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# Embryonic Development of the Rat Cerebellum. III. Regional Differences in the Time of Origin, Migration, and Settling of Purkinje Cells

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ABSTRACT

The time of origin, site of origin, migratory path and settling pattern of the Purkinje cells of the cerebellar hemispheres, anterior vermis, and posterior vermis were investigated in thymidine radiograms and plastic-embedded materials from rat embryos ranging in age from 15 to 22 days. In the hemispheres there is a rostral-to-caudal cytogenetic gradient: the Purkinje cells of lobulus simplex, crus I, and crus II are produced earlier than the Purkinje cells of the paramedian lobule and paraflocculus, followed by the Purkinje cells of the flocculus. The Purkinje cells of the vermis, in general, are generated later than those of the hemispheres, and with a reverse gradient from caudal to rostral: the Purkinje cells of the posterior vermis (lobules X–VI) being produced ahead of the Purkinje cells of the anterior posteriorly directed wedge of early-produced Purkinje cells through the vermis.

Evidence was obtained that the Purkinje cells of the hemispheres derive from the lateral cerebellar primordium capping the lateral recess of the fourth ventricle anteriorly. The Purkinje cells of the anterior vermis originate from the subisthmal cerebellar primordium medially lining the isthmal canal. The Purkinje cells of the posterior vermis originate in the postisthmal cerebellar primordium overlying the tela choroidea caudally. The young Purkinje cells migrate from the neuroepithelium to the surface of the cerebellum in a strictly caudal-to-rostral order, paralleling the spread of the EGL superficially from posteroventral to anterodorsal. This pattern is independent of the time of origin of Purkinje cells. In the posterior vermis the earliest-settling Purkinje cells of the uvula follow a short radial course, and a discrete Purkinje layer is formed 3 days after they are generated. In the anterior vermis the Purkinje cells of lobulus centralis, which follow an anterodorsal migratory course, are still settling on day E22, 7 days after their production, presumably awaiting the fusion of the cerebellar base anteriorly. The fissura prima forms medially at the interface region of Purkinje cells derived from the postisthmal and subisthmal cerebellar primordia. For 1–2 days after their settling, the Purkinje cells of the newly forming lobules can be distinguished by certain cytological criteria from the Purkinje cells in the more caudally-situated, earlier-settled lobules.

Key words: cerebellar development, thymidine autoradiography

Previous thymidine-radiographic studies (Das and Nornes, '72; Schultze et al., '74; Altman and Bayer, '78) have established that the Purkinje cells of the rat cerebellum are produced over a relatively brief time span with a peak on day E15. In these studies, carried out in adult rats, regional differences in the various lobules of the vermis and hemisphere were not examined, possibly because regional differences are not obvious in the adult rat cerebellum where the

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Purkinje cells are widely dispersed and labeled and unlabeled cells seem to alternate randomly. The possibility of regional variations was suggested by the differences in the proportion of Purkinje cells produced in three arbitrary sagittal planes transecting the core of the medial, interpositus, and lateral deep nuclei (Altman and Bayer, '78: Fig. 14). Laterally the proportion of early-produced Purkinje cells was higher than medially. This lateral-to-medial cytogenetic gradient suggested that the Purkinje cells of the hemispheres might be produced somewhat ahead of the Purkinje cells of the vermis. However, because all the Purkinje cells were counted in a given sagittal plane this approach did not produce information about specific regional differences.

In contrast to thymidine radiograms of adult rats, in which regional differences in the labeling pattern of Purkinje cells is not obvious, in perinatal rats (day E22) regional differences were obvious upon cursory examination. In rats injected on day E15, the densely packed (not yet dispersed) Purkinje cells were labeled in some regions and unlabeled in other regions. However, cursory observation also indicated that instead of a simple lateral-to-medial gradient there was regional variability within both the vermis and the hemispheres. In the present investigation we have sought to specify these regional differences in perinatal rats and made an attempt to relate these to regional differences in the migration and settling of Purkinje cells as revealed in a series of younger embryos by short-survival thymidine-radiography and methacrylate histology.

## MATERIALS AND METHODS

The material used in this study was identical with that described in detail in the first paper of this series (Altman and Bayer, '85). Special use was made of thymidine radiograms from embryos labeled with $^3$H-thymidine on day E15 and killed thereafter at daily intervals up to day E22, and of methacrylate-embedded embryos, ages E17–E22.

## RESULTS

### Regional differences in the time of origin of Purkinje cells

***Observations in thymidine radiograms sectioned in the sagittal plane.*** The labeling pattern of Purkinje cells in a rat injected with $^3$H-thymidine on day E15 and killed on day E22 is illustrated in sagittal sections from medial to lateral in Figures 1–3. Most medially (Fig. 1A) a high proportion of the Purkinje cells is labeled in the formative lobules of the anterior vermis but relatively few are labeled in the posterior vermis. In this plane the primary fissure is beginning to form at the interface of the late-generated Purkinje cells of the anterior vermis and the early-generated Purkinje cells of the posterior vermis. The pattern is somewhat different in the vermis more laterally in that the zone of labeled Purkinje cells extends beyond the primary fissure (Fig. 1B). Still more laterally in the vermis (Fig. 2A), the labeling pattern is more complex. Anteriorly, the heavily labeled zone of Purkinje cells is reduced to patches, and in a superficial position a zone of unlabeled cells appears. Posteriorly, the proportion of labeled Purkinje cells is higher than in the more medial sections (Fig. 1A, B).

The labeling pattern changes in the cerebellar hemisphere. Medially in the anterior portion of the hemisphere (Fig. 2B), presumably representing the lobulus simplex and crus I, most of the Purkinje cells are unlabeled. Posterior to the ansoparamedian fissure two Purkinje cell zones are discernible: an interior one with some labeled cells and an exterior one with very few. Since in perinatal rats (E21–P1) the paramedian lobule and crus II have not yet separated (Larsell, '52: Figs. 7–11), we assume that the zone of unlabeled cells (the one more superficially situated) represents crus II and the zone with more labeled cells the paramedian lobule. Beneath the parafloccular fissure the paraflocculus contains a few labeled Purkinje cells. In more lateral sections (Fig. 3A) the lobulus simplex, with its essentially early-produced Purkinje cells, gradually disappears. The pattern of labeling in crus II and the paramedian lobule, and crus I and the paraflocculus, is similar to that seen more medially (Fig. 2B). A fair proportion of labeled cells is present in the formative flocculus adjacent to the germinal trigone.

In some rats injected on day E15 and killed on day E22 the proportion of cells in the posterior vermis medially was higher than in the rat illustrated in Figures 1A, B. However, in spite of these apparent differences in the absolute number of labeled cells in different animals, the sequence of cell production seemed similar. For instance, in all rats the highest proportion of labeled Purkinje cells was in the anterior vermis and the lowest proportion in the lobulus simplex, crus I, and crus II. Our assumption is that the absolute differences were due to slightly different developmental ages of the embryos nominally labeled on day E15. This, coupled with the extremely rapid generation of Pur-

*Abbreviations*

| | | | | | |
|---|---|---|---|---|---|
| apm | ansoparamedian fissure | LS | lobulus simplex | pri | primary fissure |
| AV | anterior vermis | MB | marginal nucleus of the brachium conjunctivum | psu | posterior superior fissure |
| CO | copula pyramis | | | PV | posterior vermis |
| CRI | crus I | MN | medial (fastigial) nucleus | SCP | subisthmal cerebellar primordium |
| CRII | crus II | MNc | medial nucleus, caudal part | scp | superior cerebellar peduncle |
| ctz | cortical transitory zone | MNr | medial nucleus, rostral part | sec | secondary fissure |
| EGL | external germinal layer | mp | medial protuberance | tf | transversely cut fibers |
| FL | flocculus | NE | neuroepithelium | VW | vermal wedge |
| FNL | flocculonodular lobe | pce | precentral fissure | I | vermian lobule I; lingula |
| fz | fuzzy matrix of PL | PCP | postisthmal cerebellar primordium | II | vermian lobule II; lobulus centralis, ventral |
| GT | germinal trigone | pcu | preculminate fissure | III | vermian lobule III; lobulus centralis, dorsal |
| HE | cerebellar hemisphere | PF | paraflocculus | IV | vermian lobule IV; culmen, ventral |
| icp | inferior cerebellar peduncle | PFd | paraflocculus, dorsal part | V | vermian lobule V; culmen, dorsal |
| IN | interpositus nucleus | PFv | paraflocculus, ventral part | VI | vermian lobule VI; declive |
| inc | intercrural fissure | pfl | parafloccular fissure | VII | vermian lobule VII; tuber |
| LCP | lateral cerebellar primordium | PL | Purkinje cell layer | VIII | vermian lobule VIII; pyramis |
| LN | lateral (dentate) nucleus | PMD | paramedian lobule | IX | vermian lobule IX; uvula |
| LNc | lateral nucleus, caudal part | pol | posterolateral fissure | X | vermian lobule X; nodulus |
| LNr | lateral nucleus, rostral part | PPY | prepyramidal fissure | | |

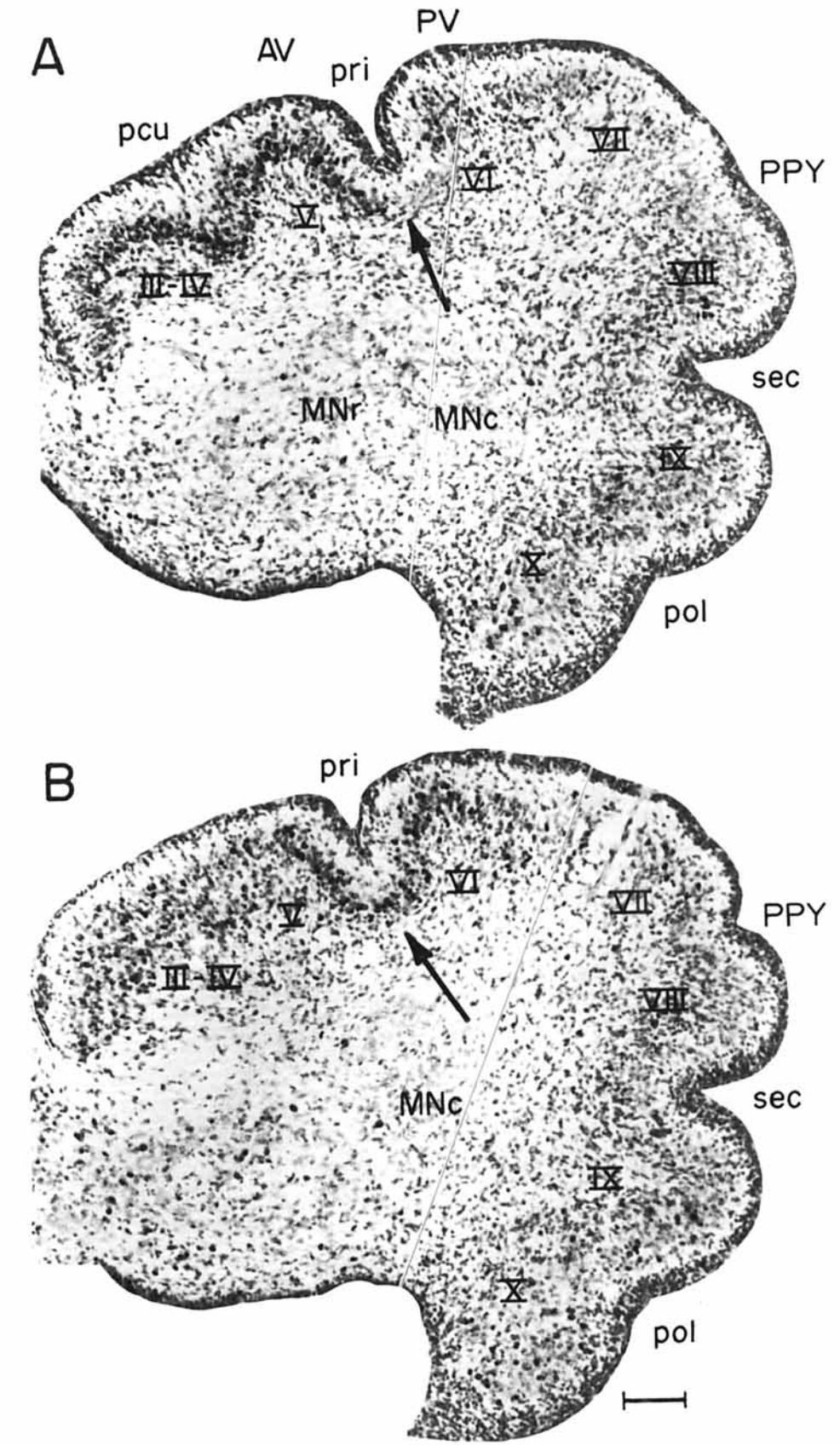


Fig. 1. A. Sagittal thymidine radiogram of the cerebellum of a rat labeled on day E15 and killed on day E22. B. A more lateral section from the same animal. Arrows point to the region where the fissura prima is deepening. Paraffin; scale: 100 $\mu$m.

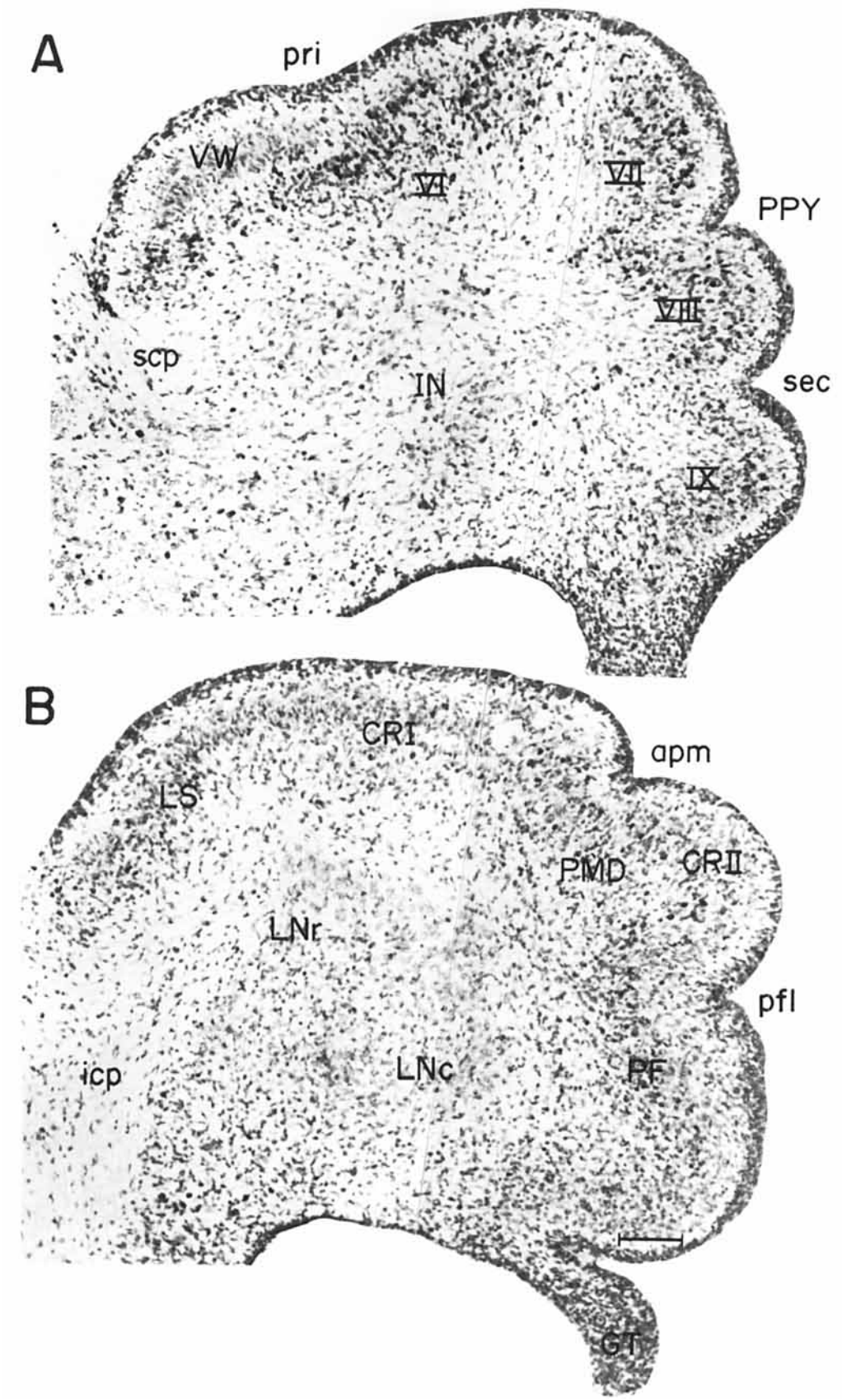


Fig. 2. Continuation, from medial to lateral, from the rat shown in Figure 1. A. The lateral portion of the vermis. Note the unlabeled cell cluster of the vermal wedge. B. The medial aspect of the cerebellar hemisphere, with a paucity of labeled Purkinje cells. Paraffin; scale: 100 $\mu$m.

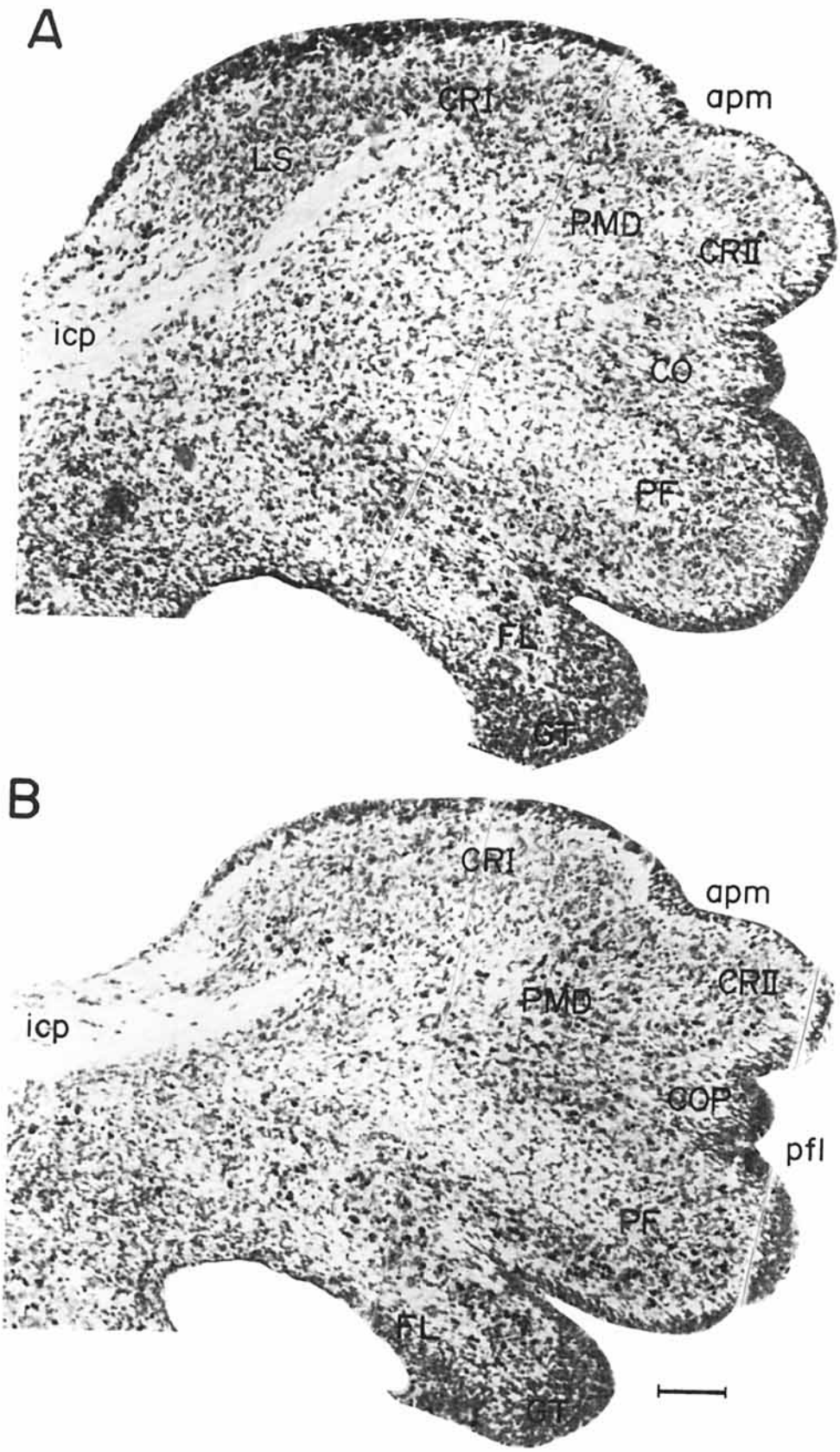


Fig. 3. Continuation, from medial to lateral, from the rat shown in Figures 1 and 2. A. Middle part of the hemisphere. There are few labeled cells in crus I and II, more in the paraflocculus and, particularly, the flocculus. B. Lateral part of the hemisphere. Note labeled cells in the paramedian lobule, the ventral paraflocculus, and the flocculus. Paraffin; scale: 100 $\mu$m.

kinje cells, led to the apparent numerical differences in the number of labeled and unlabled cells in different regions of the cerebellum.

***Observations in thymidine radiograms sectioned in the coronal plane.*** Examination of coronally sectioned thymidine radiograms from rats injected on day E15 and killed on day E22 provided some clues about the regional differences in the cytogenesis of Purkinje cells. Figure 4 illustrates the pattern in a rat that was presumably "immature" at the time of injection (note the high proportion of labeled cells in the posterior vermis; Fig. 4D). Rostrally, (Fig. 4A), the overall cytogenetic gradient is from lateral to medial, the Purkinje cells of the hemisphere originating de toto earlier than the Purkinje cells of the anterior vermis. But there are differences within these two regions. In the hemisphere there is a slight dorsal-to-ventral gradient, the proportion of labeled cells being lower in crus I than in the paramedian lobule and the rostral part of the paraflocculus. At the same level, situated over a continuous sheet of labeled cells of the anterior vermis is a superficial cluster of unlabeled Purkinje cells. A similar pattern is seen more caudally (Fig. 4B) except that the narrow zone of early-produced Purkinje cells forming a *vermal wedge* has shifted more medially. The paramedian lobule contains a high

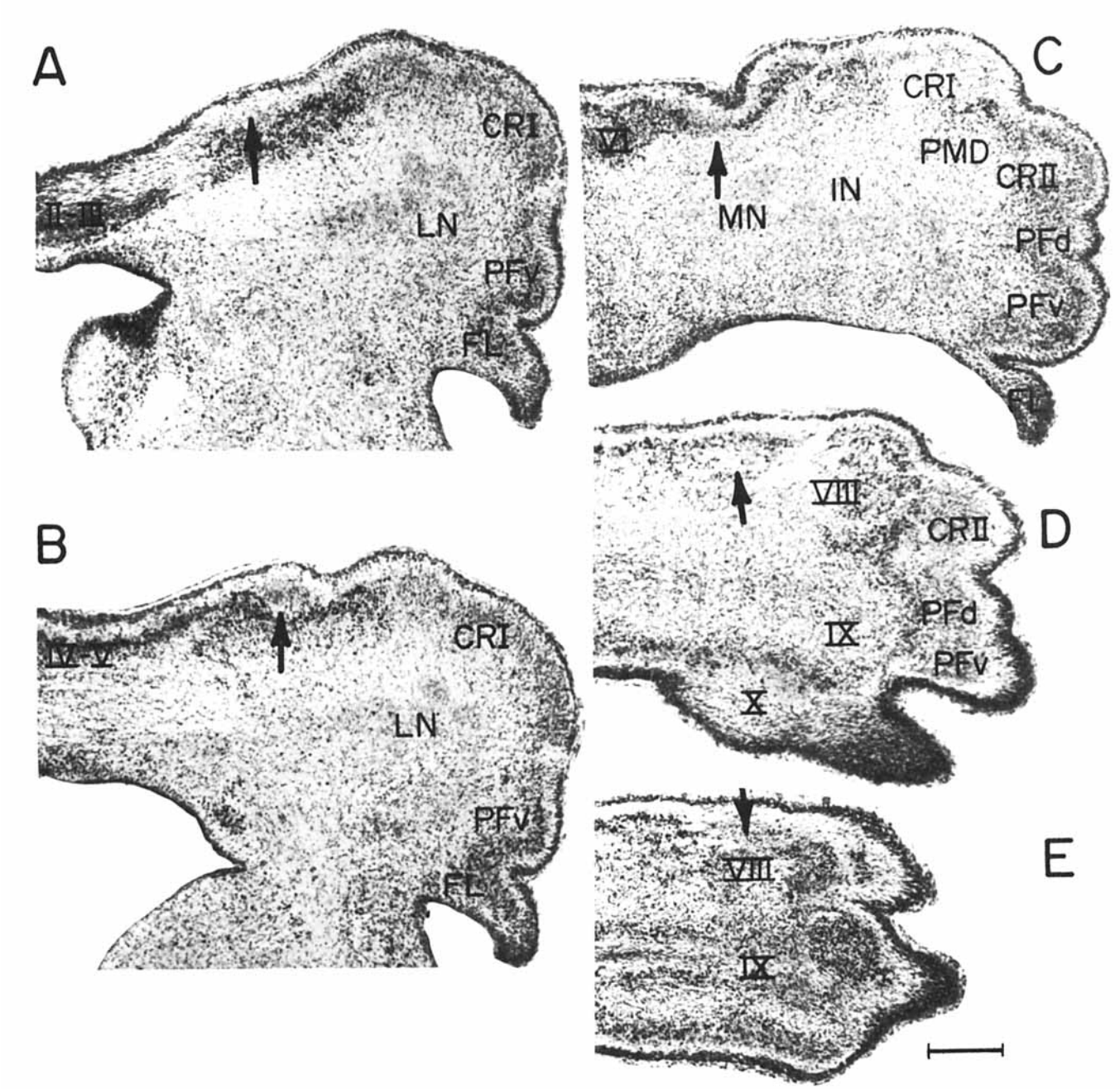


Fig. 4. Coronal thymidine radiograms from rostral to caudal (A–E), from a rat labeled on day E15 and killed on day E22. Because in this rat the Purkinje cells are labeled in the posterior vermis (lobules VII–IX; D, E) it is assumed that it was developmentally immature at the time of injection. Arrows point to the unlabeled cells of the vermal wedge. Paraffin; scale: 200 $\mu$m.

proportion of labeled Purkinje cells but more laterally crus II is largely composed of unlabeled Purkinje cells. At the rostral level of the posterior vermis (Fig. 4D) there are two zones of Purkinje cells laterally; the earlier-generated outer zone is related to the hemisphere, the inner zone to the vermis. Presumably the outer zone Purkinje cells are associated with the lateral EGL, the inner zone (compare with Fig. 4E) with the posterior EGL. In the posterior vermis the vermal wedge of early-produced Purkinje cells bisects the vermis (Fig. 4E).

Figure 5 schematically summarizes the pattern of Purkinje cell labeling in 13 roughly equidistant coronal sections. Twelve levels illustrate the pattern in the vermis and the hemispheric pattern is shown at four levels (levels 4, 7, 10, and 11 correspond to the figures shown in Fig. 4A–D, respectively). As previously seen in sagittal sections (Fig. 1A, B), the cytogenetic gradient is from caudal to rostral in the vermis. In the posterior vermis the vermal wedge of early-produced Purkinje cells is bisecting the vermis but in the anterior vermis this band shifts laterally and to a superficial position. In the hemisphere, lobulus simplex, crus I, and crus II contain few labeled cells; the proportion of labeled cells is relatively high in the paramedian lobule, while in the paraflocculus variability is evident, possibly with a caudal-to-rostral gradient.

## Regional differences in the migratory pattern of Purkinje cells

***Observations in thymidine radiograms sectioned in the sagittal plane.*** Figure 6 illustrates the apparent migratory path of Purkinje cells in the medial vermis in a series of developmentally "young" embryos labeled on day E15 and killed on various days afterward. The majority of labeled Purkinje cells are still in the neuroepithelium or in the cortical transitory zone on day E17 (Fig. 6A). By day E18 (Fig. 6B), the neuroepithelium has receded and the bulk of the labeled cells are in the cortical transitory zone. By day E19 (Fig. 6C) heavily-labeled cells are reduced in the cortical transitory zone near the neuroepithelium and the radially-migrating heavily-labeled cells approach the surface in most regions and, in the posterior vermis, settle beneath the EGL. By day E21 (Fig. 6D) all the Purkinje cells, including those settling in the anterior vermis, form a discrete Purkinje cell layer.

More laterally, where the cells of the interpositus nucleus have accumulated superficially by day E16 (Fig. 7A), the radial migratory route of Purkinje cells may be somewhat modified. At these levels the cortical transitory zone, which contains on day E17 a higher proportion of unlabeled cells laterally (Fig. 7B) than medially (Fig. 6B), the cells moving toward the anterior portion of the posterior vermis (Fig. 7D) seem to be taking a detour around the interpositus nucleus by day E19 (Fig. 7C) as the latter is translocated from a superficial to a deep position. Although the Purkinje cells of the hemispheres are, as a group, generated earlier than the Purkinje cells of the vermis (compare Figs. 7D, 6D), the radial migration of the Purkinje cells, and with it the superficial spread of the EGL in the anterior direction, is less advanced laterally by day E21 (Fig. 7D) than medially (Fig. 6D).

***Observations in thymidine radiograms sectioned in the coronal plane.*** The migratory route of the earliest-settling Purkinje cells, those that form lobule IX of the posterior vermis (Fig. 11), is a direct and short one from the neuroepithelium of the postisthmal vermis and can be conceptualized as the shortest radial route (Fig. 6) or a modification of it (Fig. 7). The route taken by Purkinje cells to the anterior vermis must be more complicated (Figs. 8, 9). This is so because anteriorly the two cerebellar halves are not fused

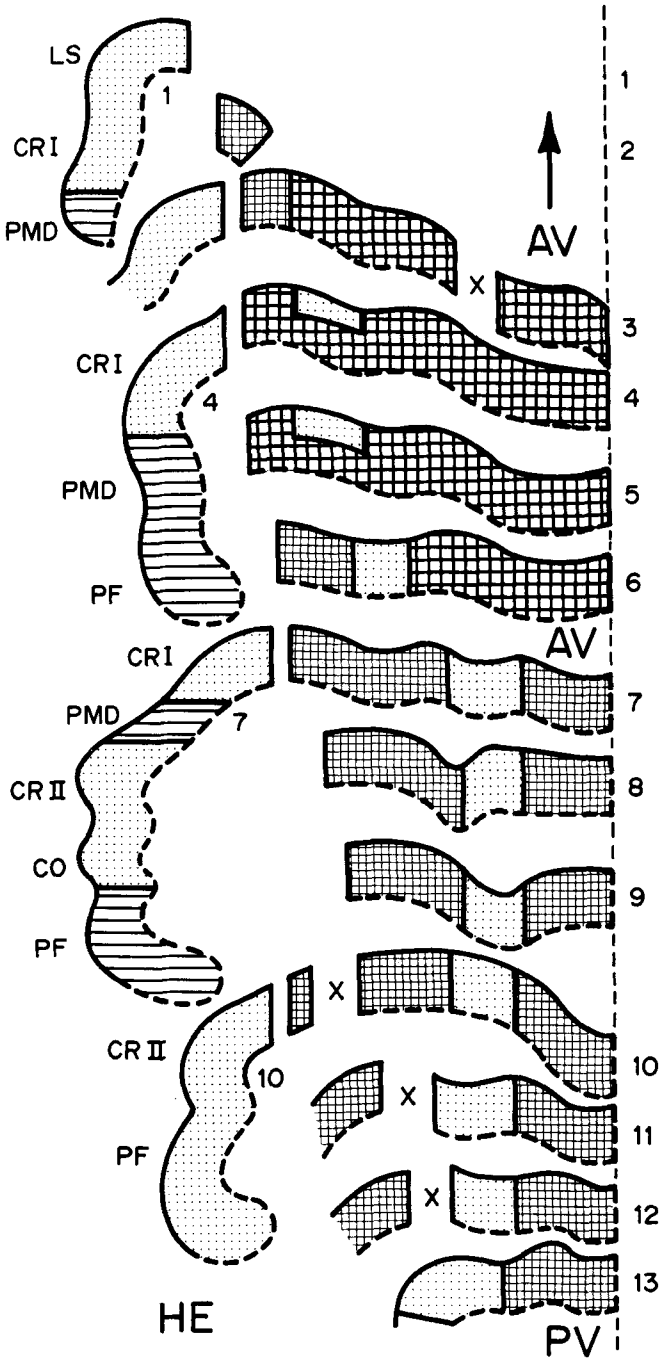


Fig. 5. Pattern of Purkinje cell labeling in 13 roughly equidistant coronal sections from a rat injected on day E15 and killed on day E22 (tracings from the "immaturely" labeled animal illustrated in Fig. 4). Areas with fine dots have few labeled cells (note the vermal wedge in the vermis); areas with horizontal lines have more labeled cells; light checkered area represents the posterior vermis, and the heavy checkered area, with the highest proportion of labeled cells, the anterior vermis. Arrow indicates that the formation of the most anterior lobules is not completed at this age; X indicates areas devoid of Purkinje cells.

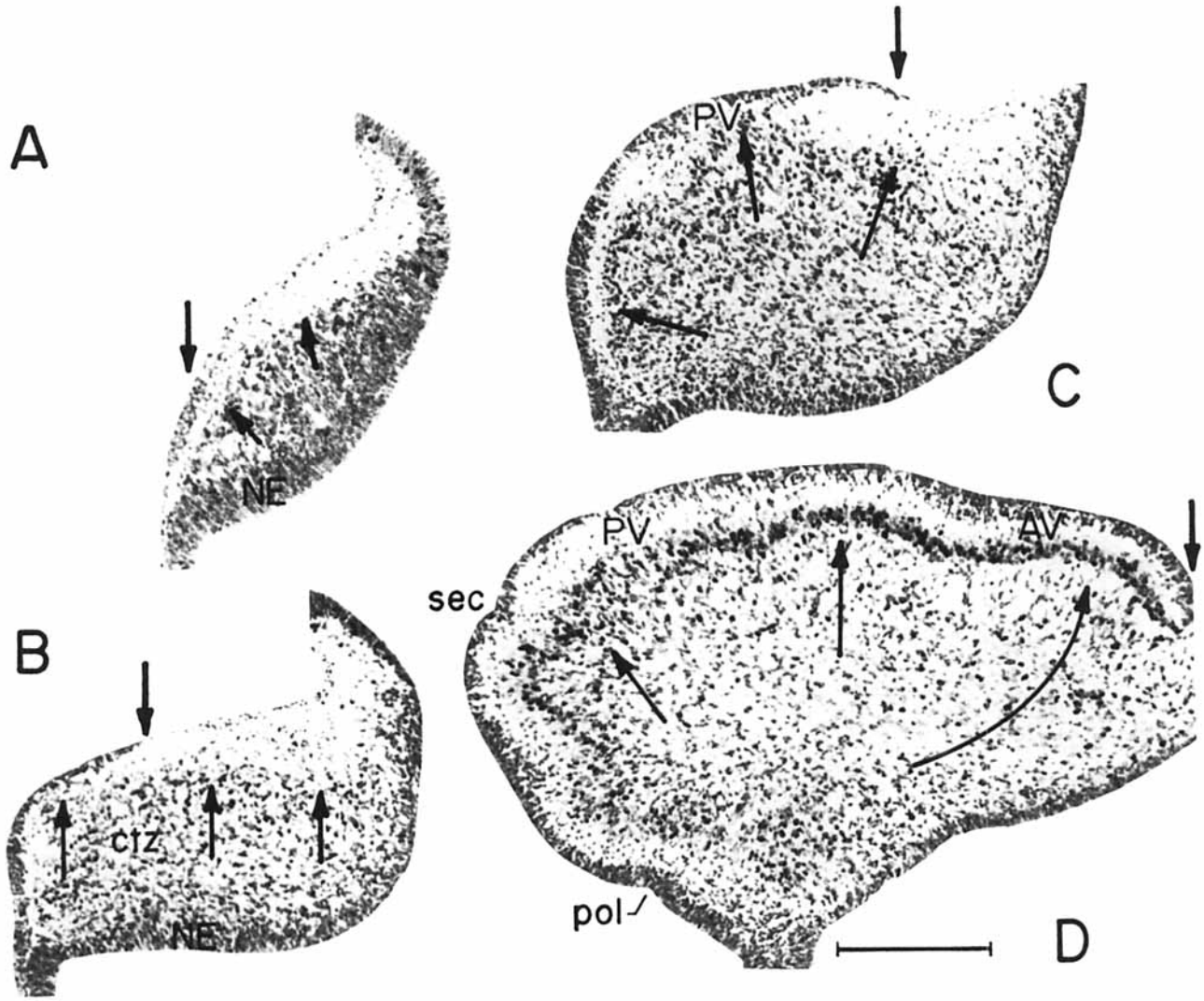


Fig. 6. Midsagittal thymidine radiograms from rats labeled on day E15 and killed on days E17 (A), E18 (B), E19 (C), and E21 (D). Outside vertical arrows indicate the anterior front of the spreading EGL; inside arrows show the migratory route of cells from the cortical transitory zone to the Purkinje cell layer. Paraffin; scale: 200 $\mu$m.

until day E20 (Fig. 8B, C) and there is no cerebellar primordium (neuroepithelium) present locally that could be the source of Purkinje cells. A Purkinje cell layer does not appear at the caudal levels of the anterior vermis until day E21 (Fig. 8D), at which time it is forming a small medial patch beneath a similar, isolated medial patch of the EGL. This is about 4 days after the Purkinje cell layer is beginning to form in the caudal region of the posterior vermis (Fig. 11). At caudal levels of the anterior vermis the Purkinje cell layer has spread across the entire vermis to join the hemispheric Purkinje cell layer by day E22 (Fig. 8E), but more anteriorly this process is not completed at this time (Fig. 5).

The best reconstruction of the site of origin and migratory route of the late-generated Purkinje cells of the anterior vermis (and this will be supported below by observations in methacrylate sections) is as follows. In rats injected on day E15 and killed on days E16 (Fig. 9A) and E17 (Fig. 9B) an extensive neuroepithelium is present medially in the subisthmal cerebellar primordium. By day E18 (Fig. 9C) the dispersing cells of this region seem to reach more rostral levels where the cerebellar halves begin to fuse. This fusion proceeds in an anterior direction but the site where the Purkinje cells of the anterior vermis will settle will not be fused until 2–3 more days (Fig. 8C, D). These observations suggest that unlike the Purkinje cells of the posterior vermis, which derive from the nearby postisthmal cerebellar primordium, the Purkinje cells of the anterior vermis originate in the neuroepithelium of the subisthmal cerebellum. The earliest-settling Purkinje cells reach the midline region of the anterior vermis caudally after the cerebellar halves have fused here, about 6 days after their production (day E21; Fig. 8D). It is only on the succeeding day that the settling Purkinje cells spread in the lateral direction in the caudal region of the anterior vermis. The migratory path, therefore, is a modified anterodorsal radial route and it

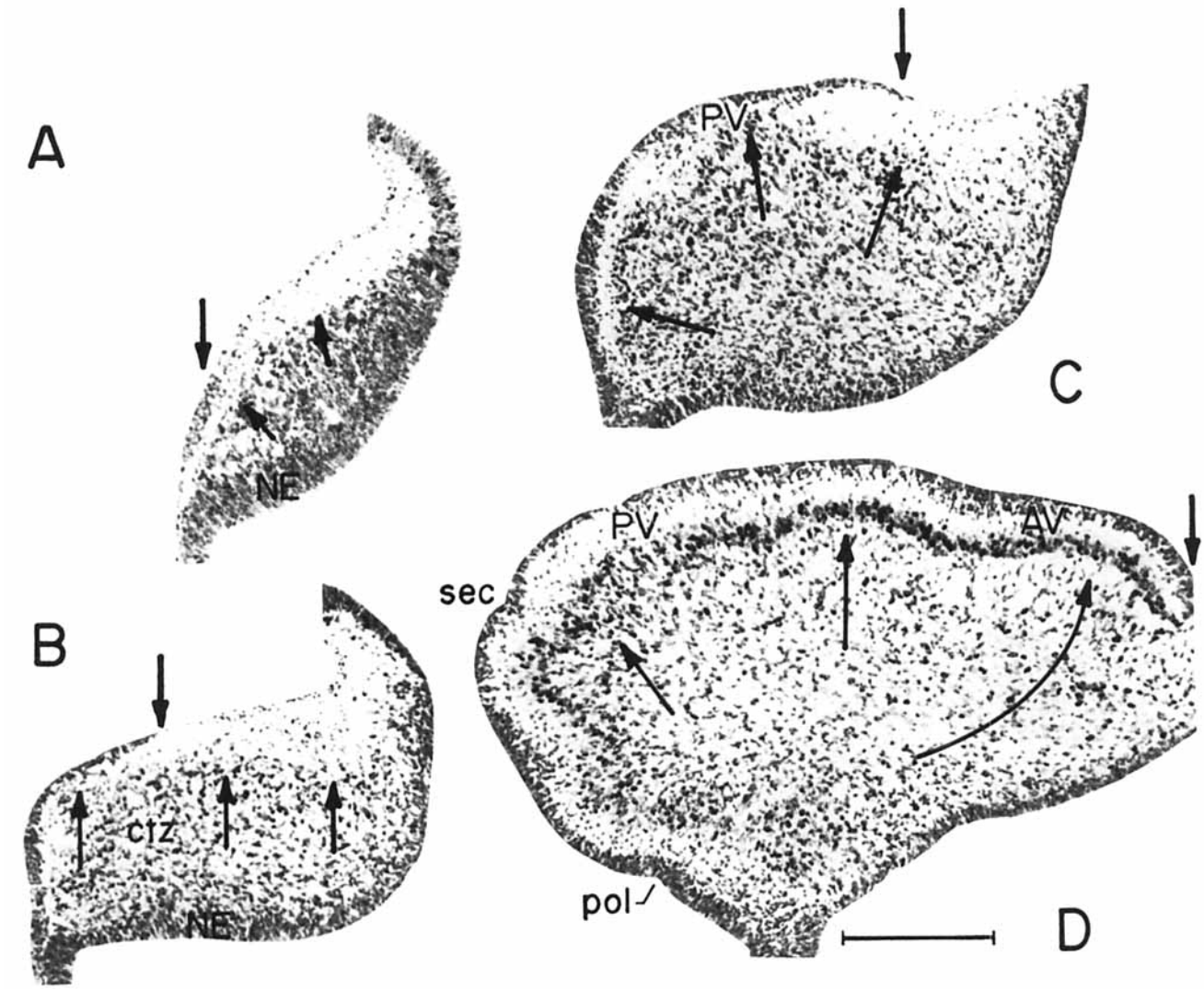


Fig. 7. Sagittal thymidine radiograms through the cerebellar hemisphere of rats labeled on day E15 and killed on days E16 (A), E17 (B), E19 (C), and E21 (D). Arrows as in Figure 6. Paraffin; scale: 200 $\mu$m.

must be a relatively long one because during the period between day E15 and E21 the cerebellum is increasing considerably in volume.

The migratory route of Purkinje cells was difficult to follow in the lateral vermis and the hemisphere. The early-produced Purkinje cells of the vermal wedge (Fig. 5) could not be recognized until they settled dorsally on day E20 (Fig. 10). This was partly because these cells were difficult to distinguish from the unlabeled cells of the interpositus nucleus (Fig. 10A). The presence of a germinal trigone in the anterior cerebellum ventromedially (Fig. 10A) suggested the possibility that this is the source of the isolated, medially emerging EGL on day E21 (Fig. 8D). In general, the vermis with its relatively late-produced Purkinje cells was morphogenetically developing ahead of the hemisphere (Fig. 10A). The possibility that the paraflocculus arises in the posterior vermal region by migration laterally was indicated. It is likely, however, that most of the hemispheric Purkinje cells derive from the lateral cerebellar primordium.

***Observations in methacrylate-embedded specimens.*** In the posterior vermis, the EGL has spread early on day E17 from its posteroventral source, the germinal trigone, in an anterodorsal direction to the point where the cerebellum curves anteriorly (Fig. 11). Beneath the EGL a multiple-cell thick Purkinje cell layer is forming, presumably constituting the uvula (lobule IX). These just-settled cells are tightly packed and are associated with a superficial fuzzy matrix (Fig. 12). Throughout the depth of the cerebellum spindle-shaped cells abound ahead of this region, together with vertically-oriented fibers. These are interpreted to be Purkinje cells with leading and trailing processes which are in the process of migration to the surface. By day E18 (Fig. 13A) the EGL and the Purkinje cell layer have further

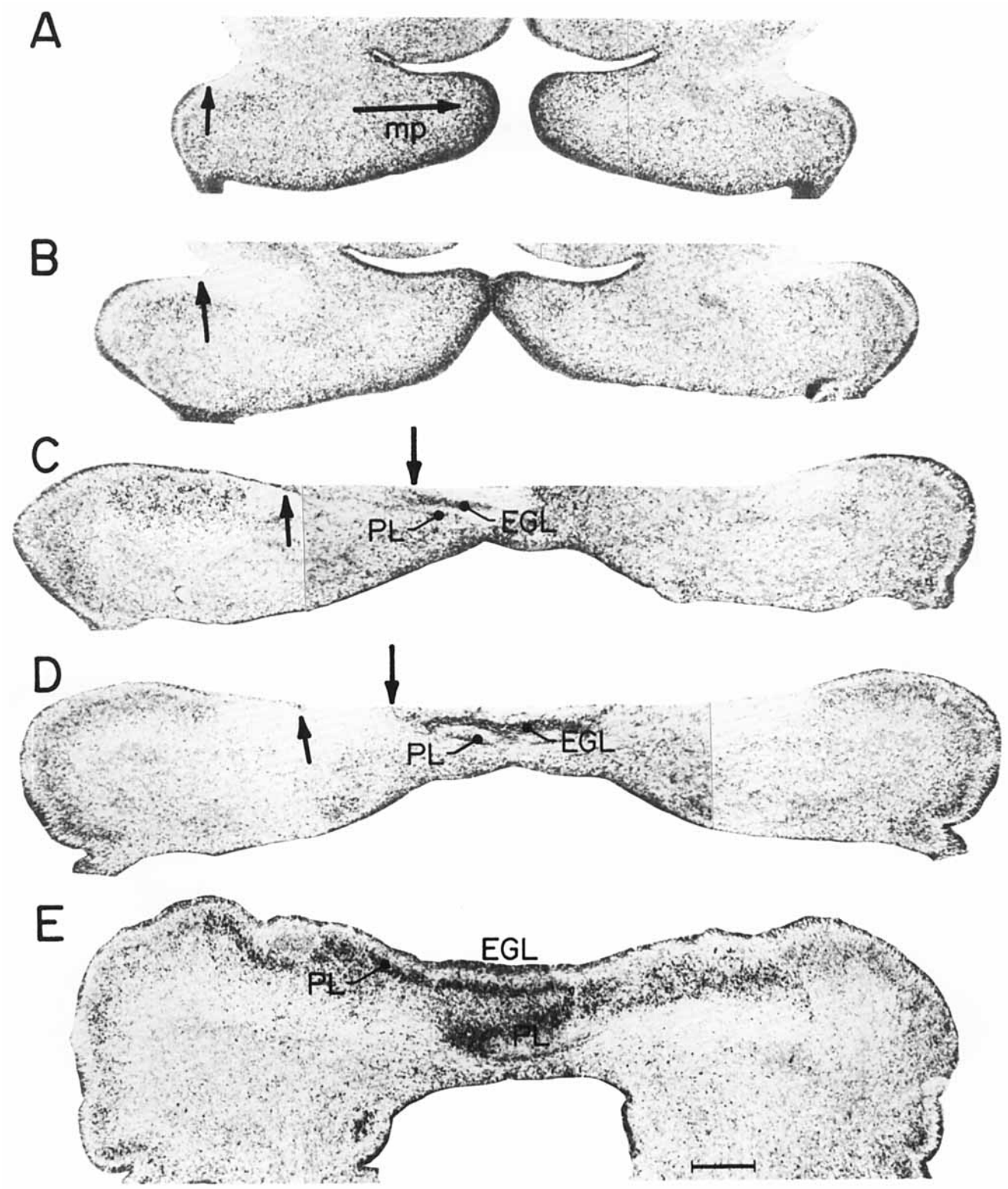


Fig. 8. Coronal thymidine radiograms through the area of the caudal region of the formative anterior vermis, from rats labeled on day E15 and killed on days E18 (A), E19 (B), E20 (C), E21 (D), and E22 (E). Fusion of the medial protuberance (base of the cerebellum) occurs between days E19 and E20 (B, C), and the first signs of the medial penetration of the Purkinje cell layer on days E20 and E21 (C, D). Upward arrows point to the medial spread of the lateral EGL, downward arrows to the lateral spread of the medial EGL. Fusion of the cortex at this level occurs on day E22 (E). Paraffin; scale: 250 $\mu$m.

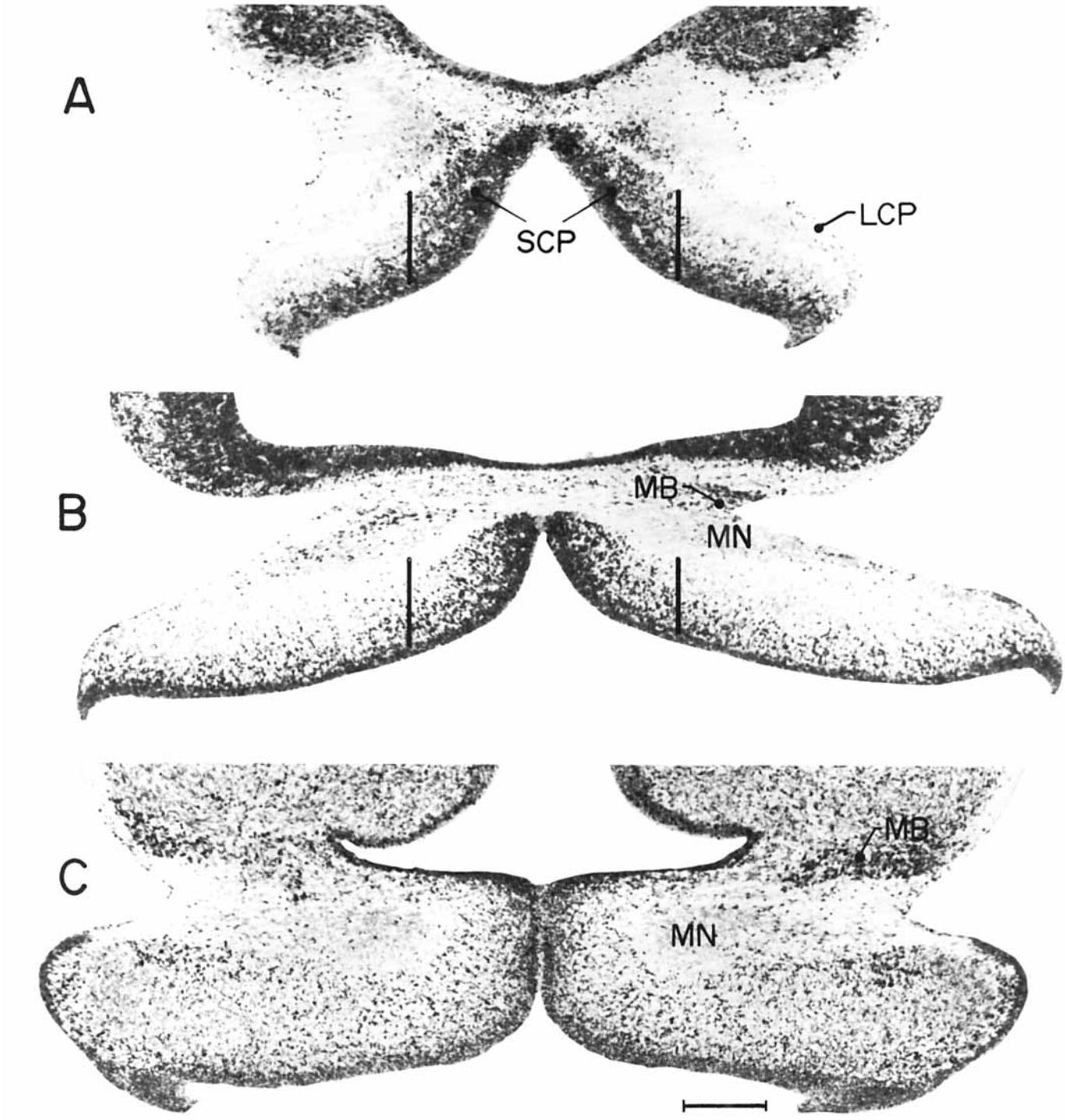


Fig. 9. Coronal thymidine radiograms from rats labeled on day E15 and killed on days E16 (A), E17 (B), and E18 (C). Vertical lines enclose the subisthmal primordium with a high concentratrion of primitive cells on days E16 and E17 (A, B). By day E18 the dispersing cells reach a more rostral level (C) where the cerebellar halves begin to fuse. Paraffin; scale: 250 $\mu$m.

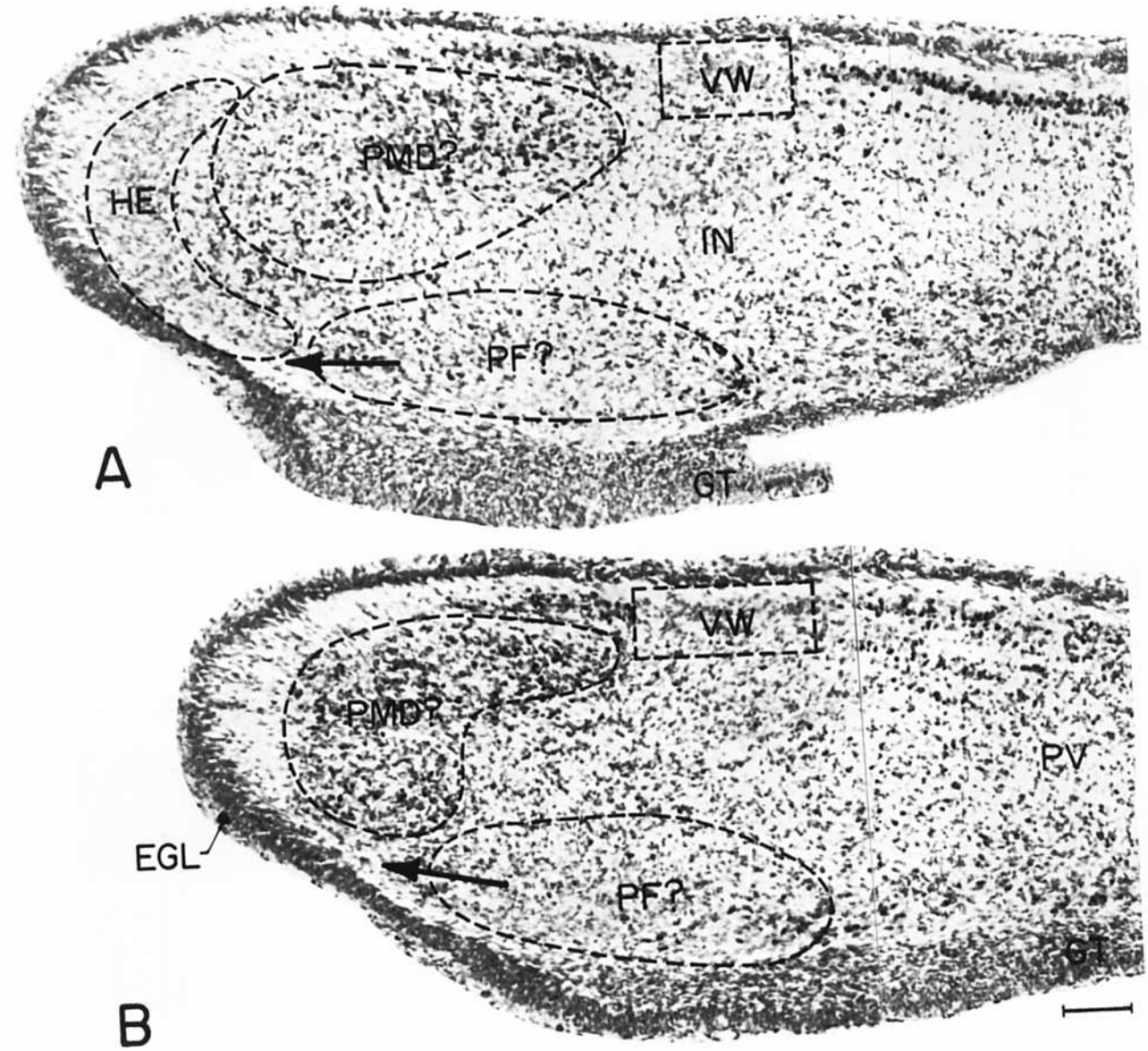


Fig. 10. Coronal thymidine radiograms from rostral (A) to caudal (B), from a rat labeled on day E15 and killed on day E20. By this age the early-generated Purkinje cells of the vermal wedge have settled posteriorly. The upper left enclosed cell mass, judging by its labeling pattern, may be Purkinje cells of the paramedian lobules. The possible migration of paraflocculus Purkinje cells from medial to lateral is also indicated. Paraffin; scale: 100 $\mu$m.

extended rostrally. The newly acquired complement of Purkinje cells is again distinguished by dense packing and a fuzzy matrix but, by now, these features are less evident in the earlier-settled region (Fig. 13A). The settling Purkinje cells on day E18 form the pyramis (lobule VIII). On day E19 (Fig. 13B) a transversely oriented fiber bundle appears in this medial plane above the pyramis and ahead of it are settling the Purkinje cells of the tuber (lobule VII). Now these cells display the features of newly-settled Purkinje cells while the neurons of the earlier settled uvula and pyramis have become less conspicuous. The EGL and the Purkinje cell layer extend further rostrally on day E20 (Fig. 14A). The new addition probably represents the declive (lobule VI) and the dorsal and ventral culmen (lobules V and IV). The Purkinje cells of the anterior vermis are distinguished at this stage, in addition to the fuzzy matrix, by their large size, pronounced spindle shape, and darkly staining trailing processes (Fig. 15). These Purkinje cells migrate through and settle above a matrix of transversely cut fibers (Fig. 15). In coronal sections of day E20 rats (Fig. 16) these fibers are seen to form a relatively large bundle that traverses the midline where the caudal portion of the anterior vermis has fused on the previous day (compare with Fig. 8B, C). The fuzzy matrix is not obvious where the Purkinje cells have not yet reached their settling site (Fig. 16B). The new additions by day E21 (Fig. 14B) are the settling Purkinje cells of the lobulus centralis (lobule III; Fig. 17B). By this time the cells of the culmen are no longer

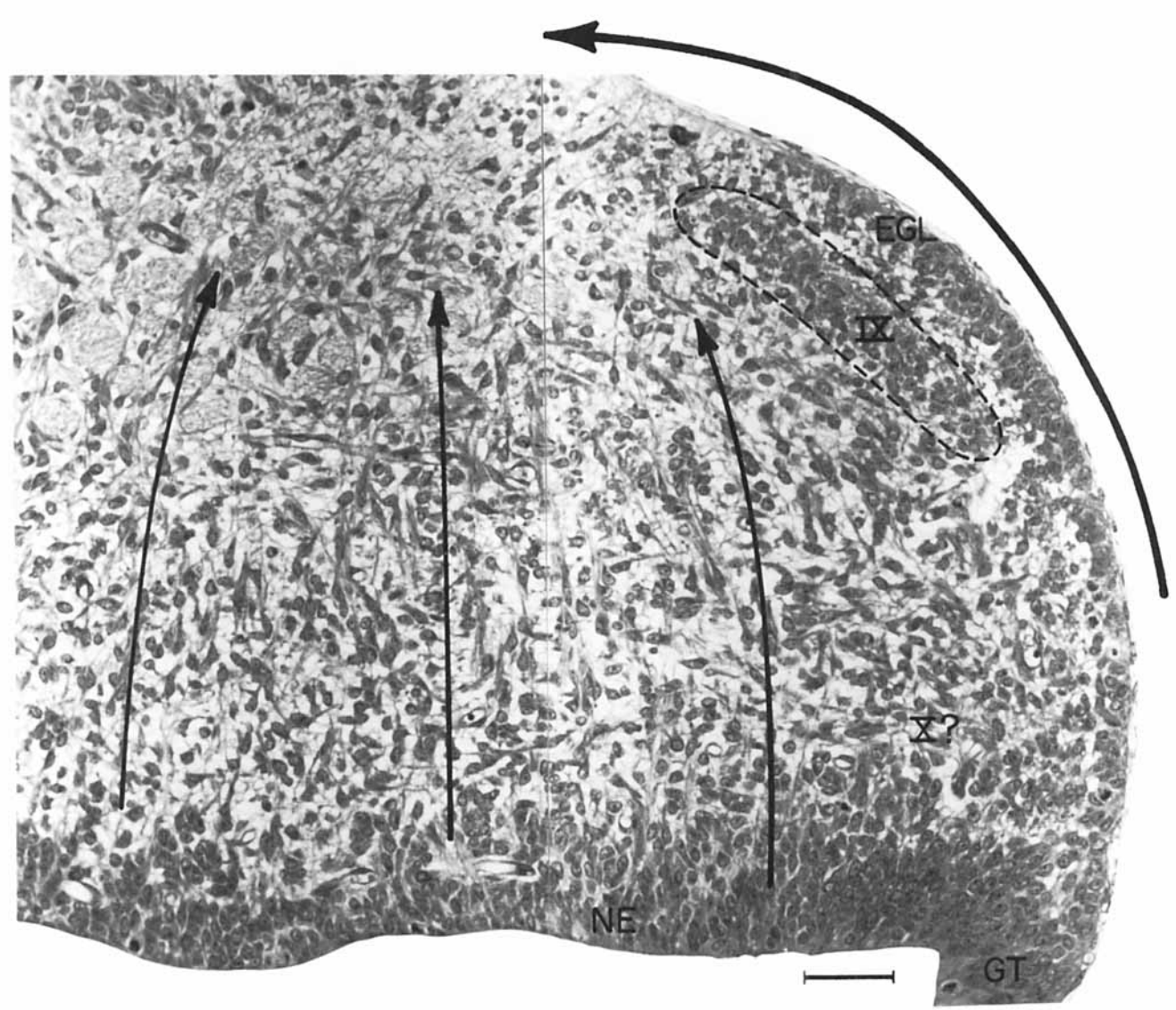


Fig. 11. The Purkinje cells of uvula (lobule IX) are settling by day E17. Outer arrow indicates the dispersal of the EGL; inner arrows indicate the radially oriented spindle-shaped Purkinje cells (and their processes) which are apparently migrating to form the pyramis (lobule VIII). Methacrylate; scale: 50 $\mu$m.

spindle-shaped and the cells of the posterior vermis, behind the developing fissura prima, have become less conspicuous. By day E22 (Fig. 18) all the Purkinje cells of the medial vermis have assumed an inconspicuous appearance that characterizes the cerebellum of the perinatal rat. These alternating phases of the conspicuous appearance of the just-settling Purkinje cells and their subsequent regression could be seen throughout the cerebellum and is illustrated in the lateral cerebellum for lobulus simplex and crus I between days E20 (Fig. 19A) and E22 (Fig. 19C).

## DISCUSSION

### Regional differences in the time of origin of Purkinje cells

In rats labeled with $^3$H-thymidine on day E15 and killed on day E22 systematic regional differences were seen in the time of origin of Purkinje cells (Fig. 5). Generally speaking, the anterior vermis was mostly composed of late-produced (labeled) Purkinje cells; the posterior vermis contained an admixture of late-produced and early-produced (unlabeled) Purkinje cells (with variability from animal to animal), while several hemispheric lobules were composed predominantly of early-produced Purkinje cells. A caudal-to-rostral gradient in the production of Purkinje cells in the vermis was previously observed in the mouse by Andreoli et al. ('73) and a lateral-to-medial gradient (from hemisphere to vermis) by Inouye and Murakami ('80). This simple pattern is relatable to our hypothesis (to be discussed further below) that these three subdivisions of the cerebellum derive from three distinguishable cerebellar primordia (Altman and Bayer, '85) characterized by sequential spurts of proliferative activity: the hemisphere from the lateral primordium,

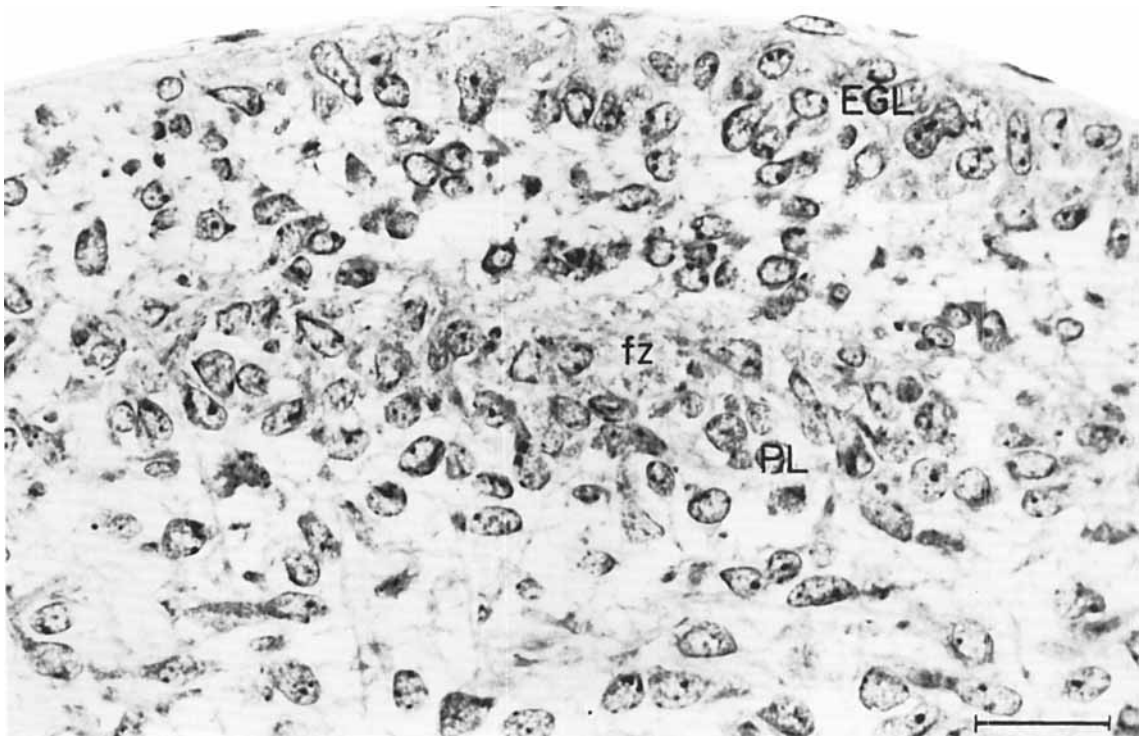


Fig. 12. The "fuzzy" matrix overlying the formative Purkinje cell layer in a day E18 rat. Methacrylate, sagittal section; scale: 25 μm.

the posterior vermis from the postisthmal primordium, and the anterior vermis from the subisthmal primordium. However, there are exceptions to this generalization about the labeling pattern of the three cerebellar lobes. First, the posterior vermis is bisected by a narrow longitudinal strip of early-produced Purkinje cells, what we called the vermal wedge. This band could be traced some distance into the anterior vermis. Second, several hemispheric lobules contained a fair proportion of late-produced Purkinje cells: specifically, the flocculus, the paraflocculus, and the paramedian lobule. Of these, the flocculus contained labeled cells most consistently and in the highest numbers (Fig. 3), exceeding perhaps all the lobules of the posterior vermis except the nodulus (lobulus X, Fig. 1) with which it is often associated as the flocculonodular lobe (Larsell, '52). The paraflocculus was more variable (compare Figs. 2B and 3B or Fig. 4B and C), reflecting, possibly, differences between its ventral and dorsal lobules.

Empirical evidence is not presently available that the pattern of distribution of Purkinje cells persists unchanged into adulthoood. If that were the case, the pattern seen in day E22 rats would appear in the adult cerebellar cortex as shown in Figure 20. The region containing the highest proportion of early-produced Purkinje cells consists of the anterolaterally situated lobulus simplex, crus I, and crus II. Next in line with more late-produced Purkinje cells is the paramedian lobule and the paraflocculus. The flocculus, posterolaterally with still more late-produced Purkinje cells, is comparable to the pattern seen in the lobules of the posterior vermis. Finally the anterior vermis anteromedially is composed predominantly of late-produced Purkinje cells. Thus, there is a rostral-to-caudal cytogenetic gradient in the hemisphere and a reverse caudal-to-rostral gradient in the vermis. In addition, if we may disregard the flocculus, there is also a gradient from lateral to medial. But overriding this pattern is the vermal wedge which bisects the vermis with its longitudinal band of early-produced Purkinje cells. This developmental pattern partially supports Korneliussen's ('68) idea of cerebellar development in terms of longitudinal zones, since the vermis can be looked upon as consisting of three longitudinal zones. However, the developmental organization of the hemisphere is easier to describe as a transverse one consisting of three cytogenetically distinguishable parts, the hemisphere proper (lobulus simplex, crus I, and crus II), the paramedian lobule, and the paraflocculus, and the flocculus (Fig. 20).

We have entertained the possibility that the onset of fissurization of the cerebellar cortex, an event which begins with the primary fissure on day E21 (Fig. 14B), might be associated with the differences in the time of origin of Purkinje cells in the posterior and anterior vermis. Medially (Fig. 1A), the primary fissure is forming at the interface of the two zones of Purkinje cells, those generated before and those after the morning of day E15. Insofar as there is some evidence that the cells of lobule VI of the posterior vermis derive from the postisthmal cerebellar primordium, whereas the cells of lobule V of the anterior vermis (those characterized by a pronounced spindle shape and late arrival; Figs. 14–16) originate in the subisthmal cerebellar primordium (see below), mechanical factors might be at work at the meeting point of the two zones to favor fissurization. However, in the series of illustrated radiograms the interface of labeled and unlabeled Purkinje cells did not coincide with the primary fissure some distance from the midline (Fig. 1B). This observation permits two interpretations. First, since we have noted that the

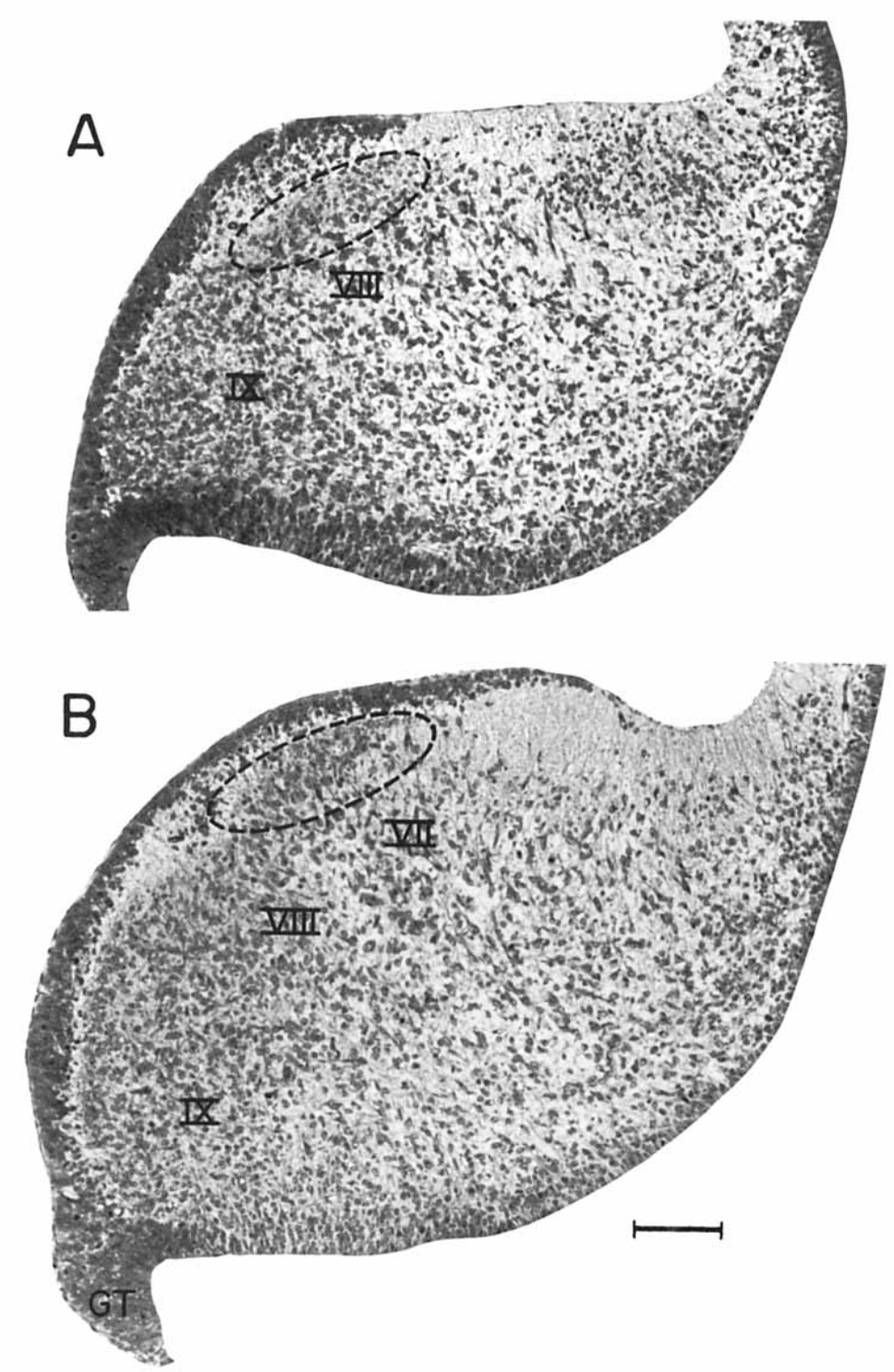


Fig. 13. Sagittal cerebellar sections from days E18 (A) and E19 (B) rats, showing the shifting of the conspicuous, newly formed Purkinje cell layer (dashed circles) from posteroventral to anterodorsal. Methacrylate; scale: 100 $\mu$m.

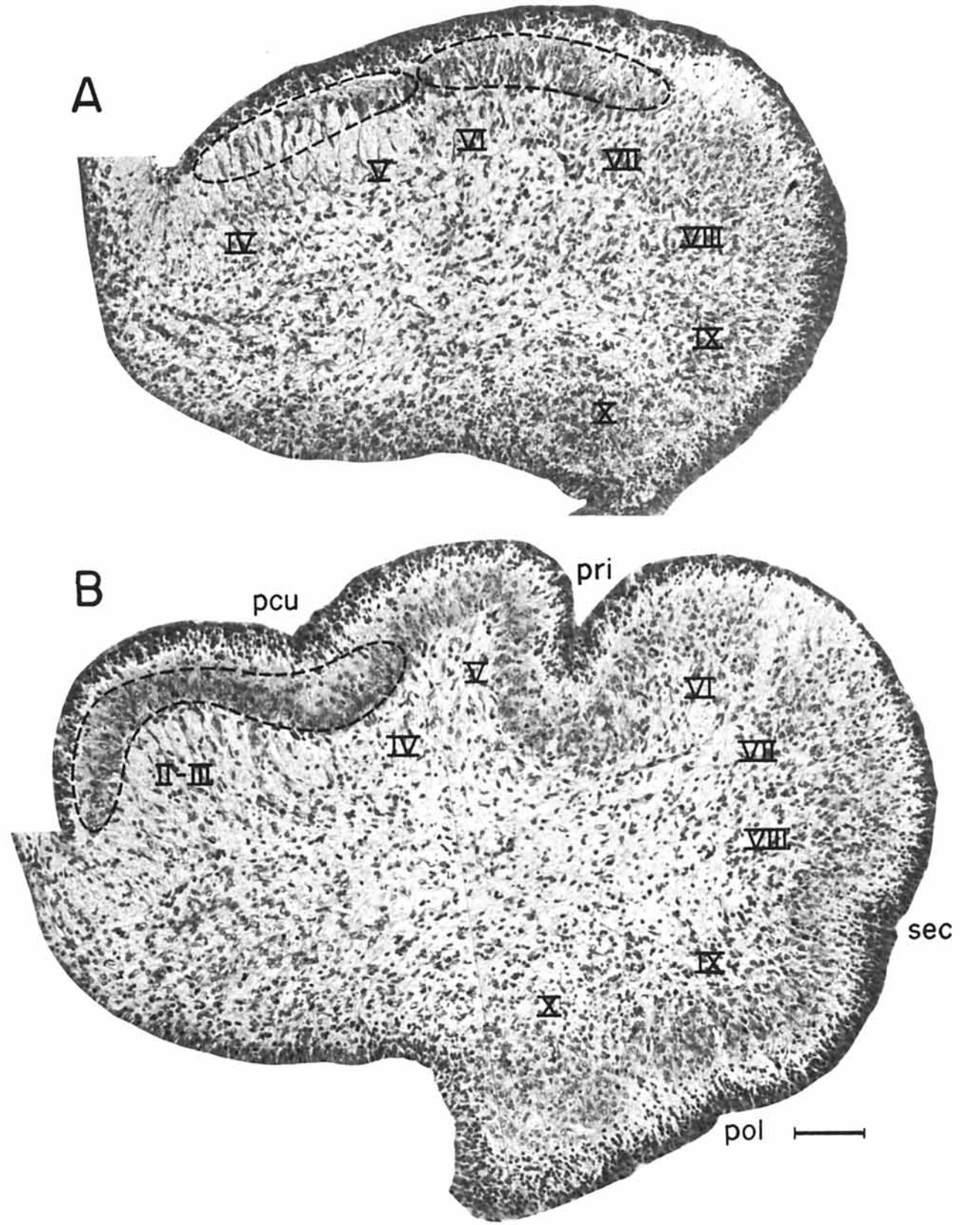


Fig. 14. Sagittal cerebellar sections from day E20 (A) and E21 (B) rats. New additions on day E20 are the conspicuous Purkinje cell layers of declive (lobule VI) and the just-forming dorsal culmen (lobule V). It is on this day that lobule X (nodulus) is first recognizable. Lobulus centralis (lobule III and II) is added on day E21. Methacrylate; scale: 100 $\mu$m.

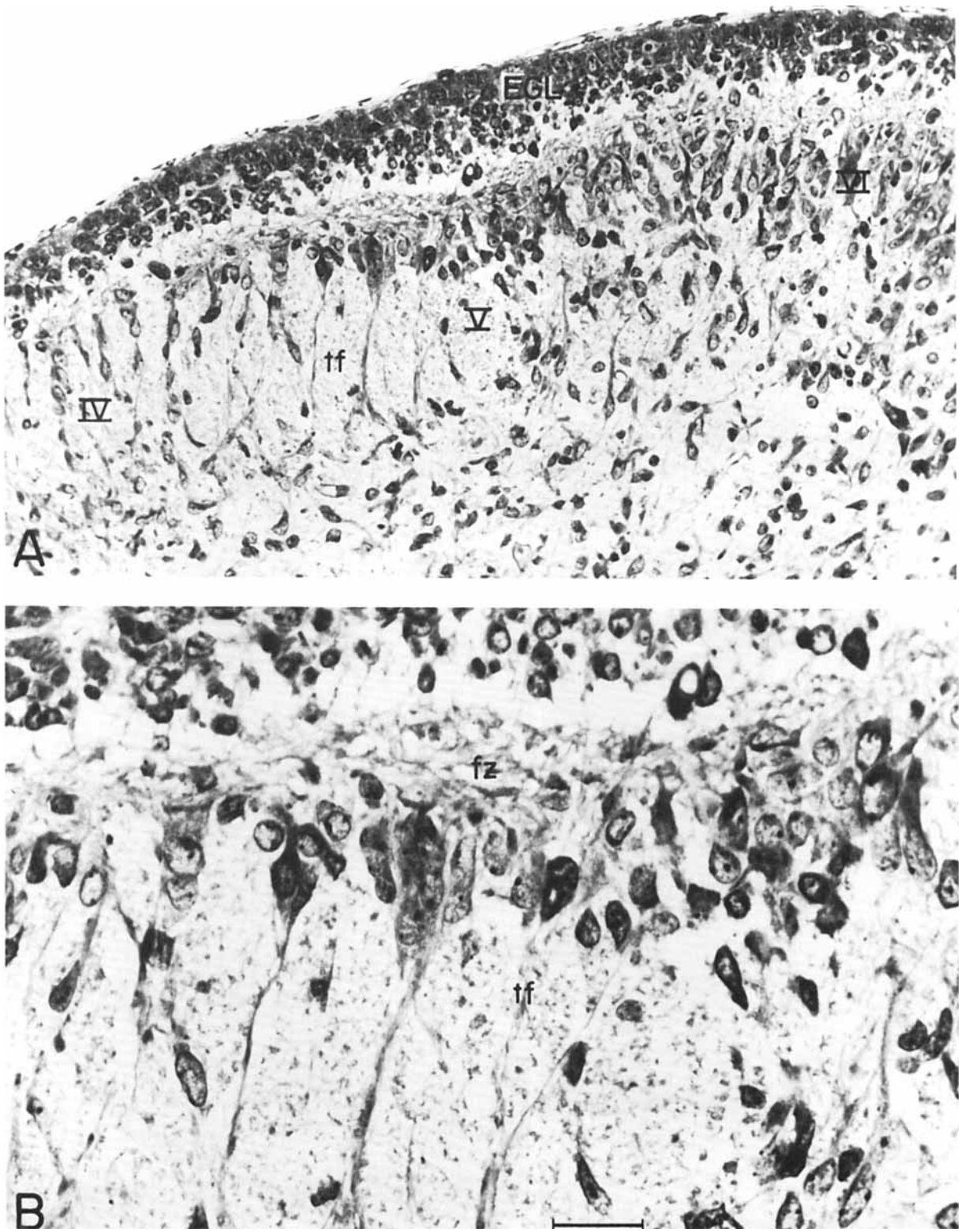


Fig. 15. A. The apical "fuzz" of lobules V and IV, and radially oriented Purkinje cells and trailing fibers, in a sagittal section of a day E20 rat. B. At higher magnification the trailing fibers, and a cell with leading fiber (lower right) is seen to be embedded in a matrix of transversely cut fibers. Methacrylate; scales: A, 50 $\mu$m; B, 20 $\mu$m.


Fig. 16. A. The migrating (between arrows) and settled Purkinje cells of lobule V in a coronal section from a day E20 rat. The migrating cells are embedded in transversely oriented fiber bundle. B. The settled cells (right) are associated with an apical fuzz. Arrows follow spindle-shaped, migrating cells. Methacrylate; scales: A, 50 $\mu$m; B, 20 $\mu$m.

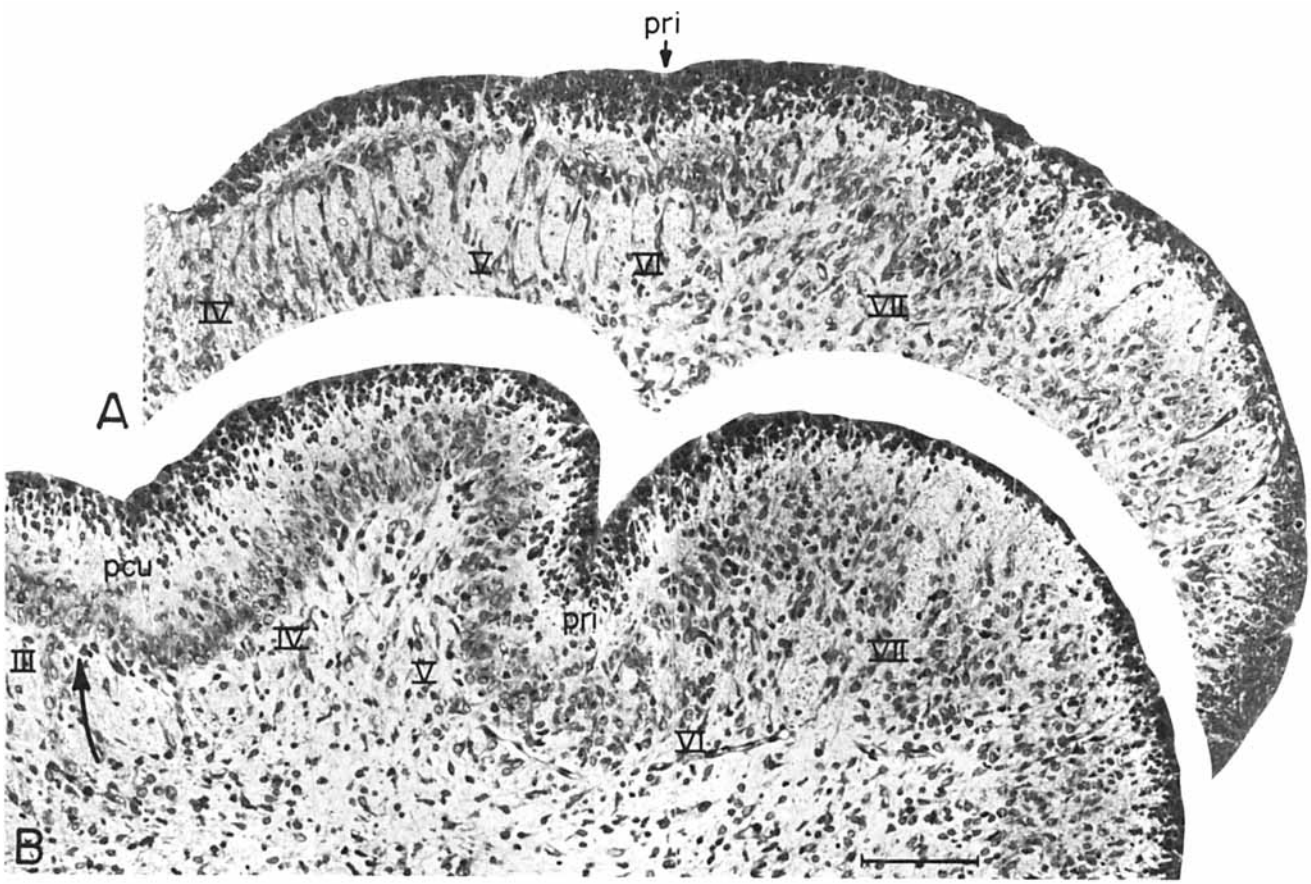


Fig. 17. The spindle-shaped Purkinje cells of the dorsal and ventral culmen (lobules V and IV) of the day E20 rat (A) are transformed into roundish cells by day E21 (B). Less conspicuous spindle-shaped cells (arrow in B) are settling in the day E21 rat rostral to the preculminate fissure. Methacrylate; scale: 100 $\mu$m.

first-arriving lobule V Purkinje cells are situated on either side of the fusing midline (Figs. 8C, D, 16A), it is conceivable that it is at these sites medially where the fissura prima will form but that beyond these two points laterally the fissura prima does not rigidly follow the interface of the two zones. (The onset of the formation of the fissura prima medially is suggested by comparing Figs. 1A and 2A.) Alternatively, the fissura prima does form rigidly along the interface of the two cytogenetic zones but our labeling procedure does not allow us to distinguish the two zones by a dichotomous pattern along the entire course of the fissura prima. Possibly the generation times of the Purkinje cells of the two components differ only by a few hours and it is fortuitous to find an animal with no labeled Purkinje cells on one side of the meeting point and many on the other side throughout the entire fissura prima from medial to lateral. This interpretation is supported by the observation that in some rats labeled on day E15 not only the Purkinje cells of the anterior vermis but also those of the posterior vermis were labeled (Fig. 6D). We made the assumption that the animals showing this pattern were developmentally less mature, or "young," at the time of injection.

## Regional differences in the migratory pattern of Purkinje cells

The long-held view that the Purkinje cells take a radial course from the ventricular neuroepithelium to the surface of the cerebellar cortex is supported by the present observations with two qualifications. The first is a minor one, namely, that the Purkinje cells seem to take a detour where the deep nuclei are present (Fig. 7C, D). The second is the observation that the Purkinje cells of the anterior vermis, which originate caudally in the subisthmal cerebellar primordium (Fig. 9) before the cerebellum has fused anteriorly to provide a settling site (Fig. 8), take a relatively long anterodorsal rather than a strictly dorsal route (Fig. 21).

There is considerable regional variability in the time taken by Purkinje cells to settle and form a Purkinje cell layer. The Purkinje cells of lobule IX in the posterior vermis settle as early as day E17 (Fig. 11). Assuming that the majority of these cells are generated late on day E14 (Fig. 1), this represents a transit time of about 3 days. At the other extreme, the Purkinje cells of lobulus centralis (lobules II and III) in the anterior vermis settle on day E21

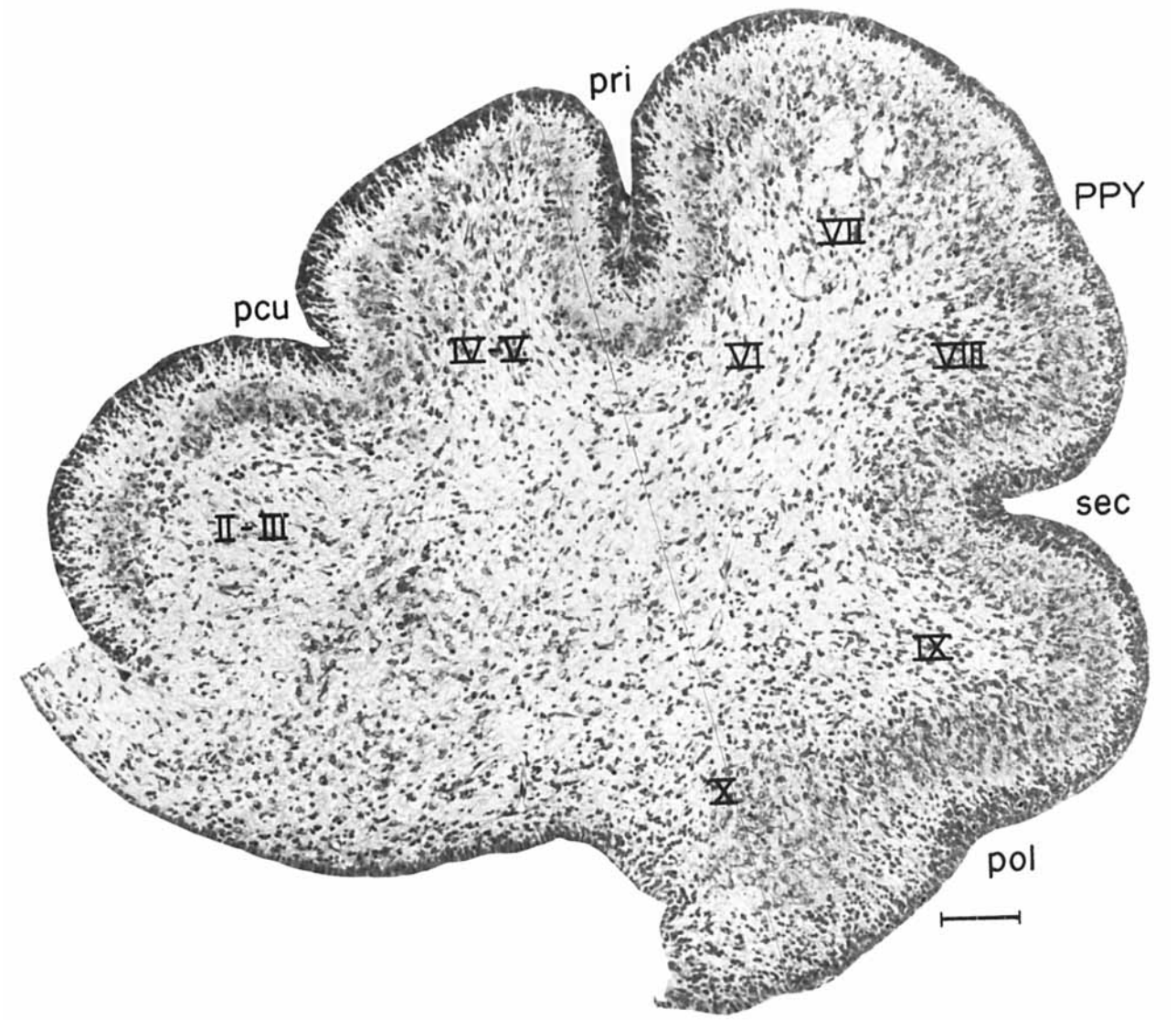


Fig. 18. The inconspicuous appearance of the Purkinje cells throughout the vermis of a day E22 rat. Methacrylate; scale: 100 μm.

(Figs. 14B, 17B). Assuming that the majority of these cells are generated late on day E15 (they are no longer labeled with injections on day E16), this represents a transit time of about 6 days. This difference is not likely to be due entirely to the lengthened migratory path. It is more likely that either the Purkinje cells destined for the anterior vermis move very slowly or stop in their path while the fusion of the cerebellar halves is in progress (Fig. 8).

Although both the cytogenetic gradient and migratory gradient are parallel (caudal to rostral) in the vermis, the two are probably independent and disosciable processes. This is indicated by several observations. First, the earlier-produced Purkinje cells of the hemisphere settle later than the later-produced Purkinje cells of the vermis (compare Fig. 14A, B with Fig. 19A, B). Second, whereas the gradient of Purkinje cell production is rostral-to-caudal in the hemisphere (Fig. 20) the formation of the Purkinje cell layer is caudal-to-rostral throughout the cerebellum (Figs. 6, 7, 19). Finally, while there are no indications for a cytogenetic gradient *within* either the lobules of the posterior vermis or the lobules of the anterior vermis, the settling of Purkinje cells takes place in a strictly caudal-to-rostral sequence over a protracted period. Lobule IX forms on days E17 (Fig. 11), lobule VIII by day E18 (Fig. 13A) and lobule VII by day E19 (Fig. 13B). By day E20 lobules V and IV of the anterior vermis have formed (Fig. 14A) and by day E21 the Purkinje cells of lobules III and II are settling (Fig. 14B). This caudal-to-rostral settling gradient is evidently not directly related to the gradient of Purkinje cell production but rather to the spread of the EGL over the cerebellar surface in a caudorostral direction (Figs. 6, 7).

In many instances the leading front of the dispersing EGL was ahead of the Purkinje cells arriving at the surface of the cerebellum (Figs. 6C, 7C, 13B), but this was not a consistent observation (Figs. 7D, 11). Therefore, instead of an inductive influence exerted by the EGL on the migration of Purkinje cells it is more likely that two events are independently synchronized. The EGL originates in the ger-

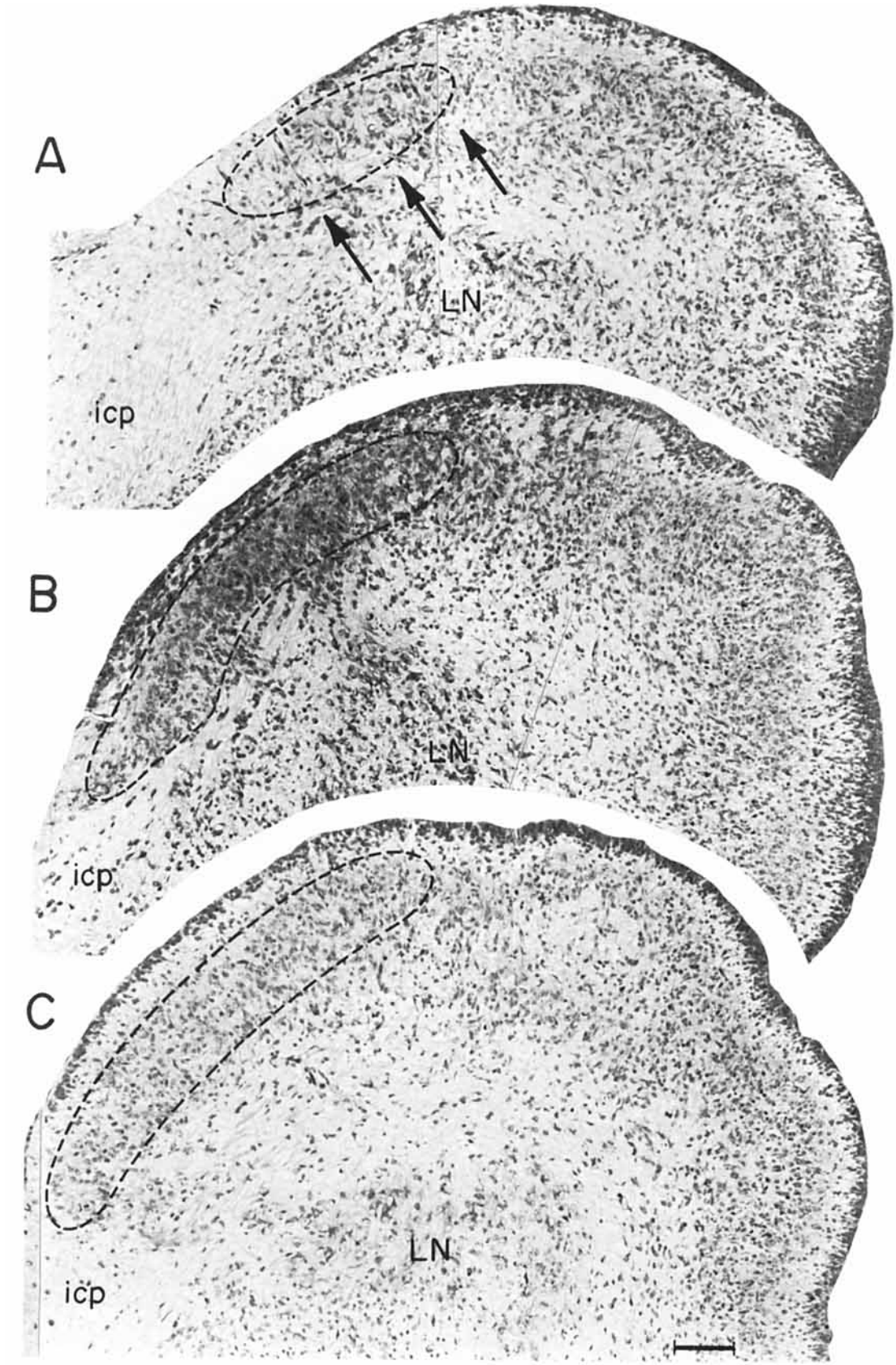


Fig. 19. The caudal-to-rostral sequence of the settling of Purkinje cells in the cerebellar hemispheres of days E20 (A), E21 (B), and E22 (C) rats. Arrows in A point to the just-arriving Purkinje cells. Dashed circles in B and C surround the recently-settled Purkinje cells. The Purkinje cell layer in this region is most conspicuous on day E21. Methacrylate; scale: 100 $\mu$m.

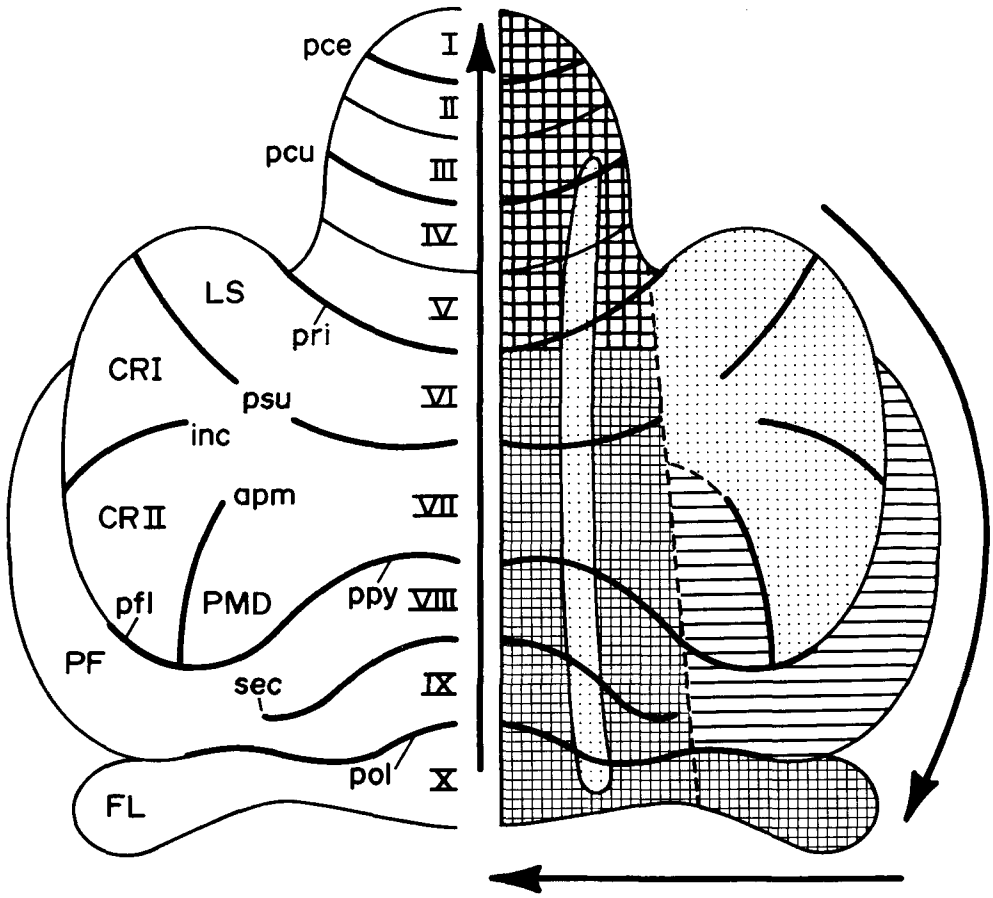


Fig. 20. The extrapolated neurogenetic organization of the adult rat cerebellum. The left half is a planar representation of the cerebellum, with the designation of the major lobules and fissures based mainly on Larsell's ('52) classification. On the right side the sequential order of Purkinje cell production is shown in four patterns: dots (earliest), horizontal lines, light checkered, and heavy checkered (last). With the exception of the vermal wedge, the gradient is from rostral-to-caudal in the hemisphere, and from caudal-to-rostral in the vermis.

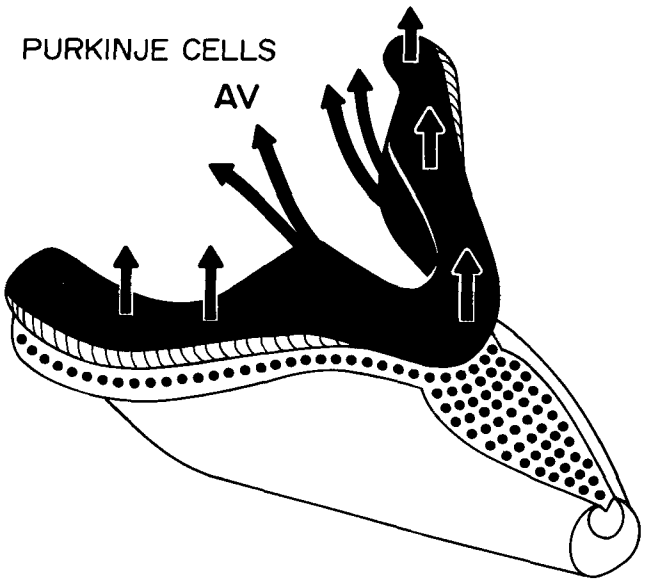


Fig. 21. Two patterns of Purkinje cell migration: radial and short in the hemispheres and posterior vermis, and anterodorsal and long in the anterior vermis. The Purkinje cells derived from the subisthmal primordium (see Altman and Bayer, '84a: Fig. 24) have to await the fusion of the base of the cerebellum rostrally.

minal trigone of the vermis caudally and in the lateral cerebellum laterally (Altman and Bayer, '78). From this crescent-shaped source the EGL spreads anterodorsally and lateromedially, as previously illustrated (Altman and Bayer, '85: Fig. 27). Our present observations clarify how these two components fuse to form a continuous covering over the perinatally and postnatally developing cerebellar cortex. In the fusing halves of the lateral cerebellum the EGL is restricted to the vicinity of its source ventrolaterally (Fig. 8A, B). The EGL of the midline vermis reaches the fusion point apparently spreading independently caudorostrally (Fig. 8C, D); then it spreads laterally to meet the more slowly dispersing lateral cerebellar EGL (Fig. 8E). The process is schematically illustrated in Figure 22. The cytological composition of the cerebellar tissue that fuses prior to the spread of the EGL and the Purkinje cells (Fig. 8) is not known.

Examination of plastic sections revealed that the just-settling or settled Purkinje cells have a characteristic cytological appearance and can be distinguished from the Purkinje cells that have been settled for a few days. The perikarya of these cells is surrounded by a "fuzz" (Figs. 12, 15) of an as yet undetermined ultrastructural organization. The conspicuous appearance of the recently-settled Purkinje cells may be due to the metabolic activity associated with migration and settling. Interestingly there were regional differences in the appearance of these just-settled

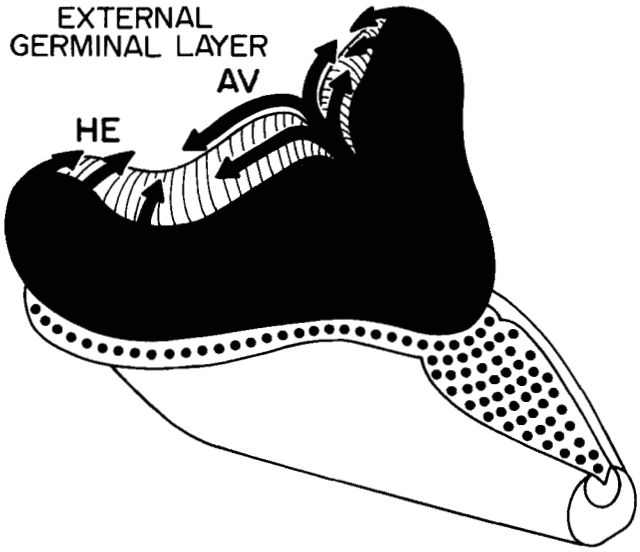


Fig. 22. Three directions in the dispersal of the EGL over the surface of the cerebellum: lateromedial in the hemispheres, caudorostral in the posterior vermis (see Altman and Bayer, '84a: Fig. 27), and mediolateral in the anterior vermis.

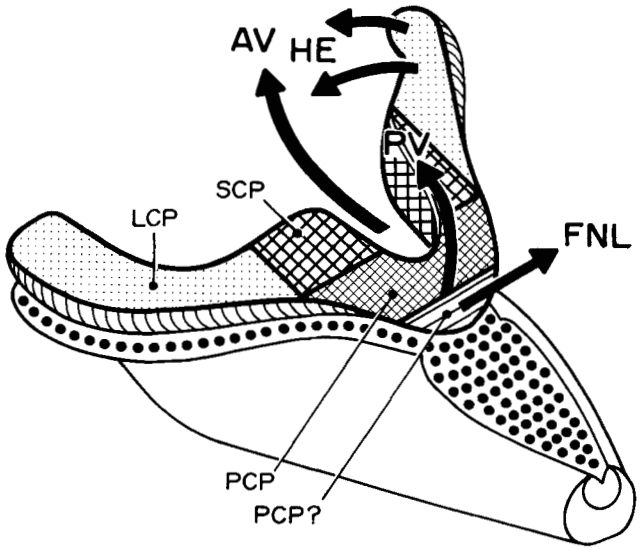


Fig. 23. The hypothesized differential fate of the three cerebellar primordia: the lateral primordium (dotted area) giving rise to the hemisphere, the postisthmal primordium (lightly checkered area) to the posterior vermis, and the subisthmal primordium (heavily checkered area) to the anterior vermis. The possible separate derivation of the flocculonodular lobe is conjectural.

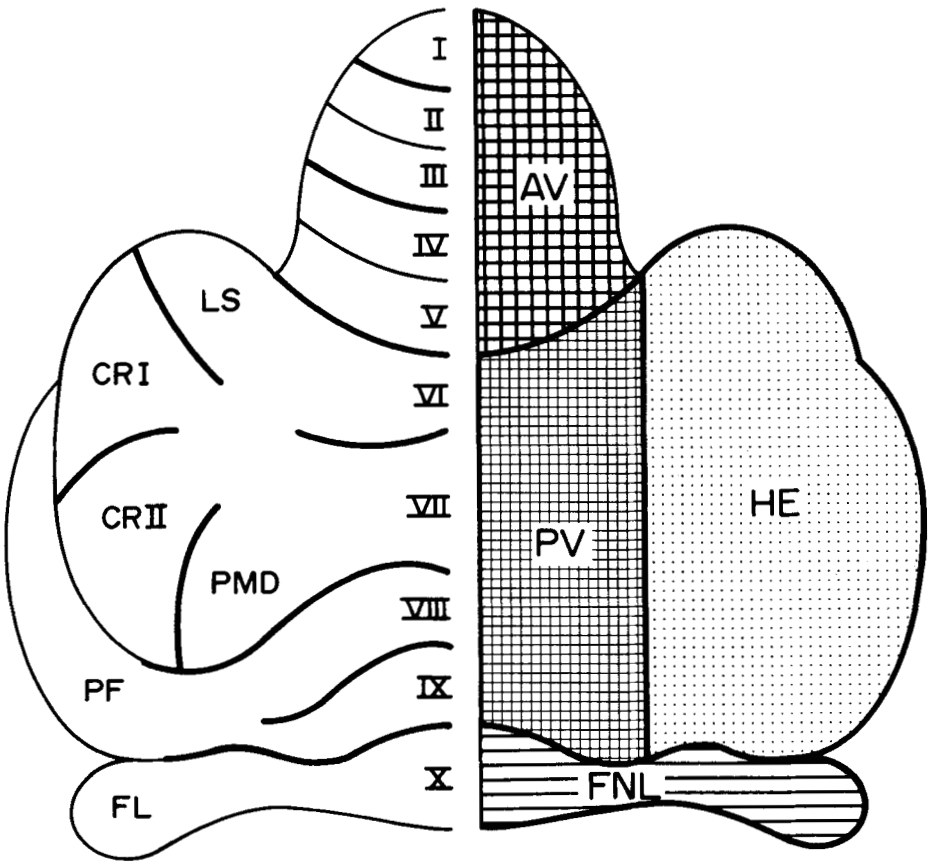


Fig. 24. The extrapolated derivation of the three major components of the adult rat cerebellem from the three cerebellar primordia: the hemisphere from the lateral primordium (dotted area), the posterior vermis from the postisthmal primordium (lightly checkered area), and the anterior vermis from the subisthmal primordium. The separate derivation of the flocculonodular lobe is conjectural.

cells with the conspicuousness of the cells increasing from caudal to rostral (Figs. 11, 13–17). Particularly outstanding were the Purkinje cells of lobules IV and V with their large size, spindle shape, and trailing processes. Perhaps the size of the settling cells reflects the distance that they had to traverse to reach the cerebellar anlage.

In an earlier study (Altman, '72) we described several stages in the postnatal maturation of the Purkinje cells of the rat cerebellum, most of which were relatable to their dendritic development. Observations in the developing mouse (Hendelman and Aggerwal, '80), opossum (Laxson and King, '83), and the newborn rat (Sotelo et al., '84) have revealed an earlier immature stage, characterized by long apical processes. Laxson and King hypothesized that the apical processes may be involved in the migration of Purkinje cells. The recently-settled conspicuous cells observed in this study may be identical to the immature Purkinje cells described in the mouse and the opossum. It is not likely that the "fuzz" observed is related to dendritic development. In the rat the first phase of dendritic development is the formation of an apical cap and of perisomatic processes; in the posterior vermis this occurred on day P5 (Altman, '72: Fig. 3). On the preceding postnatal days the Purkinje cells were relatively small with little cytoplasm. Conceivably this early postnatal stage is identical to the postmigratory "dormant" stage seen throughout the vermis in day E22 rats (Fig. 18).

### Site of origin of components of the cerebellar cortex

In the first paper of this series (Altman and Bayer, '85) we proposed a subdivision of the cerebellar primordium into three components: the lateral, subisthmal, and postisthmal primordia. The lateral cerebellar primordium caps the lateral recess of the fourth ventricle; it is contiguous with the pons medially and is separated ventrally from the anlage of the cochlear nuclei by the lateral branch of the tela choroidea. The subisthmal cerebellar primordium is situated beneath the isthmus, lining medially the isthmus canal. Laterally and posteriorly it is continuous with the lateral and postisthmal primordia. The postisthmal cerebellar primordium caps the postisthmal recess of the fourth ventricle and extends caudally to the medullary fourth ventricle proper covered by the medial branch of the tela choroidea (Altman and Bayer, '85: Figs. 12, 13, 24). The observations made in the present study support the hypothesis (Altman and Bayer, '85) that these three embryonic subdivisions of the cerebellum are sources of the Purkinje cells of the hemispheres, the anterior vermis and the posterior vermis, respectively (Fig. 23). The relatively early-produced Purkinje cells of the hemispheres could be traced from the lateral primordium to translocate with the spreading medial protuberance of the embryonic cerebellum in a medial direction (Fig. 8; compare with Altman and Bayer, '85: Fig. 14). The medial, subisthmal origin of the late-produced and late-settling Purkinje cells of the anterior vermis was supported by both radiographic (Fig. 9) and cytological (Figs. 14–17) observations. The same applies for the likely origin of the relatively early-produced and early-settling Purkinje cells of the posterior vermis from the nearby postisthmal neuroepithelium (Figs. 6, 11). But this simple tripartite scheme leaves unanswered the source of the Purkinje cells of the vermal wedge which, in terms of time of origin, resemble the Purkinje cells of the cerebellar hemispheres. Also, the site of origin and migratory route of the Purkinje cells of the flocculonodular lobe appear ambiguous. In terms of cytogenesis the flocculus resembles the posterior vermis more than the hemisphere, whereas the Purkinje cells of the nodulus, in terms of their late settling (Figs. 11, 13, 14), are unlike the other lobules of the posterior vermis. We shall tentatively assume that the flocculonodular lobe represents a fourth component of the developing cerebellum (Fig. 24).

## ACKNOWLEDGMENTS

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