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Embryonic Origins of Cells in the Leech *Helobdella triserialis*

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To ascertain the embryonic origins of the cells in various tissues of the leech *Helobdella triserialis*, horseradish peroxidase (HRP) was injected as a cell lineage tracer into all identified blastomeres of the early embryo in turn, except for a few of the micromeres, and the resulting distribution of HRP-labeled cells was then examined in the late embryo. In this way it was found that in every body segment a topographically characteristic set of neurons in the ganglion and body wall and a characteristic territory of the epidermis is derived from each of the four paired ectodermal teloblasts N, O/P, O/P, and Q, whereas the muscles, nephridia, and connective tissue, as well as a few presumptive neurons in each segmental ganglion, are derived from the paired mesodermal teloblast, M. Each topographically characteristic, segmentally iterated set of neurons descended from a given teloblast is designated as a kinship group. However, the prostomial (nonsegmental) epidermis and the neurons of the supraesophageal ganglion were found to be derived from the a, b, c, and d micromere quartet to which the A, B, C, and D blastomeres give rise at the dorsal pole of the embryo. The superficial epithelium of the provisional integument, which covers the surface of the embryo midway through development and is sloughed off at the time of body closure, was found to be derived from the a, b, c, and d micromere quartet, as well as from other micromeres produced in the course of teloblast formation. The contractile fibers of the provisional integument were found to be derived from the paired M teloblast. These results demonstrate that development of the leech embryo proceeds according to a highly stereotyped pattern, in the sense that a particular identifiable blastomere of the early embryo regularly gives rise to a particular set of cells of the adult (or provisional embryonic) tissues.

INTRODUCTION

The development of intracellularly injectable cell lineage tracers has provided a new experimental approach to developmental fate mapping (Weisblat *et al.*, 1978; 1980a,b). By injecting horseradish peroxidase (HRP) as an intracellular lineage tracer in early embryos of the glossiphoniid leech *Helobdella triserialis*, and staining for its presence in late embryos, we found that the leech nervous system has several embryonic sources (Weisblat *et al.*, 1980a), in contrast to the single source proposed for it on the basis of direct observations (Schleip, 1936). In the work reported here these observations were extended by means of experiments in which the progeny distribution for every blastomere of the early leech embryo, except for some of the micromeres, was determined using HRP as a cell lineage tracer. Patterns of HRP stain were examined in both neural and non-neural tissues in the late embryo.

The results of these experiments are presented in terms of the nomenclature and staging scheme put forward previously for the development of glossiphoniid leeches (Weisblat *et al.*, 1980a; Fernandez, 1980; Stent *et al.*, 1982), modified as described below and summarized in Fig. 1. By stage 4a, all four blastomeres A, B, C, and D (formed at stage 3) have cleaved unequally to yield four micromeres a, b, c, and d at the dorsal pole of their junction and four macromeres (which retain the capital letter designations). By the end of stage 6 the stereotyped sequence of early cleavages has given rise to 13 large blastomeres: macromeres A, B, and C, and five bilateral teloblast pairs, M, N, O/P, O/P, and Q, derived from macromere D after stage 4a. Previously, the two pairs of teloblasts referred to here both as O/P were designated as distinct pairs of O and P teloblasts. As will be shown here, the nominal O and P teloblasts have different fates in normal development. But they are, in fact, developmentally equipotent (Weisblat and Blair, 1984; Shankland and Weisblat, in preparation), as reflected in the revised nomenclature. During stage 7, each teloblast undergoes a series of iterated, unequal cleavages to form a bandlet of smaller primary blast cells (previously referred to as "stem cells"). On either side of the midline, the bandlets grow and merge to form right and left germinal bands overlying the macromeres. Each germinal band consists of a superficial layer provided by the four ectodermal bandlets and a deep layer provided by the mesodermal, m, bandlet (Fig. 1). Once the bandlets of the two O/P teloblasts enter the germinal band their distinct fates in normal development can be predicted unambiguously by their relative position. Thus, the O/P-derived bandlet lying next to q is designated p, that lying next to n is designated o, so that the ectodermal bandlets lie in mediolateral order q, p, o, n, within the germinal bands.

Left and right germinal bands project to the future

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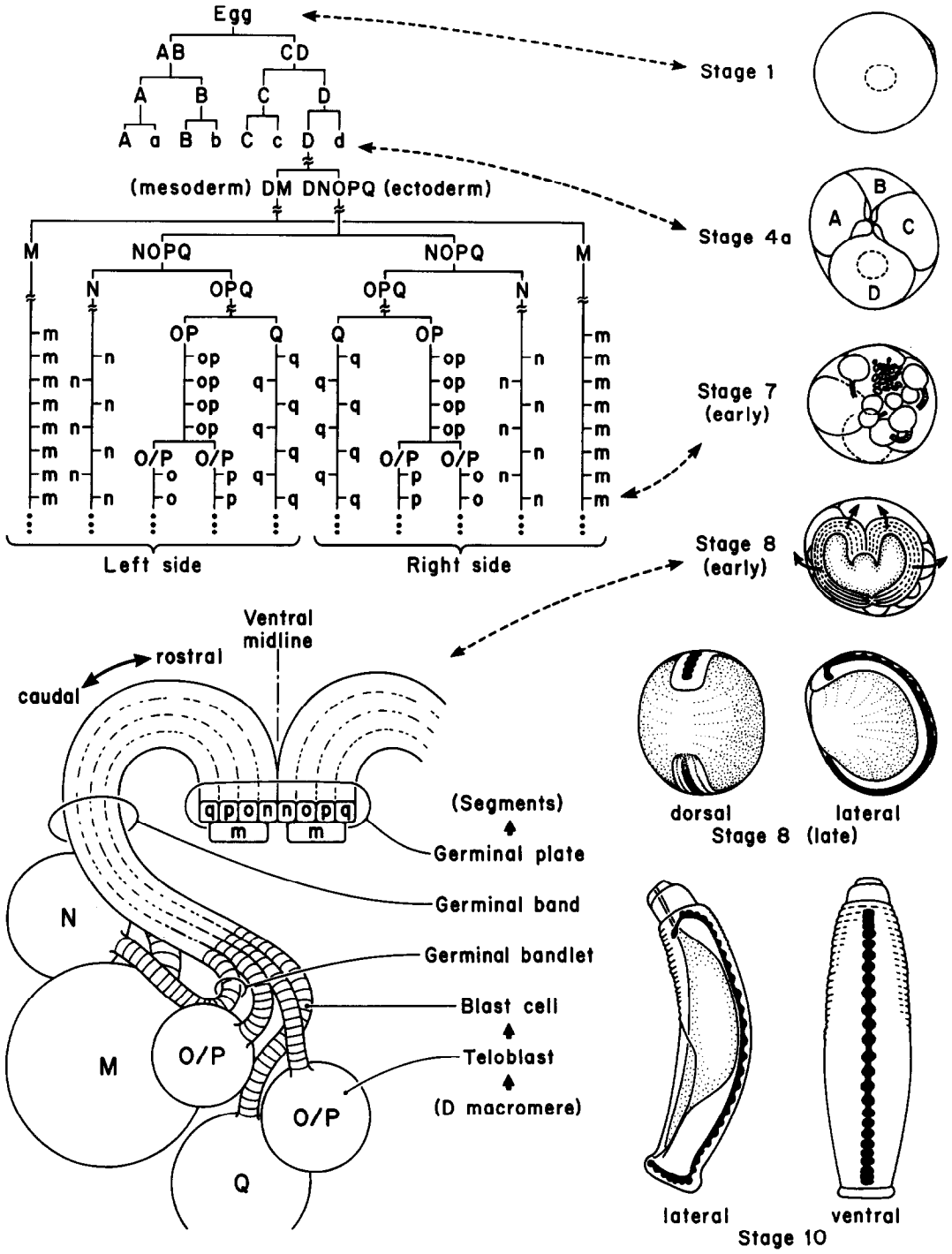

FIG. 1. Schematic summary of the development of *Helobdella triserialis*. *Upper left:* Cell pedigree leading from the uncleaved egg to the macromeres A, B, and C, the micromeres a, b, c, and d, the teloblast pairs M, N, O/P, O/P, and Q, and the paired primary blast cell bandlets. Breaks in the lineage indicate points where additional micromeres may be produced. The number of op blast cells produced prior to cleavage of proteloblast OP varies from four to seven (Shankland, unpublished observations). *Lower left:* Hemilateral disposition of the teloblasts and their primary blast cell bandlets within the germinal band and germinal plate. *Right margin:* Diagrammatic views of the embryo at various stages. The dashed circle in the uncleaved egg (stage 1) signifies the teloplasm, which is passed on mainly to the D macromere (stage 4a). In the stage 7 embryo the dashed circle signifies the right M teloblast (which is invisible from the dorsal aspect) and the many small, closed contours in the upper midportion indicate the micromere cap. In the stage 8 (early) embryo, the heart-shaped germinal bands migrate over the surface of the embryo in the directions indicated by the arrows. The incipient larval integument is shown as a stippled area lying between the germinal bands. In the stage 8 (late) embryo the germinal plate is shown to be on the ventral midline, with the nascent ventral nerve cord and its ganglia and ganglionic primordia indicated in black. The stippled larval integument covers the entire embryo, from one edge of the germinal plate to the other. In the stage 10 embryo shown, body closure is nearly complete. Here, the stippled areas signify the yolky remnant of the macromeres and teloblasts, now enclosed in the gut of the embryo. The chain of ganglia linked via connectives, shown in black, already closely resembles the adult nerve cord.

dorsal surface, and the first-born blast cells converge at the site of the future head. With ongoing production of primary blast cells, the bands lengthen and their midportions move circumferentially onto the future ventral surface (stage 8). Gradually, right and left germinal bands meet and coalesce along the ventral midline. Coalescence begins at the future head and continues rearward like a zipper. As they coalesce, the germinal bands form the *germinal plate* which straddles the ventral midline (stage 8). The circumferential migration of the germinal bands leading to their coalescence inverts the mediolateral order of the blast cell bandlets, which is therefore n, o, p, q in the germinal plate (Fig. 1). In subsequent development, the blast cells of the germinal plate proliferate, ultimately producing the segmental tissues of the adult. Blast cell proliferation results in a gradual thickening and circumferential expansion of the germinal plate over the surface of the embryo back into dorsal territory (stage 9). Eventually, right and left leading edges of the expanding germinal plate meet and fuse on the future dorsal midline, thus closing the leech body (stage 10).

The cell lineage tracer experiments to be reported here showed that the following fates can be assigned to the large blastomeres of the stage 6 glossiphoniid leech embryo. Each of the four paired ectodermal teloblasts, N, O/P, O/P, and Q, makes a topographically stereotyped contribution to the ipsilateral segmental nervous system and epidermis. The mesodermal teloblast, M, gives rise to the longitudinal, circular and oblique muscle fibers of the body wall, in addition to the longitudinal muscle fibers in the nerve cord connective and to a few presumptive neurons within the segmental ganglion. The a, b, c, and d micromeres are the source of the prostomial tissues that lie in front of the metameric segmental structures and of most of the transient epithelial layer of the provisional integument that comes to cover the surface of the embryo during stage 8. Moreover, the M teloblast also gives rise to an array of circumferentially oriented contractile fibers beneath the transient epithelial layer of the provisional integument, which are presumably responsible for the peristaltic movement of the embryo prior to development of the definitive circular muscles and nervous system.

MATERIALS AND METHODS

The procedures for laboratory culture of *H. triserialis* and for growing individual embryos from uncleaved egg to maturity *in vitro* are described elsewhere (Blair and Weisblat, 1984). The procedures for HRP injection of blastomeres, histochemical processing of HRP-injected embryos, and observing the distribution of stain either in wholemounts of the embryos or in serial thick sections of Epon-embedded specimens have also been described previously (Weisblat *et al.*, 1978; 1980a), except for the following modifications. (1) Fast green dye (Sigma) was added (0.5 to 1.0% final concentration) to the HRP solution prior to blastomere injection, in order to allow visual monitoring of the progress of the injection (Parnas and Bowling, 1977; Blair, 1982). The greater control over the injection afforded by visual monitoring increased the success rate of the lineage tracer experiments by lowering the incidence of under- as well as (lethally) over-injected embryos. (2) In some experiments fixation was carried out in sodium cacodylate buffer (0.1 *M*, pH 7.4), instead of phosphate buffer, with no change in results. (3) The duration of glutaraldehyde fixation of stage 10 embryos was reduced from 10–15 to 1–2 hr at 4°C, and the amount of H_2O_2 added per milliliter of saturated benzidine dihydrochloride solution was reduced from 50–100 μl of a 3% solution to 10–50 μl of 0.3% solution. These modifications raised the sensitivity of HRP detection, especially in so far as they permit the visualization of HRP stain in fine neuronal processes and in epidermal cells. (4) For preparation of serial thick (1–3 μm) sections, the fixed and stained embryos were embedded in a softer Epon formulation (mixture A from Luft, 1961) and sectioned with glass knives using a Sorvall JB4 microtome.

RESULTS

To ascertain the contribution of each of the five paired teloblasts' progeny cells to the tissues of *Helobdella*, HRP was injected into one particular teloblast of stage 6 or 7 embryos. After further development had proceeded to stage 10, the embryos were fixed and stained. The HRP distribution in the stained embryos was examined either in wholemounts or in serially sectioned material. As shown by the wholemounts of Fig. 2 and 3, five distinct, segmentally repeated, unilateral labeling patterns are obtained in this way, each pattern being characteristic of a particular teloblast. The stained cells lie on the same side of the midline as the injected teloblast. In exception to this statement it should be noted that a background stain is sometimes detected (even in uninjected specimens), in the botryoidal tissue, a group of large cells distributed *bilaterally* along the gut (Figs. 3 and 10). The staining of these cells (Fischer *et al.*, 1976) means that *their* embryonic origins cannot be determined using HRP as the injected cell lineage tracer, but it does not otherwise interfere with the interpretation of the staining patterns. Except for the left Q and right M teloblasts, as noted below, each teloblast has been successfully injected dozens of times. (The survival rate of injected embryos varies between experiments from 50 to 80%. Of surviving embryos, between 50 and 100%

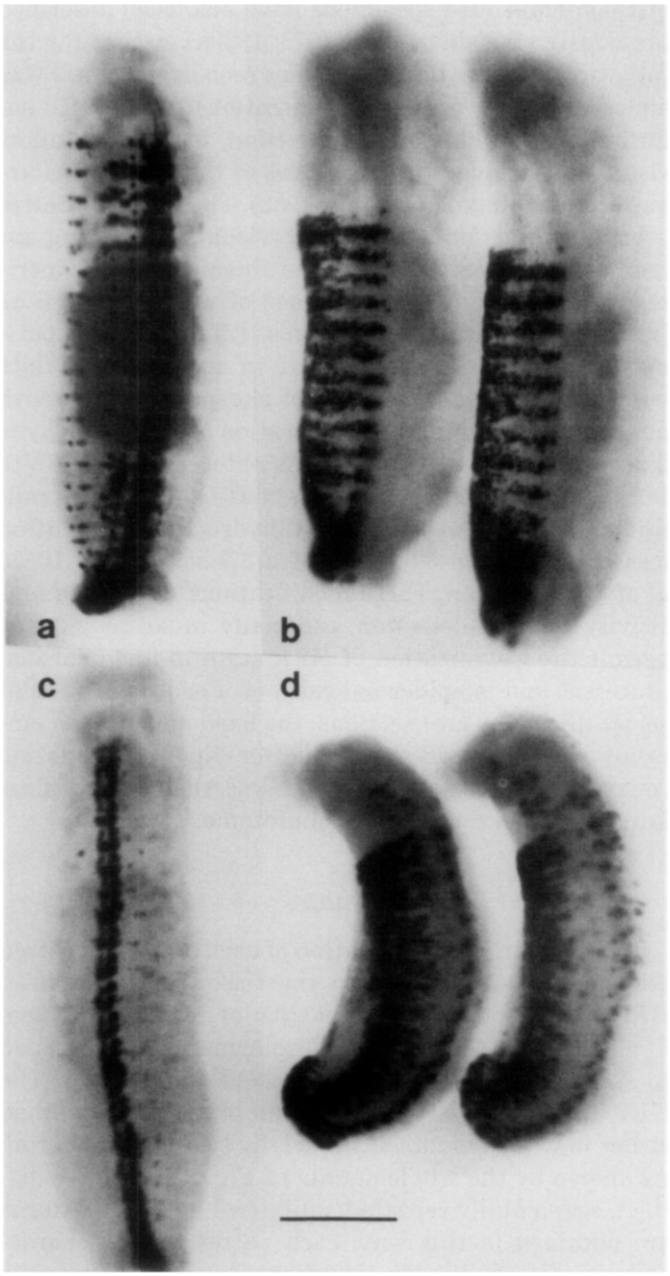

FIG. 2. Segmentally iterated contributions of the ectodermal teloblasts to nervous system and epidermis. Labeling patterns of wholemounted stage 10 *Helobdella* embryos in which one teloblast had been injected with HRP at stage 7. Since prior to injection the teloblast had already produced some blast cells, the frontmost body segments are unlabeled. All labeled cell bodies lie on the same side of the embryo as the injected teloblast. Anterior is up; scale bar 200 μm. (a) Right O/P teloblast injected; O pattern obtained. Labeled cell processes can be seen to project frontward through the connective nerves from the anteriormost labeled ganglion. (b) Right O/P teloblast injected (in two replicate embryos); P pattern obtained. (c) Left N teloblast injected. (d) Right Q teloblast injected (in two replicate embryos). (a–c): Ventral views; (d): lateral views. Ventral midline of embryo is marked by apparent right edge of labeling pattern in (a), (b), and (d) and by apparent left edge in (c). In (d), leading edge of expanding germinal plate is marked by apparent left edge of labeling pattern.

give good staining; most of the remainder either show a staining pattern too faint to be useful, or have suffered the death of the injected cell.) To determine the domains occupied by the stained cells within the ganglia, a series of from five to eight midbody ganglia were examined in each of two or more horizontally sectioned embryos for each HRP-injected teloblast.

Before interpreting these results it must be noted that the disposition of the teloblast pairs in the stage 7 embryo is not bilaterally symmetric. For instance, whereas the left M teloblast can always be identified and impaled because of its relatively large size and superficial position at the left edge of the future dorsal aspect of the embryo, the right M teloblast is more difficult to locate and impale because it usually lies deep within the embryo, submerged in the surrounding macromere. The left Q teloblast is similarly difficult to locate and impale. However, the right M and left Q teloblasts have each been successfully injected a few times, resulting in labeling patterns (not shown) which are mirror symmetric to those resulting from injection of their contralateral homologs. Thus the asymmetric disposition of the teloblasts does not lead to an asymmetric fate of their progeny.

Many of the present findings contradict conclusions reached by the pioneer analysts of cell lineage in glossiphoniid leech development (for a summary see Schleip, 1936), who observed developing embryos without the aid of tracers. According to the most widely accepted of these earlier views, the nervous system is descended entirely from the n bandlets, the o, p, and q bandlets are the precursors of the circular muscles [for which reason Müller (1932) designated their parent teloblasts as M^1, M^2, and M^3], and the epidermis is derived from the expanding cell sheet designated as the micromere cap (Fig. 1). In fact, as will be shown further below, the circular muscles actually derive from the M teloblast (referred to as My by Müller), and all but the most rostral area of the micromere cap is a provisional embryonic integument, rather than the precursor of the definitive epidermis of the mature leech. It should be noted that, by way of dissent from these earlier mainline views, Apathy (1889) had maintained that the nervous system is derived from the n, o, and p bandlets, with each bandlet making a distinct contribution, a view which is closer to the truth but for which he was ridiculed by Bergh (1891).

Segmental Tissues: Ectodermal Teloblasts

We have found that all four of the ectodermal teloblasts N, O/P, O/P, and Q, make segmentally iterated progeny cell contributions both to the nervous system and to non-neural ectoderm, mainly epidermis (Fig. 2).

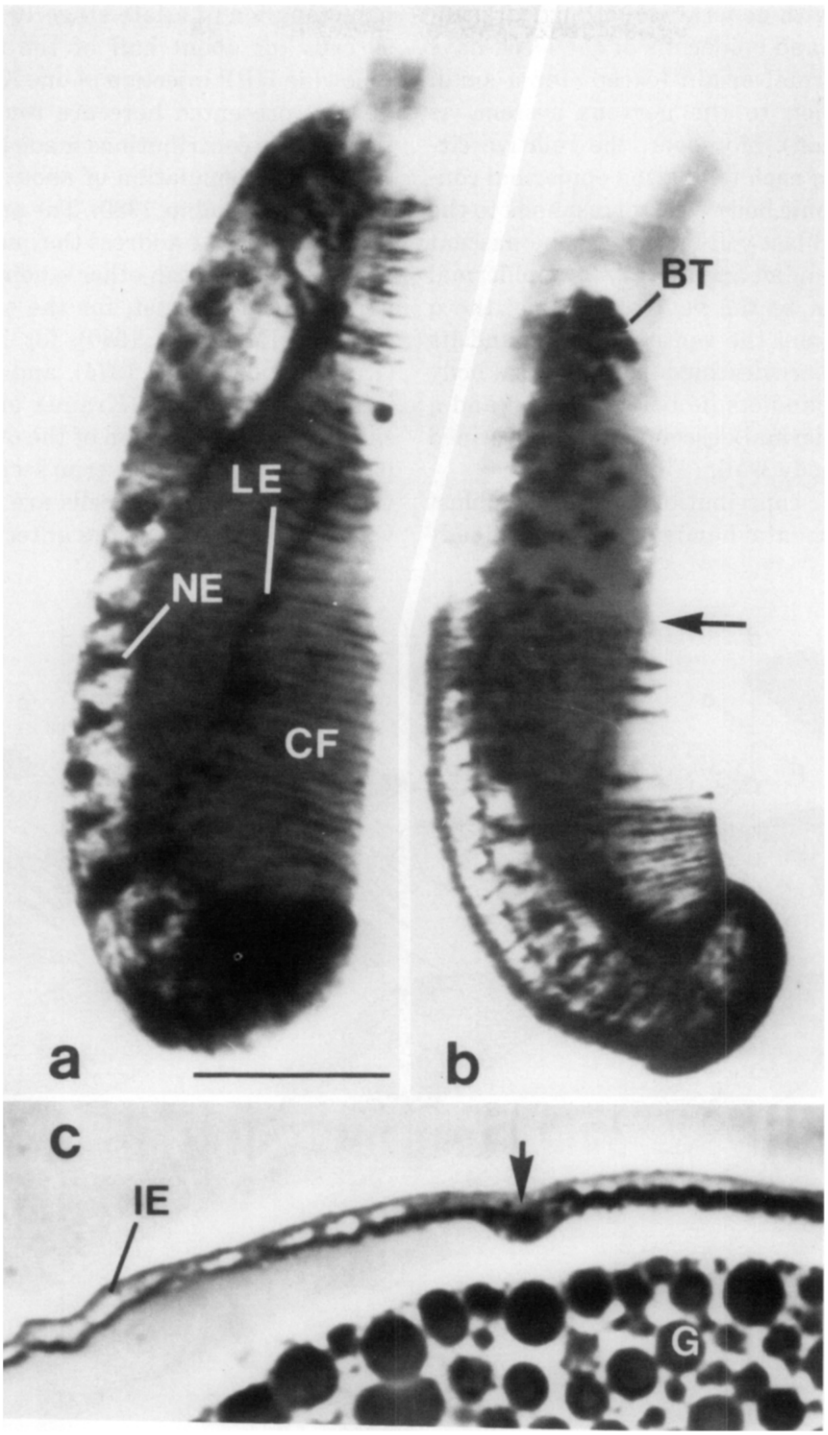

FIG. 3. Contribution of the mesodermal M teloblast to the body wall and provisional integument. Labeling pattern of stage 10 embryos whose left M teloblast had been injected with HRP. (a) Injection at stage 6; dorsolateral aspect, venter to the left. The leading edge (LE) of the germinal plate is visible as a dark waving line lying over the gut (dark gray, tear-shaped profile). The segmental repeating unit is indicated by labeled nephridia (NE). Circumferentially oriented contractile fibers (CF) extend from the dorsal midline of the provisional integument into the germinal plate. (b) Injection at stage 7, when m blast cell production is well under way; lateral aspect, venter to the left. Labeled, segmentally iterated cells are confined to the sector of the germinal plate posterior to the arrow. The boundary between labeled and unlabeled cells is the same for the contractile fibers of the provisional integument as for the germinal plate tissues, indicating that the former are also derived from the m blast cell bandlet. Background staining is seen in botryoidal tissue (BT) anterior to the boundary marked by the arrow. These cells also stain on the right (unlabeled) side of the embryo, and in embryos which were not injected with HRP at all. (c) Transverse section through the provisional integument of an embryo similar to that shown in (a). Labeled cell body (arrow) and processes of integumental contractile fibers lie in close apposition to the unlabeled squamous epithelium (IE). The yolk-filled gut (G) lies below, separated from the integument by a fluid-filled space. Scale bar 200 μm in (a) and (b); 20 μm in (c).

Thus in conformance with general embryological principles, nervous system and epidermis of the leech have a common, i.e., ectodermal origin (except for a small mesodermal contribution to the nervous system, as shown in a later section). Moreover, the relative circumferential position of each teloblast's epidermal contribution to the embryonic body wall corresponds to the relative position of its blast cell bandlet in the nascent germinal plate: the n bandlet and its very few epidermal descendants lie nearest to the ventral midline; the q bandlet lies furthest from the ventral midline and its epidermal descendants are destined for the dorsal body wall; and the o and p bandlets lie between the n and q bandlets and their epidermal descendants are destined for the ventrolateral body wall.

The principal cellular contribution of the N teloblast is to the ipsilateral segmental hemiganglion. A midbody hemiganglion of a late stage 10 embryo contains about 90 cells (or about half of the total) that are labeled following HRP injection of one N teloblast. (The number of cells presented here are rough estimates based on the relative contributions made by each teloblast, scaled to the total population of about 190 neurons per hemiganglion (Macagno, 1980). The present estimates are not precise enough to address the question of developmental variability, although other studies have shown that such variability does exist, for the overall cell number per ganglion (Macagno, 1980), for identified neuron types (Kuffler and Muller, 1974), and for the descendants of individual teloblasts (Kramer and Weisblat, 1984).) As shown by reconstruction of the overall neuronal labeling pattern (Fig. 4) seen in serial sections of labeled embryos (Fig. 5), the N-derived cells are arranged as two transverse slabs of cells in the anterior and posterior parts

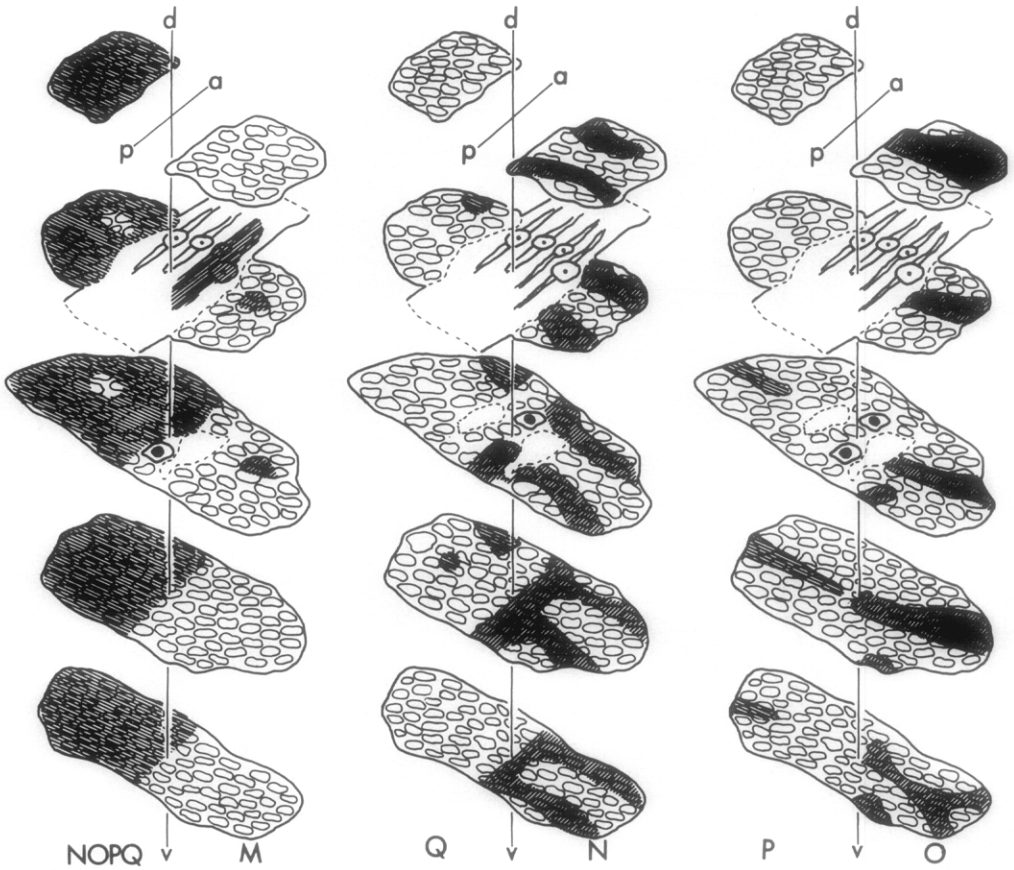

FIG. 4. Embryonic origins of the cells of the *Helobdella* segmental ganglion. The drawing shows five horizontal sections through a set of schematized midbody segmental ganglia of a mid-stage 10 embryo (d = dorsal, v = ventral, a = anterior, p = posterior). In each half-ganglion, crosshatched domains indicate the disposition of the kinship group descended from the blastomere or teloblast designated at the bottom of the figure. The two pairs of nucleated, elongated contours in the center of the second section from the top represent the longitudinal muscle cells in the connective nerve tract. The two dark nucleated contours in the third section represent the identifiable giant neuropil glia. The faint contours do not correspond to identified cells (and thus are drawn without regard for domain boundaries), but merely indicate the approximate size, disposition and number of neurons in the ganglion. The overlap that would obtain if the M, N, O, P, and Q kinship groups in this figure were all projected onto the same half-ganglion reflects the variability in the boundary of any given kinship group from segment to segment.

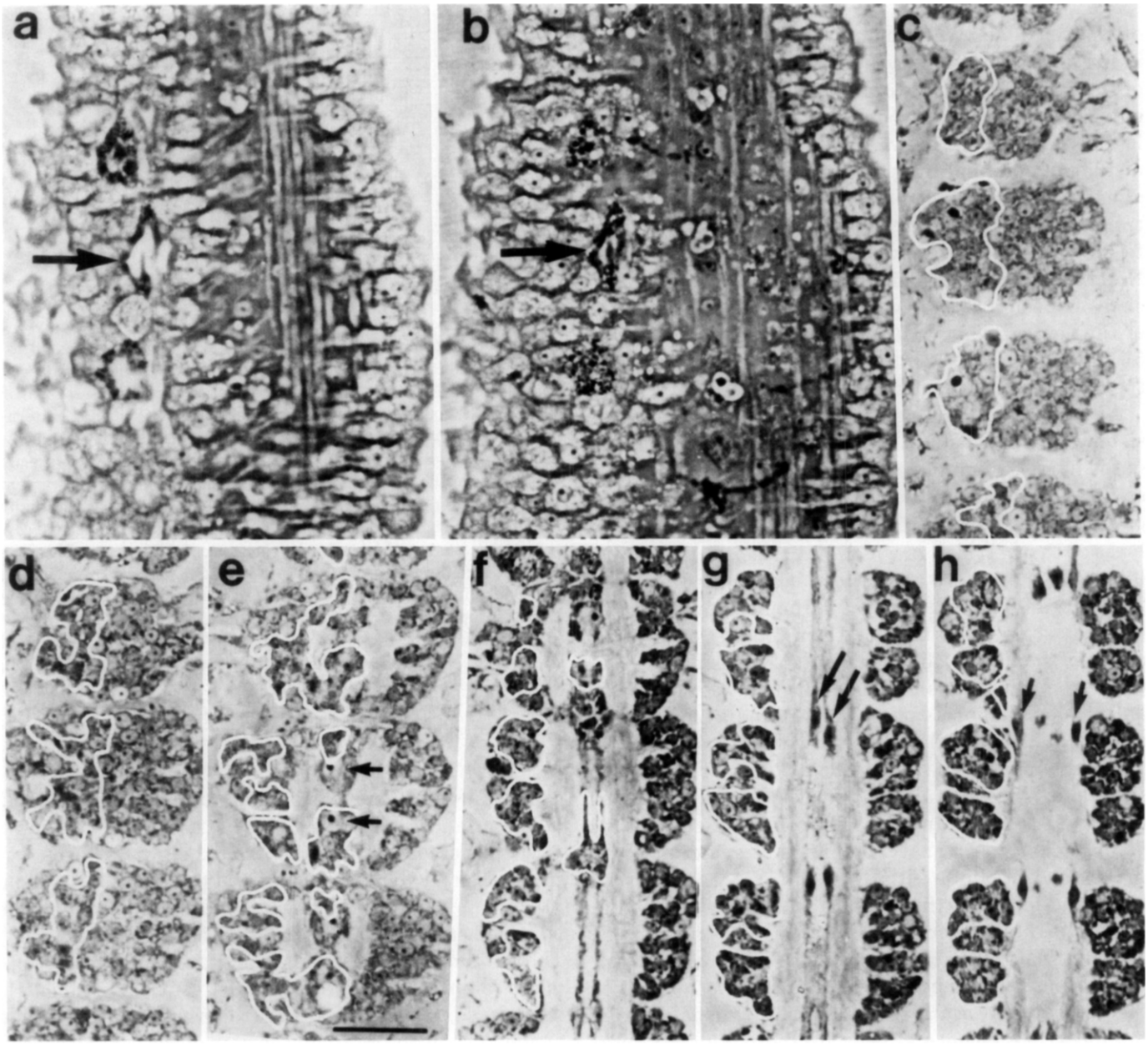

FIG. 5. Horizontal sections through three segments of the germinal plate of a stage 10 embryo whose N teloblast had been injected with HRP at stage 6. Eight 3-μm-thick sections are shown, from a series of serial sections, proceeding from the ventral body wall (a) to the dorsal surface of the segmental ganglia (h). In (a) and (b), which show two successive sections through the ventral body wall, labeled (arrows) and unlabeled epidermal cells are visible in each segment, as are unlabeled circular, oblique, and longitudinal muscles. In panels (c)–(h), which show representative sections through the nerve cord, domains of labeled cell bodies are outlined in each ganglion; HRP-labeled cells are identified in the original sections as yellow or light brown, as distinct from the other cells which are counterstained blue. These differences are not apparent in black and white photographs except when the stain is especially heavy or vessiculated (as in the epidermal cells of this preparation). The morphologically distinct cell bodies of the giant neuropil glia are seen in (e (arrows)) and (f). Neuropil is visible in (e)–(g), interganglionic connections in (f)–(h), and connective muscle cells (arrows) in (g) and (h). Scale bar: 25 μm.

of the hemiganglion, and as a longitudinal band of cells near the midline on the ventral aspect of the ganglion. In agreement with previous findings (Weisblat *et al.*, 1980a; Blair and Weisblat, 1982), this band of cells always includes either the anterior or the posterior, but never both, of the two morphologically distinguishable neuropil glia, whose cell bodies are arrayed anteroposteriorly along the ventral midline of the ganglionic neuropil. Only a few N-derived cells lie outside the hemiganglion; these include one or a few cells located in the body wall lateral to the posterior portion of the ganglion (Fig. 2). These cells had been previously observed in

stage 9 embryos and tentatively proposed as precursors of the sensillae (Weisblat *et al.*, 1978). However, even in the late stage 10 embryos (near to the point of closure of the body tube) shown in Fig. 2, there is only one such extraganglionic N-derived cell or cell cluster, whereas leeches typically have several bilateral pairs of sensillae distributed circumferentially over the central annulus of each midbody segment (Grassé, 1959). Hence the extraganglionic N-derived cells are unlikely to give rise to all sensillae, if to any at all. The remainder of the N-derived extraganglionic, segmental contribution consists of one or a few cells per segment in the outer layer of the ventral body wall, adjacent to the midline (Fig. 5). On the basis of their flat, block-shaped morphology, they appear to be epidermal cells.

Although it is not possible to distinguish between sister O/P teloblasts at the time of their impalement in the living embryo, two distinct labeling patterns nevertheless result from their HRP injection. One of these patterns predominates following injection of that teloblast of the O/P sisters which lies closer to the N teloblast. This pattern has been designated as the "O pattern." The contribution to the hemiganglion of a midbody segment by the O pattern consists of about 60 cells, arrayed as an oblique column traversing the ganglion from the midportion of its ventral aspect to the anterior portion of its dorsal aspect and a small group of cells at the caudal end of the ventral aspect of the ganglion (Fig. 4). The O pattern also comprises a substantial extraganglionic contribution to the ipsilateral body wall (Figs. 2 and 6). This contribution consists predominantly of transverse fingers of epidermal cells that extend circumferentially into the ventral body wall about one-fourth of the distance from the ventral midline to the future lateral edge, between adjacent ganglia, and cover part of the hemiganglion. The extraganglionic component of the O pattern also includes a few cells in the ventral body wall which are distinguishable from the block-like epidermal cells by their punctate morphology and more intense HRP staining. Thus they appear as a regular array of dots (each consisting of one or a very few cells) in the body wall, underlying, and partially obscured by, the O pattern epidermis (Fig. 2). For that reason, the inventory of nonepidermal extraganglionic cells presented here is tentative, pending serial reconstruction of the labeled body wall. Two dots per segment are located just lateral to the nerve cord, one within and the other outside the circumferentially projecting finger. A larger dot is located at the anterior edge of the lateral end of the finger and a group of dots is located just beyond the lateral end of that finger. It has not yet been determined whether these dots consist of more than one cell, but they are likely to contain the cell bodies of some peripheral neurons (Blair, 1983; Weisblat and Blair, 1984; Stuart *et al.*, in preparation).

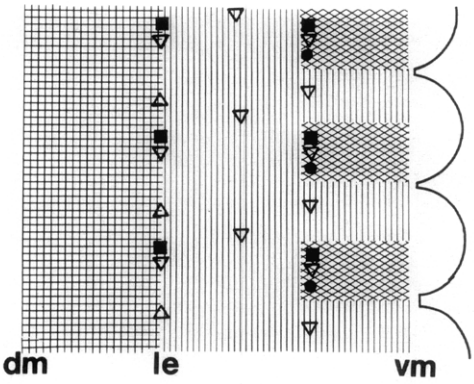

FIG. 6. Schematic representation of the segmentally iterated extraganglionic contributions of the N, O, P, and Q labeling patterns, as abstracted from cell lineage tracer data such as those shown in Fig. 2. Epidermal territories derived from O, P, and Q labeling patterns are indicated by diamond, parallel, and square hatching, respectively. (The few, N-derived epidermal cells are not shown). The precise boundaries of the various epidermal contributions vary from segment to segment (Fig. 2; see also Blair and Weisblat, 1984) but are mutually complementary, as indicated here, to provide complete epidermal coverage (unpublished observations). The approximate relative locations of some peripheral neurons, or neuron clusters derived from N, O, P, and Q labeling patterns, are indicated by black circles, down-pointing triangles, up-pointing triangles, and black squares, respectively. Not all peripheral neurons are accounted for in the schema. The width of the dorsal body wall, from dorsal midline (dm) to lateral edge (le), has been reduced in this drawing relative to that of the ventral body wall. The outlines of the contralateral half-ganglia are indicated next to the ventral midline (vm).

The other of the two distinct labeling patterns for the O/P sister teloblasts, obtained predominantly upon injection of that sister which is more distant from the N teloblast, has been designated as the "P pattern." The contribution to the hemiganglion of a midbody segment comprised by the P pattern consists of about a dozen cells, arrayed as a thin transverse strip coursing mediolaterally in the middle portion of the ventral aspect (Fig. 4). Like the O pattern, the P pattern also comprises a substantial contribution to the ipsilateral body wall outside the ganglion (Figs. 2 and 6). The epidermal component of this extraganglionic part of the P pattern lies within the ventral body wall, where it is essentially complementary to the O pattern, so that between them, the O and P patterns account for the bulk of the future ventral epidermis. Specifically, the epidermal P pattern consists of a longitudinal band of cells coursing about halfway between the nerve cord and the future lateral edge. A finger of epidermal cells projects circumferentially from this band to the ganglion, where it appears to merge with the thin transverse strip of P pattern neurons within the ganglion. The epidermal fingers of

the O and P patterns interdigitate. The nonepidermal, extraganglionic component of the P pattern is more extensively obscured by epidermis than that of the O pattern, but it has also been seen to include at least one dot per segment of nonepidermal cells aligned with the most lateral of the nonepidermal dots of the O pattern (Fig. 6). The lateral dot in the P pattern is also likely to contain one or more peripheral neuronal cell bodies (Blair, 1983; Weisblat and Blair, 1984; Stuart *et al.*, in preparation).

Of the four ectodermal teloblasts, the Q teloblast, with the most lateral blast cell bandlet in the nascent germinal plate, also makes the smallest contribution, about 10 cells, to the ipsilateral segmental hemiganglion. These cells are arrayed as two clusters, a small one near the midline at the anterior border of the ganglion and an even smaller one in the middle portion, halfway to the lateral border on the ventral aspect (Fig. 4). Following injection of the Q teloblast, a small amount of HRP label is consistently seen also at the posterior border of the ipsilateral hemiganglion. However, since in the stage 10 embryos examined in this study, the segmental ganglia are still in close apposition, it was difficult to ascertain whether this small patch of label is actually contained in cell bodies. In fact, it is probably attributable to the growth of HRP labeled processes into the ganglion from the Q-derived connective glial cells at the anterior border of the next posterior ganglion (Kramer and Weisblat, 1984). The principal contribution of the Q teloblast is to the epidermis of the future dorsal body wall, in the form of a broad, ragged longitudinal band of cells extending from the leading edge of the circumferentially expanding germinal plate (the future dorsal midline) to the future lateral edge (Figs. 2 and 6). The extraganglionic component of the Q teloblast contribution also includes a segmental complement of nonepidermal cells. Among these is a cluster of cells which lies just outside the lateral border at the middle of the segmental ganglion (Fig. 7) that is likely to include a peripheral dopamine-containing neuron cell body (Stuart *et al.*, in preparation). Additional Q-derived, nonepidermal, extraganglionic cells can be seen at the medial edge of the Q-derived epidermis, i.e., apparently in line with the most lateral O/P-derived dots at the future lateral edge.

The o, p, and q blast cell bandlets are separated from the ventral midline by the n blast cell bandlet in the nascent germinal plate, yet they contribute to the segmental ganglion. This indicates that in the process of gangliogenesis the blast cell progeny of one bandlet migrate towards the ventral midline past those of another bandlet. This medial migration is most apparent for the cells contributed to the ganglion (and to the cell cluster just outside the ganglion) by the q bandlet, since these cells move further and therefore separate more clearly from their lateral siblings than those of any other bandlet (Fig. 7).

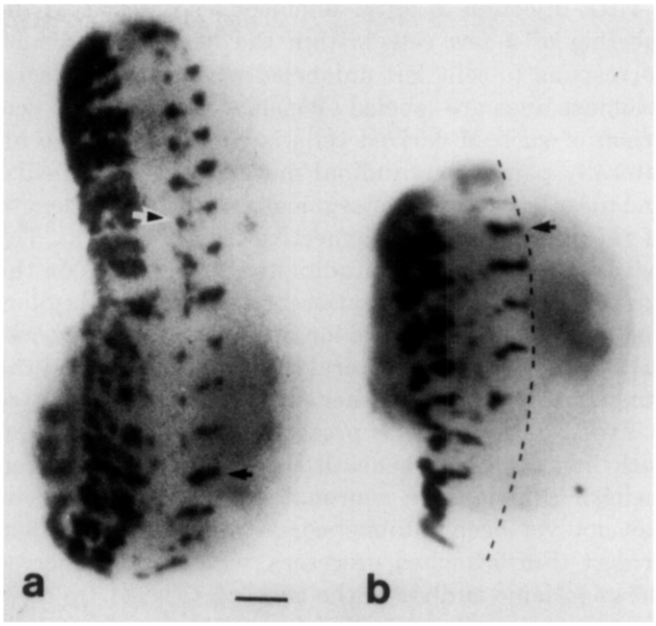

FIG. 7. Migration toward the ventral midline of neuroblasts derived from a lateral blast cell bandlet. Ventral aspects of the labeling pattern of a wholemounted, late-stage 9 embryo, whose right Q teloblast had been injected with HRP at stage 6. The entire germinal plate cannot be shown in a single photograph, because the embryo is curved. (a) View of the developmentally more advanced rostral and midbody segments; (b) view of the developmentally less advanced caudal segments. In the frontmost segments seen in (a), the neuroblasts have reached their ultimate destinations, including those within the ganglion, whereas in the rearmost segments seen in (b), migration toward the midline has barely begun. A single cluster of labeled neuroblasts at the anterior edge of a ganglion (cf. Fig. 4), is indicated by a black arrow in (a) and (b). A cluster of labeled neuroblasts just outside a ganglion is indicated by a black and white arrow in (a). The extensive labeling in the lateral half of the germinal plate is in the future dorsal epidermis. The ventral midline is indicated by a dashed line in (b). Anterior is up; scale bar 50 μm.

Segmental Tissues: Mesodermal Teloblasts

Following HRP-injection of an M teloblast, labeled cells are distributed throughout the ipsilateral body wall of the stage 10 embryos (Fig. 3). The most prominent of these labeled cells comprise the longitudinal, circular and oblique muscle fibers of the body wall, as well as the nephridia (of which one bilateral pair is present in most midbody segments). Moreover, in sectioned embryos, cells lining and demarcating the boundaries of various tissues are also labeled. Thus, again in conformance with general embryological principles, musculature, nephridia, and connective tissue of the leech are of common, mesodermal provenance.

HRP-injection of an M teloblast also results in the labeling of a few cells within the nerve cord, which correspond to cells left unlabeled when all ectodermal teloblast lines are labeled (Weisblat *et al.*, 1980a). One group of such M-derived cells is composed of two bilaterally paired longitudinal muscle cells (one medial and the other lateral) per segment that course the length of the interganglionic connective nerves (Fig. 8). The cell bodies of these longitudinal muscle cells lie on the dorsal aspect at the anterior border of the ganglion, and their processes extend longitudinally along the dorsal surfaces of the ipsilateral connective nerve and the ganglionic neuropil. Another small group of about three to five M-derived cells is present in about the center of each hemiganglion, beneath the ventral layer of cell bodies. Although the neuronal identity of these cells has not yet been established, at least some of them project thin branched processes, which extend across the ganglionic midline in the neuropil (Fig. 8). On morphological grounds, these M-derived intraganglionic cells are indistinguishable from the presumptive neurons derived from the N, O/P, and Q teloblasts, and physiological studies of the homologous cells in the related leech *Haementeria ghilianii* indicate that they are in fact a discreet set of segmental interneurons (Kramer and Weisblat, 1984). If these M-derived cells are indeed neurons then they would present the unusual but not unheard of (Sulston *et al.*, 1983) case of nervous tissue of mesodermal provenance.

Prostomium

In addition to the 32 metameric segments and their ganglia, the leech body has a nonmetameric prostomium at its rostral end. The prostomial structures include the supraesophageal ganglion of the head brain and the anteriormost body wall. (The subesophageal ganglion of the head brain arises by fusion of the frontmost four segmental ganglia of the ventral nerve cord.) The supraesophageal ganglion is bilaterally symmetric; it lies dorsal to the subesophageal ganglion and the esophagus, which it partially envelops (Livanow, 1904; Mann, 1953; Hagadorn, 1958) (Fig. 9). Supra- and subesophageal ganglia are joined at their anterior ends by a pair of circumesophageal connectives. We estimate that in the stage 10 embryos examined in this study there are between 150 and 300 cell bodies in the cortex of the supraesophageal ganglion and along the circumesophageal connectives, outside a well-defined neuropil. There is no clear anatomical boundary between supra- and subesophageal ganglia at this stage, the neuronal cell bodies not yet being segregated into distinct cell packets.

Previous experiments showed (Weisblat *et al.*, 1980a) that the neurons of the supraesophageal ganglion, as well as the cells of the prostomial skin, are not derived from any of the ectodermal teloblasts N, O/P, or Q, nor from the mesodermal teloblast M. These prostomial tissues do not, therefore, develop from the blast cells of the germinal bands. Rather, as was found following HRP injection of an A, B, or C blastomere of a stage 3 (i.e., four-cell) embryo, the prostomial tissues are derived in part from one or more of these cells.

The present experiments examine the origins of the nonmetameric prostomial tissues in greater detail. For this purpose, identified blastomeres of a series of stage 3 embryos were HRP-injected. After further development to stage 10, the embryos were fixed and stained for HRP and the distribution of label was examined either in wholemounts (Figs. 10 and 11) or in sectioned preparations (Fig. 12). It was found in this way (Fig. 9) that the A (four successful injections from 25 embryos) and B (three successful injections from 35 embryos) blastomeres contribute cells primarily to the left and right sides, respectively, in the anteromedial part of the supraesophageal ganglion, and that the C blastomere (three successful injections from 19 embryos) contributes cells primarily to the right side of the ganglion, providing for most of the cells in the posterior part of that side. The progeny of the D blastomere (two successful injections from 5 embryos) were not examined in section since D is the precursor of the germinal bands and hence the source also of all metameric (i.e., nonprostomial) ecto- and mesoderm as well (Fig. 11). However, since following HRP-injection of the A, B, or C blastomere, cells in the left side of the posterior part of the supraesophageal ganglion remained unlabeled, it is likely that they are derived from the D blastomere.

Examination of the prostomial body wall of these same embryos following HRP injection of the A, B, or C blastomere showed extensive labeling there as well. The prostomial body wall of the stage 10 embryos examined here protrudes anteriorly, creating a dolphin-like profile for the head of the embryo. The subsequent morphogenetic movements responsible for converting this structure into the anterior sucker of the mature leech have not been studied. Thus, the definitive fate of the A, B, and C blastomere-derived prostomial epidermis is not known.

Since there is only one prostomium per embryo available for analysis (as compared to the 32 metameric segments per embryo), it has not yet been possible to examine a sufficient number of cases to ascertain whether the domains of the cellular contribution of each of the A, B, C, and D blastomeres to the prostomium are as stereotyped topographically as are the domains contributed by each teloblast to the metameric tissues.

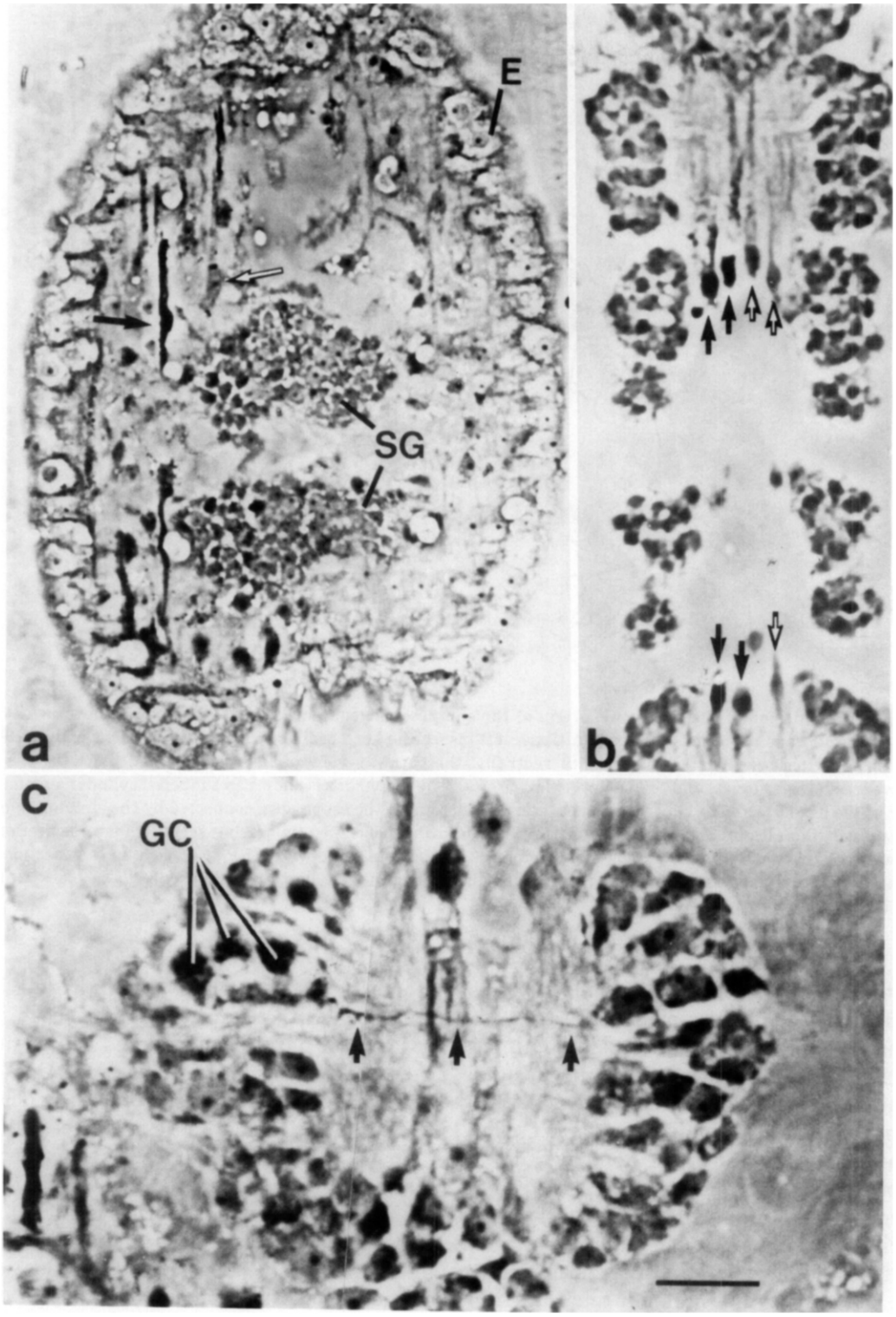

FIG. 8. Contribution of the M teloblast to the musculature and the nervous system. Horizontal sections through a stage 10 embryo whose left M teloblast has been injected with HRP at stage 7. Anterior is up. (a) View of an entire section cut near the curved ventral surface, through two segmental ganglia (SG) and the epidermis (E), just posterior to the boundary between anterior unlabeled and posterior labeled segments. Labeled (black arrow) and unlabeled (white arrow) longitudinal muscle fibers of the body wall can be seen. (b) Section through parts of five ganglia. Because of the curvature of the germinal plate, the middle ganglia are sectioned at a more dorsal level than the anterior and posteria ganglia. At the anterior edge of the third and fifth ganglia, cell bodies of labeled (black arrows) and unlabeled (white arrows) longitudinal muscles of the interganglionic connectives are seen. (c) Oblique horizontal section cutting dorsoventrally (from top to bottom of picture) through one ganglion. Here, in addition to labeled and unlabeled cell bodies of the medial pair of longitudinal connective muscles and labeled body wall longitudinal muscle fibers, three labeled ganglionic cell bodies (GC) are indicated. Segmental nerve roots and neuropil are visible in the ganglion, including a labeled cell process (arrows) which crosses the midline within the neuropil. Scale bar 25 μm in (a), 20 μm in (b), and 10 μm in (c).

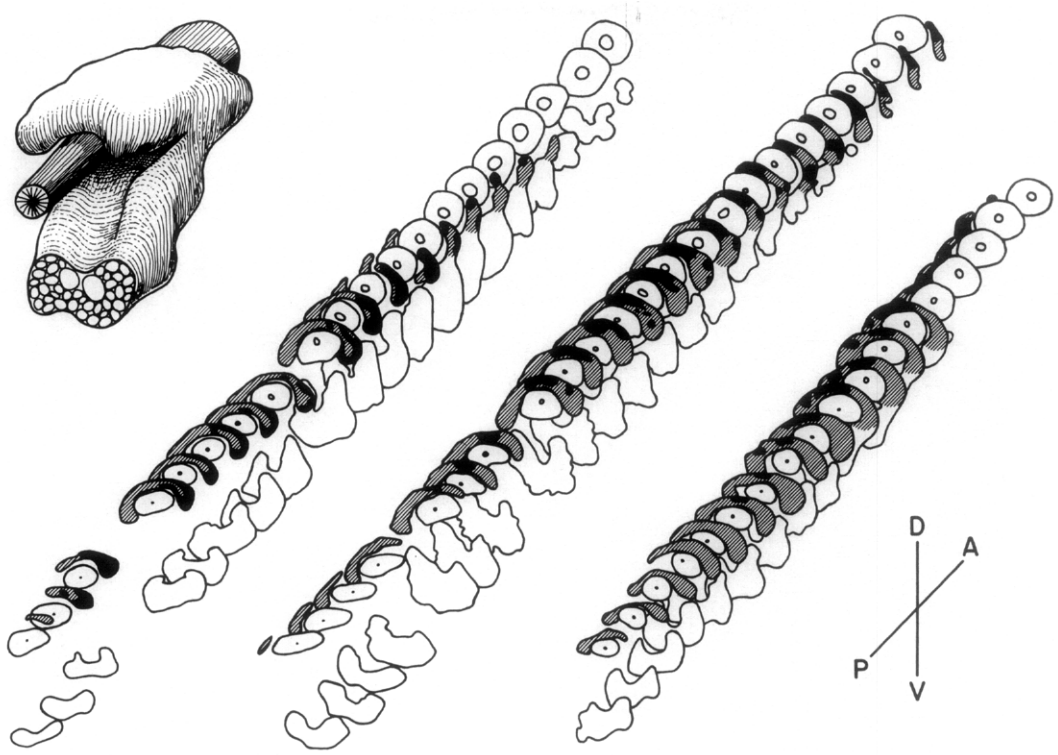

FIG. 9. Schematic representation of the embryonic origin of the supraesophageal ganglion. Oblique views, shown with dorsal up, ventral down, anterior to upper right and posterior to lower left. Upper left insert: the head brain of a stage 10 *Helobdella* embryo. The subesophageal ganglion, comprising four fused segmental ganglia, lies ventrally; the cutaway view of its posterior end indicates the relative size and distribution of nerve tract (large circles) and cell bodies (small circles). The esophagus is shown as a tapered cylinder, extending longitudinally through the center of the head brain. The supraesophageal ganglion lies above the esophagus, connected by the circumesophageal connectives to the subesophageal ganglion. Right: Exploded view of three head brains, generated by tracing serial transverse sections cut from early stage 10 embryos whose A (right), B (middle), or C (left) blastomere had been injected with HRP at stage 3. In each section, the approximate domain of the supraesophageal ganglion is indicated by parallel hatching, and the domain of labeled cells is blackened. Neuropil is not indicated in these drawings.

Nevertheless, it seems clear that of the four blastomeres, A and (presumably) D contribute primarily to the left side of the prostomium, while B and C contribute primarily to the right side of the prostomium in the stage 10 embryo. The laterality of these contributions may seem surprising because, although the A and C blastomeres appear to be left and right homologs in the stage 3 embryos, the B and D blastomeres each straddle the midline. Thus, it is not immediately apparent either why the contribution from A to the supraesophageal ganglion is anterior while that from C is posterior, or why that from B is only to the right side and that from D only to the left. Here we must recall, however, that the spiral cleavages by which the A, B, C, and D blastomeres give rise to the a, b, c, and d micromeres at the dorsal pole of their junction during stage 4a (Fig. 1) insure that each micromere will come to lie in the furrow between its parent blastomere and that of the next (clockwise) adjacent blastomere. It thus becomes apparent that, in contrast to their blastomere parents, micromeres a and d do lie wholly to the left and micromeres b and c wholly to the right of the midline. Moreover, with regard to their position in the embryo, both a and b can be considered contralaterally homologous, as can d and c. In view of this fact, it seems highly likely that the prostomium is derived from the A, B, C, and D blastomeres via their micromeres.

It should be noted that, as previously observed (Weisblat *et al.*, 1980a), the bulk of the HRP label in A, B, or C blastomere-injected embryos is, in fact, found in their gut. There the stain is more or less uniformly distributed, in a pattern that is largely independent of the identity of the injected blastomere. This aspect of the staining pattern is undoubtedly attributable to the incorporation of blastomere remnants, particularly of their yolk, into the gut of stage 10 embryos for eventual digestion during stage 11. However, in the experiments reported here, we have been unable to identify embryonic endoderm unambiguously and therefore have yet to test the classical notion of its derivation from the A, B, and C blastomeres. (The origins of the esophagus and germ line also remain unknown as yet.)

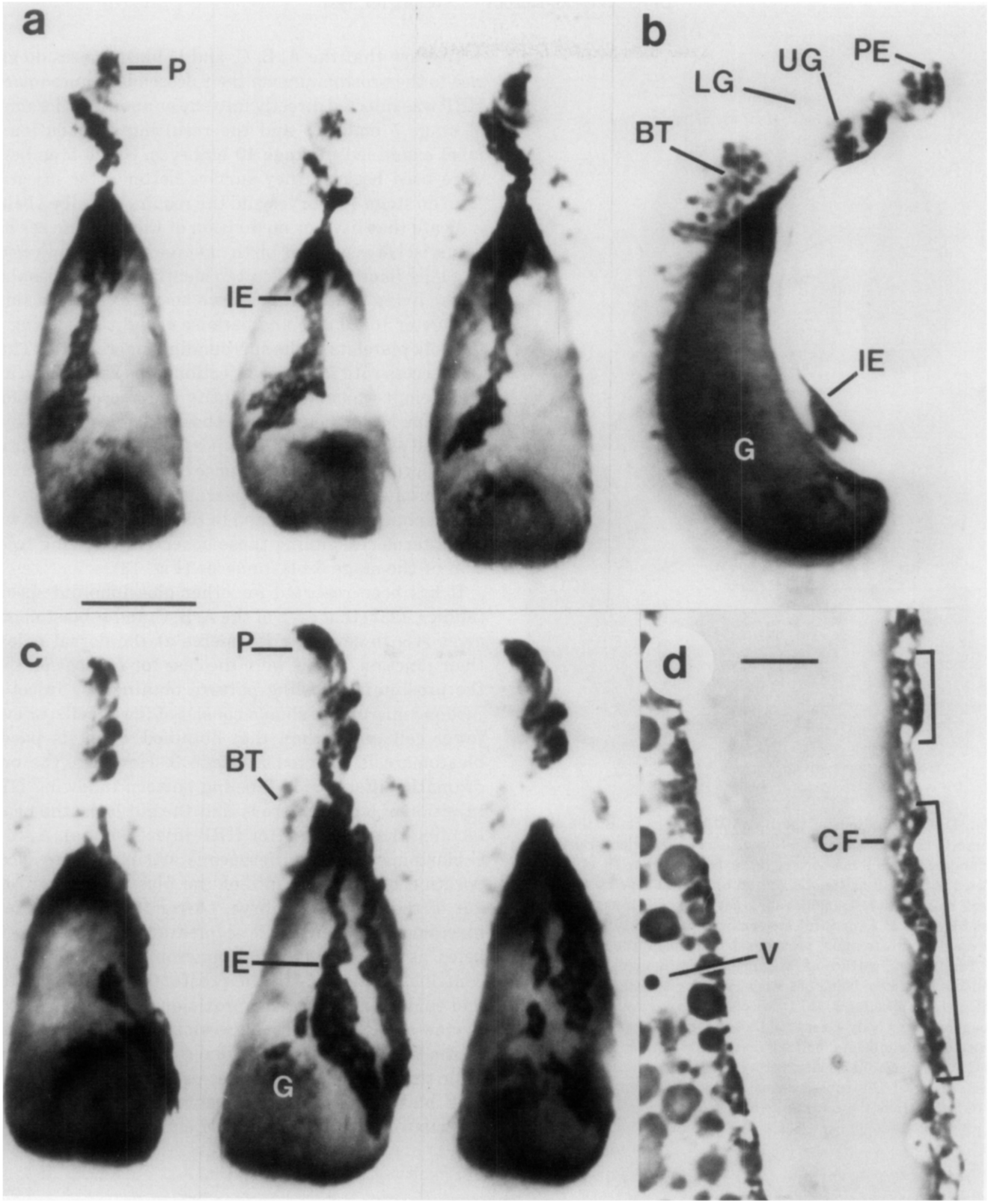

FIG. 10. Contributions of the A and C blastomeres of the stage 3 embryo to the prostomium and provisional integument. Early stage 10 embryos whose A blastomere (a, b) or C blastomere (c, d) had been injected with HRP at stage 3. (a–c) Wholemounts; anterior up; scale bar 200 μm. (a and c) Dorsal aspects of six embryos. Because of the high contrast, the perimeter of the (unlabeled) body of the embryos is mostly invisible. Although there is considerable variation in the distribution of label in these specimens, the label is confined mainly to the left side of the A-injected embryos and to the right side of the C-injected embryos. At the anterior end of each embryo are labeled cells in the prostomial ectoderm (P). Posterior to the head are patches of labeled cells in the epithelium (IE) of the provisional integument, lying over the dark, tear-shaped outline of the gut (which contains the bulk of the HRP derived from breakdown of the injected blastomere). Bilaterally

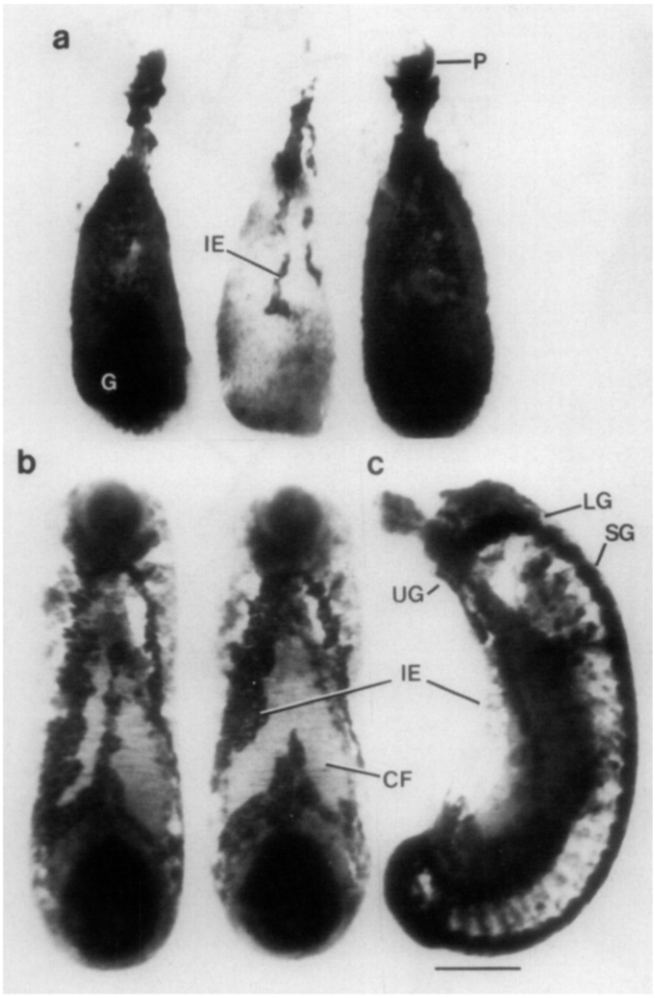

FIG. 11. Contributions of the B and D blastomeres of the stage 3 embryo to the prostomium and provisional integument. Early stage 10 embryos whose B blastomere (a) or D blastomere (b, c) had been injected with HRP at stage 3. (a and b): Dorsal aspects. (c) Lateral aspect, dorsum to left. (a) Labeling patterns of B-injected embryos resemble those of A- or C-injected embryos (cf. Fig. 10), in that label is present in prostomium, provisional integumental epithelium and gut. The labeling pattern of integumental epithelium is visible only in middle specimen, being obscured by heavy staining in the gut of right and left specimens. (b) D-injected embryos are labeled almost throughout their body except for those parts of the prostomium and provisional integumental epithelium that are derived from the A, B, and C blastomeres. Labeled structures indicated are prostomium (P), supraesophageal (UG) subesophageal (LG) and segmental (SG) ganglia, gut (G), as well as the contractile fibers (CF) and epithelium (IE) of the provisional integument. Scale bar 200 μm.

To show that the A, B, C, and D blastomeres do give rise to the prostomium via their descendant micromeres, HRP was injected directly into one or another micromere of stage 5 embryos and the resulting distribution of label examined in stage 10 embryos. Stage 5 embryos were used because they survive better after injection than do stage 4 embryos and the results described below indicate that little or no division of the micromeres had occurred between their birth at stage 4 and the injection. The micromeres are difficult to identify, or even visualize, in the living *Helobdella* embryo because they are small (diameter 10–20 μm) and because of light scattering by the yolk platelets of the surrounding macromeres. Thus, the success rate for these injections was rather low, and no attempt was made to identify the micromere before injection. Nevertheless, from about 120 injected embryos in three experiments, it was possible to obtain 16 stage 10 embryos which gave evidence of successful injections of micromeres, by the appearance of label in the supraesophageal ganglion and in the prostomial body wall in patterns resembling those observed following injection of the stage 3 blastomeres (Fig. 13).

It has been reported for other glossiphoniid species (Müller, 1932) that each of the A, B, C, and D blastomeres gives rise to several micromeres at the dorsal pole of their junction. If this were the case for *Helobdella*, then the prostomial labeling pattern obtained by injecting just one micromere should consist of fewer cells, or even fewer cell types, than that obtained when its parent blastomere is injected at stage 3. However, the only dramatic difference in labeling pattern following HRP injection of a micromere is that the gut lacks the heavy labeling that is seen after HRP-injection of an A, B, or C blastomere. That difference is attributable to incorporation of the remnants of the blastomeres into the gut of the stage 10 embryo. (After injection of the D blastomere, of course, all segmental structures are labeled as well.) Thus, single micromeres in *Helobdella* contribute cells to both the prostomial nervous system and epidermis, and to the provisional integument. And in view of the similarity between the extent of the stain in the blastomere and micromere labeling pattern, we infer that further micromeres produced from the A, B, or C blastomeres, if any, must make relatively minor contributions to the *Helobdella* embryo.

distributed, stained botryoidal tissue (BT) cells (are also visible near the anterior end of the gut. (b) Lateral aspect, dorsum to the right. Labeled supraesophageal ganglion (UG), prostomial epidermis (PE), provisional integumental epithelium (IE), and gut (G) are indicated as is (BT), and unlabeled subesophageal ganglion (LG). (d) Transverse section through the provisional integument, dorsum to the right. Brackets indicate the domain of labeled epithelial cells. The unlabeled cell body and process of an integumental contractile fiber (CF) lie next to the epithelium (cf. Fig. 3c). In the gut, a nucleus (V) reminiscent of the vitellophages of other annelids and arthropods (Anderson, 1973) can be seen among the yolk platelets. Scale bar 10 μm.

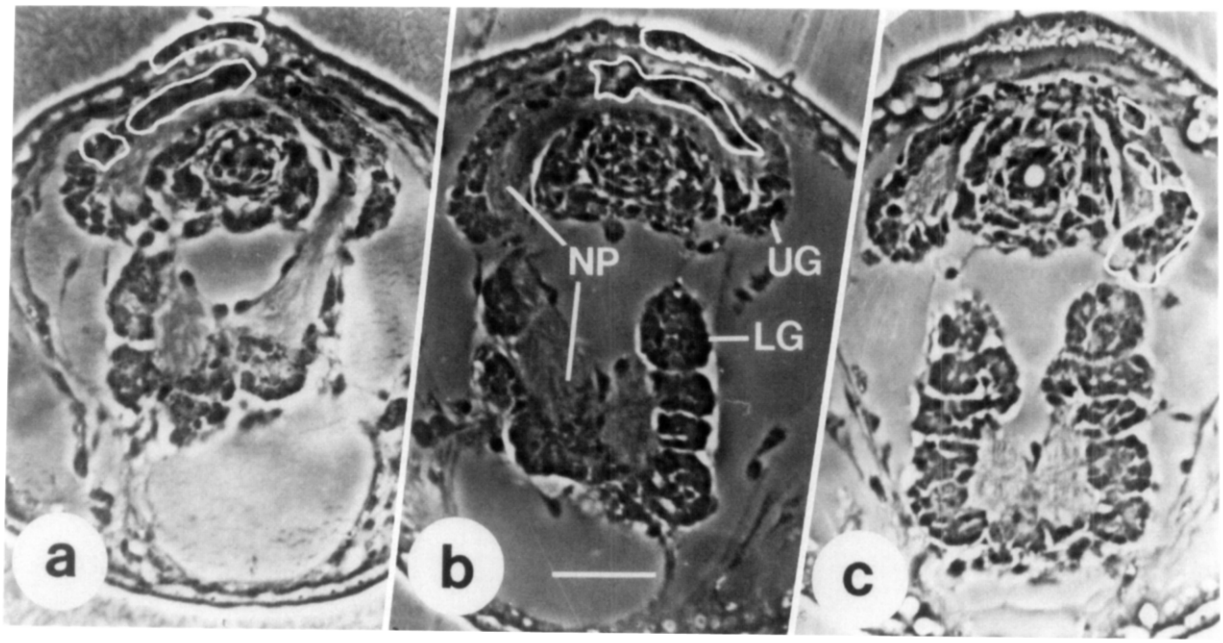

FIG. 12. Contribution of the A blastomere (a), B blastomere (b), and C blastomere (c) of the stage 3 embryo to the supraesophageal ganglion. Transverse sections through the head brain of HRP-labeled embryos such as those shown in Figs. 10 and 11. The domains of labeled cells (identified as in Fig. 5) in the epidermis and supraesophageal ganglion are indicated by outline. Neuropil (NP) and ganglion cell bodies are visible in both the subesophageal (LG) and supraesophageal ganglia (UG). These sections are representative of those on which the exploded views of Fig. 9 are based. Scale bar 25 μm.

It should be noted that the experiments reported here deal only with the origins of the prostomial ectoderm. As for the prostomial mesoderm, double-label experiments using fluorescent lineage tracers have shown that the two or three frontmost (first born) blast cells of the m bandlet directly join the early (stage 4a) micromeres at the site of the future head, in contrast to the following blast cells, each of which gives rise to one of the metameric half-somites of the leech embryo (Zackson, 1982). The eventual fate of these eldest m blast cells has not yet been ascertained, but it seems likely that they provide the mesodermal component of the prostomial tissues.

Provisional Integument

Although development of glossiphoniid leeches is direct (i.e., without passage through a distinct larval form), there nevertheless do arise some transient tissues that are not incorporated into the eventual juvenile leech. One of these is a cell sheet which begins to cover the surface of the embryo during stages 7 and 8 (Fig. 1). The origin of that expanding sheet is the dorsal pole, where the micromeres a, b, c, and d are located. For that reason this sheet has been called the micromere cap. But, as we shall show here, the micromere cap is neither homogenous with respect to its embryonic origin, nor is it homogeneous with respect to its structure and eventual fate. As was reported in the preceding, the supraesophageal ganglion and the prostomial epidermis are definitive tissues derived from the a, b, c, and d micromeres. The bulk of the micromere cap, however, is a provisional integument of the embryo, and we shall refer to it as such, all the more so because the a, b, c, and d micromeres are not its only source. The provisional integument of the stage 8–10 embryo is a two-layered structure, consisting of a superficial squamous epithelium and an underlying layer of elongated, circumferentially oriented cells. These cells extend as a fibrous mesh from the leading edge of the germinal plate to the future dorsal midline, where they interdigitate with the corresponding fibers from the other side. These circular fibers are held responsible for the peristaltic constrictions that begin to travel along the embryo at the end of stage 8 and lead to hatching from the egg membrane late in stage 9, after which peristalsis soon ceases (A. P. Kramer, personal communciation). During the formation and outgrowth of the germinal bands during stage 7, the provisional integument fills the dorsal surface of the embryo between the germinal bands. As the germinal bands migrate circumferentially and coalesce on the ventral midline to form the germinal plate during stage 8, the provisional integument expands in their wake over the future ventral surface also. By the

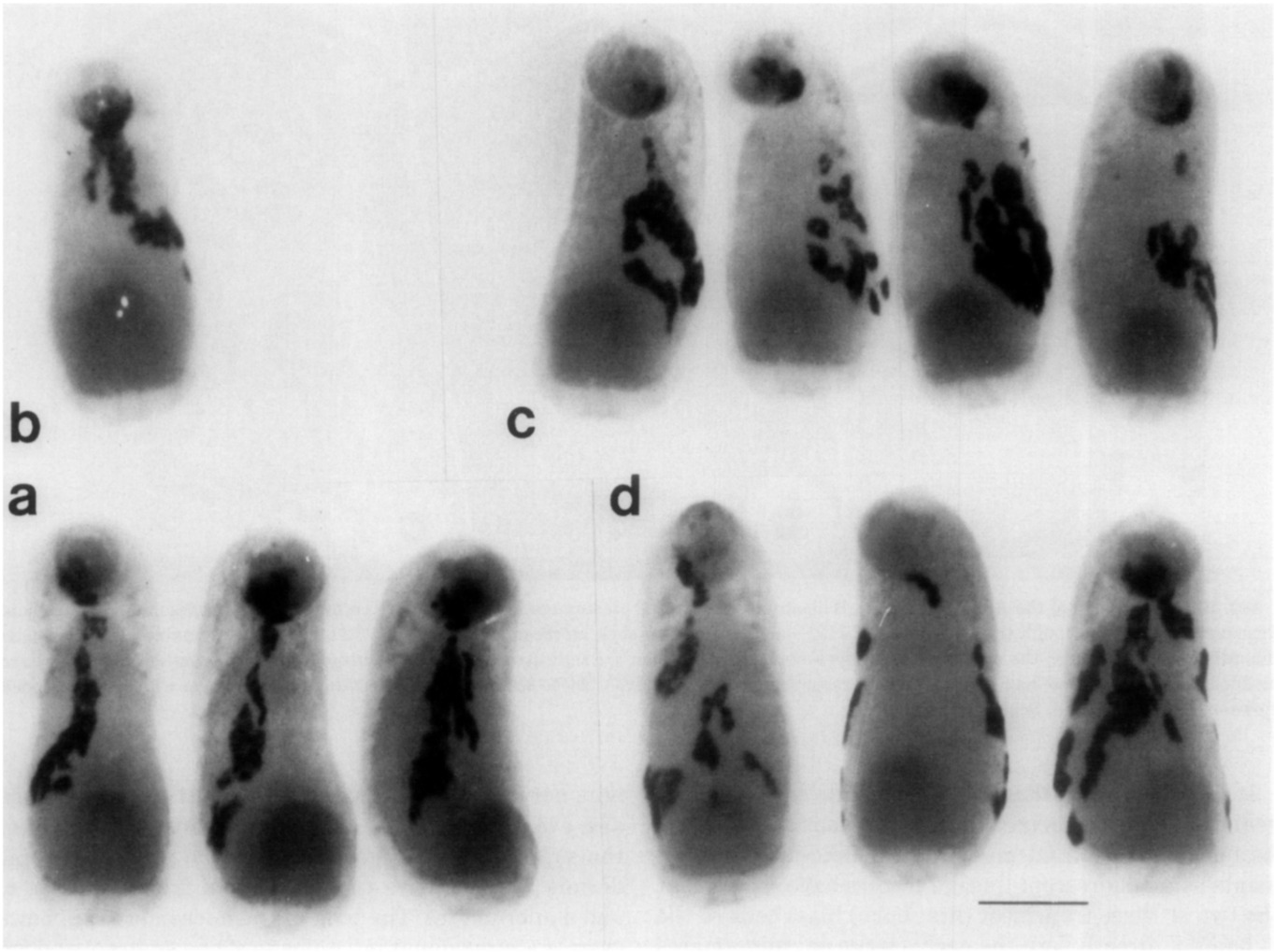

FIG. 13. Contribution of the micromere quartet to the prostomium and the provisional integument. Dorsal aspect of wholemounted early stage 10 embryos in which one (unidentified) a, b, c, or d micromere had been injected with HRP at stage 5. Labeled cells are present in the prostomium and in the provisional integumental epithelium, but, in contrast to the embryos of Figs. 10 and 11, here no label is present in the gut. Individual labeled epithelial cells can be identified; their nuclei, from which the HRP label has been excluded, appear as white dots. Labeled and unlabeled epithelial cells are evidently interspersed. On the basis of their epithelial labeling pattern, the embryos have been grouped according to the probable identity of the injected micromere. (a) Micromere a (moderate labeling, left side). (b) Micromere b (minimal labeling, both sides). (c) Micromere c (moderate labeling right side). (d) Micromere d (moderate labeling, both sides). Scale bar 200 μm.

end of stage 8, when the germinal bands have coalesced along their whole length and the germinal plate is complete, the provisional integument covers the entire surface of the embryo, in resemblance to the amnioserosa of the insect embryo. Then, as the germinal plate expands circumferentially back into dorsal territory during stages 9 and 10, the provisional integument retracts before, or is pushed back by, the leading edges of the germinal plate. By early stage 11, when the leading edges of the germinal plate have met on the dorsal midline and closed the leech body tube, the crumpled remainder of the provisional integument is sloughed off (A. P. Kramer, personal communication).

As alluded to in the previous section, it was found that upon injection of the A, B, or C blastomeres at stage 3, labeled cells are present in the squamous epithelium of the provisional integument (Figs. 10 and 11). The pattern of distribution of these labeled cells following injection of a given blastomere varied from specimen to specimen with respect to size and location of the patch of labeled epithelium. Moreover, the irregularly shaped patterns were not confined exclusively to one side of the dorsal midline. Although the labeled patches resulting from injection of the A or C blastomere lay mostly to the left or right sides of the dorsal midline respectively, the (generally smaller) patch of label re-

sulting from HRP-injection of the B blastomere fell on both sides of the midline. Epithelial cells of the provisional integument also become labeled, albeit with widely varying patterns, following HRP injection of unidentified micromeres at stage 5 (Fig. 13). Hence we infer that parts of the integumental epithelium derive from the A, B, C, and D blastomeres via their daughter micromeres. The variability in the labeling patterns observed in these embryos could be reduced somewhat by separating them into four groups corresponding to the tentative identity of the injected micromere, on the basis of the similarity between the labeling patterns of blastomere- and micromere-injected embryos (e.g., cf. Fig. 10a with Fig. 13a and Fig. 10c with Fig. 13c). The remaining variability within each group may reflect either a lack of stereotypy in the normal distribution of micromere progeny, or it may result from disruption of normal development by the injection.

In the case of the presumptive a, b, or c micromere injections, the labeling pattern was generally similar to that found after injection of the parent blastomere. But HRP injection of the D blastomere results in the labeling of a large, bilateral patch of the provisional integument which does not correspond to the pattern obtained from injecting any single micromere of a stage 5 embryo. This is because descendants of D can reach the integumental epithelium not only via the d micromere, but also via other micromeres in the D lineage, such as those produced by the OPQ blastomere and the N teloblast immediately after cleavage of their parent NOPQ blastomere and designated as the (opq)′ and n′ micromeres (Müller, 1932; Fernandez, 1980). (The cleavages that produce these micromeres are not shown explicitly in Fig. 1, but their occurrence in the *Helobdella* embryo has been confirmed (unpublished observations)). The (opq)′ and n′ micromeres do not join the subsequently produced n, o, p, and q blast cell bandlets in the germinal bands, and hence do not take part in forming the segmental ectoderm. In the absence of experiments in which these cells were injected directly with cell lineage tracer, their fates can be discerned by examining labeled stage 10 embryos in which an N or OPQ cell was injected with HRP early in stage 6a, prior to the production of the (opq)′ and n′ micromeres. Such embryos exhibit irregular arrays of labeled integumental epithelial cells, in addition to the segmentally iterated labeling pattern (Fig. 14a). Since these epithelial cells of the provisional integument are not labeled upon HRP injection of an N, O/P, and Q teloblast after blast cell production has begun, it would follow that they are derived from the (opq)′ and n′ micromeres. Early (and only early) injection of the N teloblast also results in the labeling of a prominent group of cells in the anterior ventral body wall (Fig. 14b). These cells are located in the sticky region of the ventral body wall by which the embryo adheres to the parental venter, after it emerges from the cocoon and before its posterior sucker becomes functional. They are evidently derived also from the n′ micromeres, or from the very first n blast cells.

Another source of the provisional integument is provided by the mesodermal teloblasts (M). HRP-injection of the left M teloblast results in the labeling of the circumferential, putatively contractile fibers underlying the squamous epithelium of the ipsilateral provisional integument of the resulting stage 8–10 embryo (Fig. 3). This indicates that these fibers are of mesodermal origin, as befits contractile elements underlying a superficial epithelium. Moreover, in embryos in which the left M teloblast was injected in stage 6 (after only a few m blast cells had been produced), nearly all the circumferential fibers in the left half of the provisional integument were labeled (Fig. 3a). But in embryos in which the left M teloblast was not injected until mid-stage 7 (by which time most of the m blast cells had been produced), only the rearmost fibers of the left provisional integument were labeled (Fig. 3b). This result indicates that the integumental contractile fibers arise from progeny of the (metameric) somite founder m blast cells, apparently being left behind by their somite siblings in the course of circumferential migration of the germinal bands.

DISCUSSION

The present results confirm and extend our previous findings (Weisblat *et al.*, 1978; 1980a) that development of the leech embryo is highly stereotyped, in the sense that a particular identifiable blastomere of the early embryo regularly gives rise to a particular part of the adult (or provisional embryonic) tissues, although the particular part contributed by a given teloblast comprises a variety of cell types. In the case of the nervous system this developmental stereotypy is such that a particular group of neurons in a particular body segment can be traced back to a particular primary blast cell in the germinal band.

Kinship Groups and Identified Neurons

Our results indicate that the neurons (and glia) of the segmental nervous system can be assigned to five bilaterally paired *kinship groups* according to their teloblasts of origin. Because the neuronal kinship groups in the embryonic ganglia and the identified neurons in adult leech ganglia occupy characteristic regions, it seems likely that in normal development each identified neuron arises from a particular teloblast. And, in fact, a number of identified neurons, in addition to the neu-

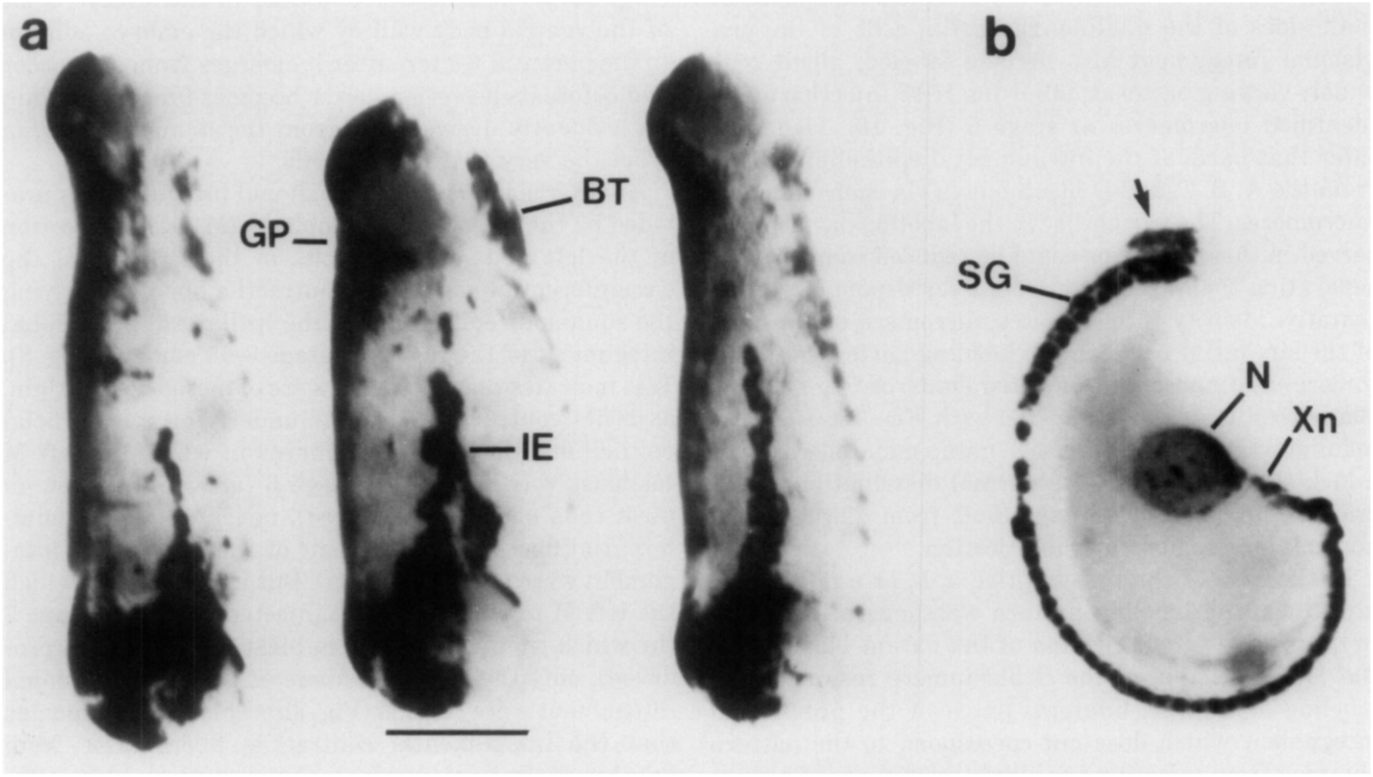

FIG. 14. Nonsegmental contribution of the OPQ blastomere and the N teloblast. (a) Dorsal aspect of three wholemounted, early stage 10 embryos whose left OPQ blastomere had been injected with HRP at stage 6a. Label can be seen in the germinal plate (GP), botryoidal tissue (BT) and provisional integumental epithelial cells (IE), presumably derived from the (opq)' micromeres. (b) Lateral aspect of a wholemounted late stage 9 embryo whose N teloblast had been injected at stage 6a. Label can be seen in the injected teloblast (N), in supernumerary blast cells (Xn), in segmental ganglia (SG), and in a patch of cells superficial to the nerve cord at the anterior end of the germinal plate (arrow). The unlabeled prostomium is not visible because of high-contrast photography. Scale bar 200 μm.

ropil glia and connective muscle cells, have been assigned to particular kinship groups (Kramer and Weisblat, 1984; Stuart *et al.*, in preparation; Shankland and Weisblat, in preparation).

The Role of Cell Lineage in Determining Cell Fate

In considering the governance of cell fate by line of descent, we note that there are three overlapping, sequential phases of cell proliferation in the development of the leech embryo. The first phase comprises the series of modified spiral cleavages marking stages 1–6, leading from the fertilized egg to the 13 large, yolky blastomeres (three macromeres plus five teloblast pairs) and the micromeres at the dorsal pole. It has been thought since Whitman that the various developmental fates of the descendants of each blastomere or micromere are attributable to an orderly segregation of intracellular determinants during this cleavage phase of embryogenesis. Similar proposals were made for the roles of early cleavages in nematodes and ascidians (Boveri, 1899; Conklin, 1905; reviewed by von Ehrenstein and Schierenberg, 1980; Whittaker, 1979). In the leech, it has long seemed plausible that the asymmetrically partitioned cell component now designated as *teloplasm* (Fernandez, 1980; Fernandez and Stent, 1980) harbors some of these determinants. During stage 1, prior to the first cleavage, teloplasm becomes concentrated at both the future dorsal and the future ventral poles of the egg. In the course of the first two (meridional, unequal) cleavages (see Fig. 1), both these teloplasms pass mainly to blastomere D. But when D cleaves in the equatorial plane (stage 4), the dorsal teloplasm passes to the ectodermal precursor DNOPQ and the ventral teloplasm to the mesodermal precursor DM. The dorsal teloplasm is then distributed among the paired ectodermal teloblasts N, O/P, O/P, and Q, whereas the ventral teloplasm is divided among the paired mesodermal teloblasts M. Each teloblast, in turn, parcels out its endowment of teloplasm to its blast cell descendants.

That the dorsal and ventral teloplasm harbor ectodermal and mesodermal determinants, respectively, is

supported by two observations. First, Müller (1932) found that when blastomere CD occasionally cleaves symmetrically (instead of asymmetrically) at stage 3, the teloplasm is distributed equally to the two daughter blastomeres, and each of these follows the cleavage pattern of the normal D blastomere. Consequently there arises a double set of five teloblast pairs, and finally a twinned embryo with a double set of ectodermal and mesodermal organs. Second, as shown by the present experiments, the micromere quartet formed at the dorsal pole of the four-cell embryo gives rise only to ectodermal tissues: the nervous system and epidermis of the prostomium and the epithelium of the provisional integument. This is consonant with the view that the A, B, and C blastomeres pass to their a, b, and, c micromere descendants what little dorsal teloplasm (with its putative ectodermal determinants) did reach them in the first two cleavages. By contrast, whatever ventral teloplasm (with its putative mesodermal determinants) reached the A, B, and C blastomeres would not appear in the micromeres, and thus m blast cells rather than micromeres provide the prostomial mesoderm and the circumferential contractile fibers of the provisional integument.

The notion that the cleavage divisions segregate tissue-specific developmental determinants seemed so plausible to Whitman and his followers that they thought that it also applies to the cleavage of the NOPQ ectodermal precursor blastomeres into four distinct pairs of ectodermal teloblasts. Our work has shown that such generalized tissue-specific segregation among the daughter teloblasts of the NOPQ blastomere is not the case and that even the presumptive mesodermal line generates some neurons. Similar observations of the lack of tissue-specific segregation of cell fates in the initial cleavages have been made for nematodes and ascidians (von Ehrenstein *et al.*, 1980; Nishida and Satoh, 1983).

The second phase of cell proliferation comprises the series of iterative, stem-cell-like divisions of the five teloblast pairs beginning during stage 6 and ending during stage 8, by which the teloblasts produce their bandlets of primary blast cells. Since each primary blast cell (in the case of n and of q, a *doublet* of primary blast cells) gives rise to the characteristic metameric mesodermal or ectodermal structures of a half segment (Weisblat *et al.*, 1978; Zackson, 1982, 1984; Weisblat, in preparation), it would appear that in the course of that stem-cell-like division mode all primary blast cells (perhaps alternate blast cells in the case of n and q) are endowed with homologous sets of fate-determining factors by their parent teloblast.

The third phase of cell proliferation comprises the divisions of the primary blast cells and their descendants in the germinal band and germinal plate during stages 7–10. This proliferation eventually gives rise to the differentiated (and mainly postmitotic) cells of the adult tissues. Assuming that the fixed correspondence between identified neuron and kinship group holds true, it seems likely that the identified neurons in each kinship group will arise through a stereotyped series of cleavages in this phase as has been found in the nematode, *Caenorhabditis elegans* (Sulston and Horwitz, 1977; Sulston *et al.*, 1983). Although this notion remains to be tested, it is suggestive that the *initial* divisions of each primary m blast cell and its immediate descendants resemble the cleavage of the early blastomeres in that the cleavage planes of successive blast cell divisions follow a highly stereotyped pattern with respect to orientation and position (Weisblat *et al.*, 1980b; Zackson, 1982, 1984).

Is There Teleological Significance in the Existence of the Teloblast-Derived Kinship Groups?

Although virtually every leech neuron appears to possess a unique set of features (Muller *et al.*, 1982), one may ask whether, in view of their common descent, all the members of a neuronal kinship group share some features that set them apart from the members of the other groups. One common feature of each kinship group is that, as seen in Fig. 4, the cell bodies of its members tend to cluster in the ganglion. But are there some intrinsic cellular features other than position uniquely possessed by the members of a given kinship group, such as function, neurotransmitter, membrane properties, or morphology? Identification of some members of various kinship groups has not yet provided any clear evidence of the existence of such kinship-group-specific intrinsic features (Kramer and Weisblat, 1983). Alternatively, the teloblast-derived kinship groups may be an ontogenetic souvenir of the phylogeny of the annelid nervous system (Weisblat and Blair, 1982).

Phylogenetic Implications of the Origins of the Supraesophageal Ganglion

There is general agreement that the supraesophageal ganglion of polychaetes is a prostomial organ whose cells are derived from the micromere cap. In adult polychaetes that ganglion not only lies in a region which is clearly rostral to the metameric body segments but also arises developmentally as the neural tissue of a micromere-derived, nonsegmented larva to which the segmental ventral nerve cord is joined later during metamorphosis (Dawydoff, 1959). By contrast, the status and developmental origin of the leech's supraesophageal ganglion has long been the subject of controversy. In

adult leeches that ganglion is far displaced posteriorly and lies in a region whose body wall appears to belong to the fifth or sixth metameric segment. Moreover, since glossiphoniid leeches develop directly, there is no developmental stage during which the supraesophageal ganglion is morphologically recognizable prior to formation of the rostral end of the germinal plate-derived ventral nerve cord. Thus, it has been doubted that the leech has a nonmetameric prostomium at all (Mann, 1962). Although Whitman (1878) had initially inferred that the supraesophageal ganglion is derived from the micromeres, he later concluded that it is serially homologous with the segmental ganglia of the ventral cord and is thus not a prostomial organ (Whitman, 1892). Müller (1932), in turn, reasoned that the supraesophageal ganglion cannot be micromere-derived since the twinned embryos which he found upon symmetric cleavage of the CD blastomere seemed to possess two complete supraesophageal ganglia, even though there had arisen but a single set of micromeres. Thus the present demonstration that in leeches, too, the supraesophageal ganglion (as well as the epidermis of the frontmost body wall) *is* derived from the micromeres rather than the germinal plate, reconfirms the *a priori* plausible but previously challenged phyletic homology of the prostomial front end of the otherwise greatly diversified members of the annelid phylum.

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REFERENCES

ANDERSON, D. T. (1973). "Embryology and Phylogeny in Annelids and Arthropods." Pergamon, Oxford.

APATHY, S. (1889). Nach welcher Richtung muss die Nervenlehre reformiert werden? *Biol. Centralbl.* **9,** 600-608.

BERGH, R. S. (1891). Neue Beitraege zur Embryologie der Anneliden. II. Die Schichtenbildung im Keimstreifen der Hirudineen. *Z. Wiss. Zool.* **52,** 1-17.

BLAIR, S. S. (1982). Interactions between mesoderm and ectoderm in segment formation in the embryo of a glossiphoniid leech. *Dev. Biol.* **89,** 389-396.

BLAIR, S. S. (1983). Blastomere ablation and the developmental origin of identified monoamine-containing neurons in the leech. *Dev. Biol.* **95,** 65-72.

BLAIR, S. S., and WEISBLAT, D. A. (1982). Ectodermal interactions during neurogenesis in the glossiphoniid leech *Helobdella triserialis*. *Dev. Biol.* **91,** 64-72.

BLAIR, S. S., and WEISBLAT, D. A. (1984). Cell interactions in the developing epidermis of the leech *Helobdella triseralis*. *Dev. Biol.*, **101,** 318-325.

BOVERI, T. (1899). Die Entwicklung von *Ascaris megalocephala* mit besonderer Rücksicht auf die Kernverhältnisse. Festschrift Kupffer. pp. 383-430. G. Fischer. Jena.

CONKLIN, E. G. (1905). The organization and cell lineage of the ascidian egg. *J. Acad. Natl. Sci. (Philadelphia)* **13,** 1-119.

DAWYDOFF, C. (1959). Ontogenese des Annelides. *In* "Traite de Zoologie" (P. P. Grasse, ed.), Vol. 5, pp. 594-686. Masson, Paris.

VON EHRENSTEIN, G., and SCHIERENBERG, E. (1980). *In* "Nematodes as Biological Models" (B. M. Zuckerman, ed.), Vol. I, pp. 1-71. Academic Press, New York.

VON EHRENSTEIN, G., SULSTON, J. E., SCHIERENBERG, E., LAUFER, J. S., and COLE, T. (1980). Embryonic cell lineage and segregation of developmental potential in *Caenorhabditis elegans*. pp. 519-525. Cell Biology Congress, Berlin.

FISCHER, V. E., LOVAS, M., and NEMETH, P. (1976). Zink Porphyrinpigmente im Batryoidgewebe von *Haemopis Sanguisuga* L. und ihre Lokalisation mit der Diamino Benzidin H_2O_2 Reaktion. *Acta Histochem.* **55,** 32-41.

FERNANDEZ, J. (1980). Embryonic development of the glossiphoniid leech *Theromyzon rude:* Characterization of developmental stages. *Dev. Biol.* **76,** 245-262.

FERNANDEZ, J., and STENT, G. S. (1980). Embryonic development of the glossiphoniid leech *Theromyzon rude:* Structure and development of the germinal bands. *Dev. Biol.* **78,** 407-434.

GRASSÉ, P. P. (1959). "Traité de Zoologie Anatomie, Systématique, Biologie, Tome V, Premier Fascicule." Masson, Paris.

HAGADORN, I. R. (1958). Neurosecretion and the brain of the rhynchobdellid leech, *Theromyzon rude* (Baird, 1869). *J. Morphol.* **102,** 55-90.

KRAMER, A. P., and WEISBLAT, D. A. (1984). Developmental neural kinship groups in the leech. Manuscript submitted.

KUFFLER, D. P., and MULLER, K. J. (1974). The properties and connections of supernumerary sensory and motor nerve cells in the central nervous system of an abnormal leech. *J. Neurobiol.* **5,** 331-348.

LIVANOW, N. A. (1904). Untersuchungen zur Morphologie der Hirudineen. II. Das Nervensystem des vorderen Koerperendes und seine Metamerie. *Zool. Jahrb. (Anat.)* **20,** 153-226.

LUFT, J. M. (1961). Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* **9,** 409-414.

MACAGNO, E. R. (1980). Number and distribution of neurons in leech segmental ganglia. *J. Comp. Neurol.* **190,** 283-302.

MANN, K. H. (1953). The segmentation of leeches. *Biol. Rev.* **28,** 1-15.

MANN, K. H. (1962). "Leeches (Hirudinea)." Pergamon Press, Oxford.

MÜLLER, K. J. (1932). Ueber normale Entwicklung, inverse Asymmetrie und Doppelbildungen bei Clepsine sexoculata. *Z. Wiss. Zool.* **142,** 425-490.

MULLER, K. J., NICHOLLS, J. G., and STENT, G. S. (1982). "Neurobiology of the Leech." Cold Spring Harbor Press, New York.

NISHIDA, H., and SATOH, N. (1983). Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme. I. Up to the eight-cell stage. *Dev. Biol.* **99,** 382-394.

PARNAS, I., and BOWLING, D. (1977). Killing of single neurones by intracellular injection of proteolytic enzymes. *Nature (London)* **270,** 626-628.

SCHLEIP, W. (1936). Ontogenie der Hirudineen. *In* "Klassen und Ordnungen des Tierreichs" (H. G. Bronn, ed.), Vol. 4, Div. III, Book 4, Part 2, pp. 1-121. Akad. Verlagsgesellschaft, Leipzig.

STENT, G. S., WEISBLAT, D. A., BLAIR, S. S., and ZACKSON, S. L. (1982). Cell lineage in the development of the leech nervous system. "Neuronal Development" (N. Spitzer, ed.), pp. 1-44. Plenum, New York.

STUART, D. K., BLAIR, S. S., and WEISBLAT, D. A. (1984), in preparation.

SULSTON, J. E., HORVITZ, H. R. (1977). Post-embryonic cell lineages of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **56,** 110-156.

SULSTON, J. E., SCHIERENBERG, E., WHITE, J. G., and THOMSON, J. N.

(1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **100,** 64–119.

WEISBLAT, D. A., SAWYER, R. T., and STENT, G. S. (1978). Cell lineage analysis by intracellular injection of a tracer enzyme. *Science* **202,** 1295–1298.

WEISBLAT, D. A., HARPER, G., STENT, G. S., and SAWYER, R. T. (1980a). Embryonic cell lineage in the nervous system of the glossiphoniid leech *Helobdella triserialis*. *Dev. Biol.* **76,** 58–78.

WEISBLAT, D. A., ZACKSON, S. L., BLAIR, S. S., and YOUNG, J. D. (1980b). Cell lineage analysis by intracellular injection of fluorescent tracers. *Science* **209,** 1538–1541.

WEISBLAT, D. A., and BLAIR, S. S. (1982). Cell lineage in leech neurogenesis during normal development and after the ablation of identified blastomeres. *NRP Bull.* **20,** 783–793.

WEISBLAT, D. A., and BLAIR, S. S. (1984). Developmental indeterminacy in embryos of the leech *Helobdella triserialis*. *Dev. Biol.* **101,** 326–335.

WEISBLAT, D. A. (1984), in preparation.

WHITMAN, C. O. (1878). The embryology of Clepsine. *Quart. J. Micros. Sci. (N.S.)* **18,** 215–315.

WHITMAN, C. O. (1892). The metamerism of *Clepsine*. "Festschrift zum 70. Geburtstage R. Leuckarts," pp. 385–395. Engelmann, Leipzig.

WHITTAKER, J. R. (1979). Cytoplasmic determinants of tissue differentiation in the ascidian egg. *In* "Determinants of Spatial Organization" (S. Subtelny and I. R. Konigsberg, eds.), pp. 29–51. Academic Press, New York.

WILSON, E. B. (1892). The cell lineage of *Nereis*. *J. Morphol.* **6,** 361–480.

ZACKSON, S. L. (1982). Cell clones and segmentation in leech development. *Cell* **31,** 761–770.

ZACKSON, S. L. (1984). Cell lineage, cell-cell interaction and segment formation in the ectoderm of a glossiphoniid leech embryo. *Dev. Biol.*, in press.