volume (cell number) of four bilateral pairs of normal constituents (N-, O-, P-, and Q-derived epidermal cell lines) onto a supporting surface at given (ventromedial, ventral, and dorsal) positions in every body segment. The various fluid components (cell lines) spread to cover the

surface until they meet, establishing the normal epidermal distribution patterns as they do so. If, upon ablation of a teloblast, one of the normal fluid components (epidermal cell precursors) is missing, the nearest remaining fluid component spreads into and covers the vacant territory. Such spread creates a vacancy at the distal margin of the spreading component, which, in turn, is covered by spread of the next-but-one remaining fluid component.

The quasi-fluid mechanism of epidermal development is likely to be of adaptive value, in that it automatically provides for the filling of any gaps that would arise in the skin of the embryo as a result of accidental malfunction of one of the ectodermal teloblasts or blast cells. In support of this inference, we have encountered some "control" (i.e., nonoperated) embryos with morphologically intact skin, in which one or more labeled teloblasts had evidently failed to give rise to their normal complement of epidermal cells. If, as our numerical analysis tends to indicate, spreading is the result of an increase in the area covered by the surviving epidermal cells, then this phenomenon would constitute an instance of what Sulston and White have referred to as functional regulation in their studies of nematode development (1980).

Finally, no spread of epidermal precursor cells across the dorsal midline was found in any of the present experiments, in contrast to the readily observable spread across the ventral midline. This suggests that the epidermal precursors are not free to spread beyond the margin of the expanding germinal plate. Accordingly, by the time the right and left leading margins of the germinal plate fuse at the dorsal midline (at the end of stage 10), the spread of epidermal precursor cells into deficient territories of the contralateral half of the germinal plate would be complete, so that no opportunity for spreading across the dorsal midline exists.

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