QUANTITATIVE ANALYSIS OF CELL PROLIFERATION AND DIFFERENTIATION IN THE CORTEX OF THE POSTNATAL MOUSE CEREBELLUM

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ABSTRACT

The generation cycle of germinative cells (*external matrix cells*) in the external granular layer of the cerebellar cortex of the 10- to 11-day-old mouse was studied by radioautography following repeated injections of H^3-thymidine. The generation time is 19 hr, presynthetic time 8.5 hr, DNA-synthetic time 8 hr, postsynthetic time 2 hr, and mitotic time 0.5 hr. These proliferating cells occupy the outer half of the external granular layer and make up the external matrix layer. Neuroblasts are differentiated from the external matrix cell, migrate out from the layer and accumulate in the inner half of the external granular layer to form the *external mantle layer*. The transit time of the neuroblasts in the external mantle layer is 28 hr. Thereafter, they migrate farther into the molecular layer and the internal granular layer. By means of long-term cumulative labeling, the rate of daily production of neuroblasts from the external matrix cell is studied in quantitative terms. It becomes clear that the entire population of the inner granule neurons arises postnatally in the external granular layer between 1 and 18 days of age and that 95% of them is produced between postnatal days 4 and 15. Finally, the fate of the cells in the external granular layer at its terminal stage was studied by marking the cells with H^3-thymidine during 15–16 days of life and following their subsequent migration and developmental changes up to 21 days of life. Comparison of radioautographs taken before and after the migration disclosed that the external matrix cells give rise to a small number of neuroglia cells. This finding revealed their multipotential nature.

INTRODUCTION

The external granular layer is a temporary structure. It contributes a substantial number of cells to the cerebellar cortex, and finally disappears when the cerebellum reaches its maturity (11). A number of attempts have been made to clarify the mode of cell proliferation and differentiation in this layer and the eventual role of the cells derived from it. However, as far as one depends upon the classical histological methods, this kind of study involves serious difficulties. To study dynamic behavior of cells using stationary pictures, one must first focus attention on a certain cell or a group of cells and follow its changes, in time and space, in a series of specimens fixed at certain intervals of time. During developmental stages, however, most cells in the cerebellum lack morphological differentiation and look alike. As a result, it is extremely difficult to establish cell identification in various stages of cerebellar development, so that conclusions drawn from such a study are necessarily highly inferential. In fact, previous investigators differ in their interpretations. Although

many authors seem to agree that the external granular layer is a major source of cells in the internal granular layer, all possible varieties of interpretations concerning contribution of the external granular cells to the eventual cyto-architectonics of the cerebellar cortex have been reported (cf. 14, for a brief review).

To resolve the controversies over the role of the external granular cells, detailed study of dynamic aspects of cell proliferation, differentiation, and migration in the developing cerebellum seems to be necessary. Recently, it was found (2, 3, 6, 7) that the cumulative labeling technique with H^3-thymidine is suitable for quantitative analysis of developmental processes of the central nervous system at the cellular level. The present study is an application of the cumulative labeling technique to the *quantitative* analysis of cell proliferation and migration of the external granular cells in the postnatal mouse cerebellum. Information obtained from the present investigation is not only helpful for understanding the dynamic aspects of cell behavior in the external granular layer, but will also be useful in establishing unambiguous identification of cells of the developing cerebellum in an electron micrograph with the aid of radioautography.

MATERIALS AND METHODS

Animals used were Swiss Albino mice kept inbred at the Purdue University Animal House.

For kinetic analysis of cell proliferation in the external granular layer, five mice of a litter of 10 days of age were repeatedly injected with 2 μc/g body weight of H^3-thymidine at intervals of 2–3 hr. Animals were sacrificed 1, 2, 3, 5, and 10 hr after the first injection. Another six mice of the same litter received repeated injections of H^3-thymidine at intervals of 5–6 hr up to the time of sacrifice and were killed at 14, 20, 28, 31, 34, and 42 hr.

For quantitative analysis of inner granule cell production during postnatal days, a litter consisting of 10 mice received repeated injections of H^3-thymidine. The multiple injections started at one of each of the following days: 0, 1, 2, 3, 4, 5, 7, 10, 12, and 15 days of life. The first injection of H^3-thymidine was in the amount of 10 μc/g body weight and the following ones were in the amount of 1 μc/g body weight. The intervals between the injections were 5–6 hr for the first 5 days, and 12 hr for the following days. All the animals were sacrificed at 20 days of age.

For studies on the fate of cells which were present in the external granular layer shortly before and at the time of its disappearance, two littermates of 15 days of age were injected with H^3-thymidine four times at intervals of 5–6 hr; one was sacrificed at 1 day and the other 6 days after the first injection.

Brains were removed and fixed in 2% glutaraldehyde solution buffered with cacodylate. After small pieces were removed for electron microscopic examination, the rest of the brain was embedded in paraffin and cut into serial sections 2–5 μ in thickness. Some sections were stained by Bodian's protargol method prior to radioautographic procedure. To improve contrast of radioautographic silver grains against the background of silver impregnation, gold chloride treatment is omitted from the original Bodian's procedure and sections were left light brown in color. After intensive washing in running water, the slides were coated by dipping into Kodak NTB 3 nuclear emulsion, exposed at 4°C for 1–2 months, and developed in Dektol at 18°C for 5 min. Sections not impregnated with silver were poststained with Meyer's hematoxylin or toluidine blue.

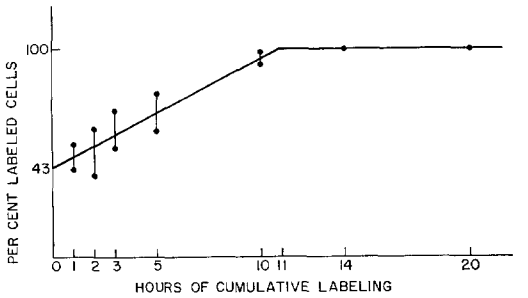

FIGURE 1 Percentage of labeled cells in the external granular layer of cerebellum of 10-day-old mice, plotted against hours of cumulative labeling.

Percentages of labeled cells were measured by counting 200 to 1,000 cells using the oil immersion system. However, in the series of 15-day-old mice in which labeled cells were sparse (0.05–2.0%) the total cells in the internal granular layer of two whole lobuli (Culmen and Central) were counted using photomicrographs. The total cells counted ranged from 10,162 to 13,297. Labeled cells were scanned, using the oil immersion system, all over the same lobuli in the external granular, molecular, and internal granular layers, respectively. The numbers of labeled cells in each layer were expressed by relative numbers per 10,000 inner granule cells of the corresponding lobuli.

RESULTS

Cell Proliferation Kinetics of 10-Day-Old Mice

The external granular layer of 10-day-old mice is composed of four to eight rows of densely packed granule cells (see Fig. 2). In the outermost two to

three rows, mitotic figures are frequent; the Mitotic Index was 2.6%.

In flash-label radioautographs, labeled cells were restricted to these outermost two to three rows. Within these rows, there was no tendency for the labeled cells to gather in any particular zone, but they were randomly scattered. No labeled cells were found in the inner half of the external granular layer. This labeling pattern indicates—

1. That cells proliferate only within the outermost zone which is called the *external matrix layer* (see Figs. 2 and 4), and
2. That differentiated cells incapable of further DNA synthesis (i.e., neuroblasts) migrate out from the external matrix layer to form the external mantle layer subjacent to it (see Figs. 2 and 4).

At various times of cumulative labeling, percentages of labeled cells were measured (Fig. 1) within the outermost two rows which are supposed to consist of a purely proliferative population. No labeled mitosis yet appeared 1 hr after injection of H^3-thymidine. 2 hr after the first injection, most mitoses became labeled and by 3 hr, no unlabeled mitosis was found. As the cumulative labeling proceeded, the percentage of labeled cells increased

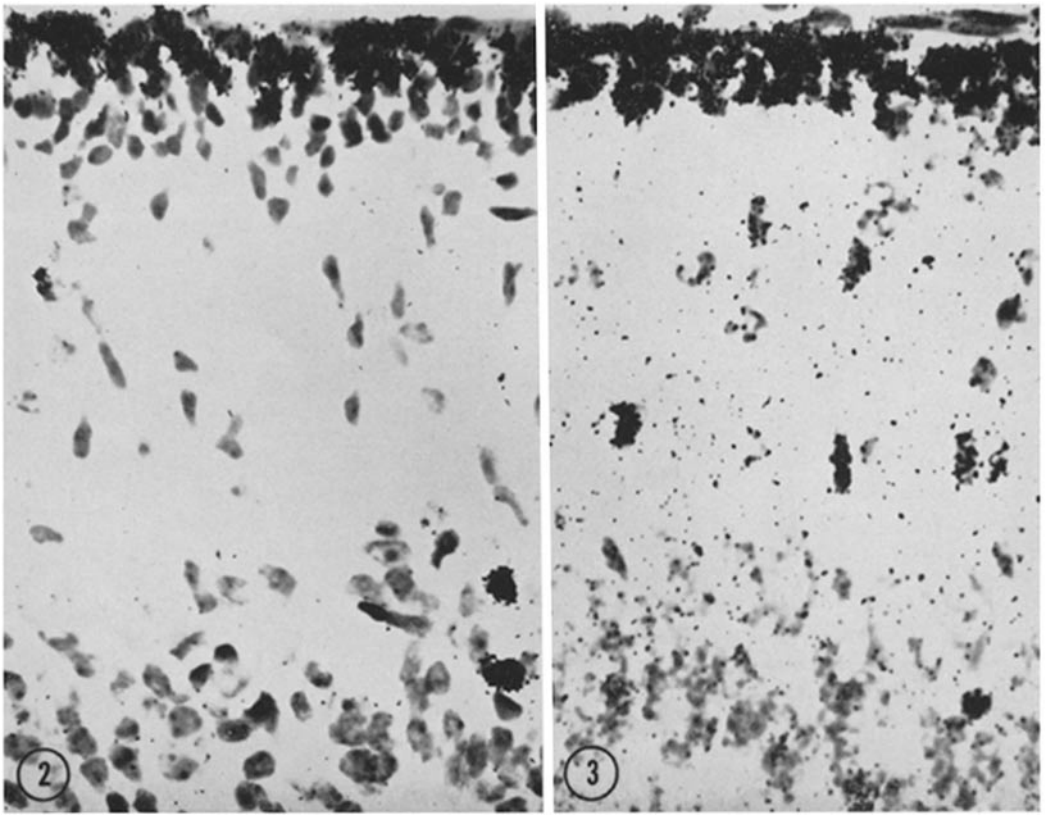

FIGURE 2 Radioautograph of 10-day-old mouse cerebellum, labeled for 10 hr. Labeled cells are prominent in the outer half of the external granular layer, but are rare in the inner half of the external granular layer. Note also rare labeled cells in molecular and internal granular layers. × 400.

FIGURE 3 Radioautograph of 10-day-old mouse cerebellum, from a littermate of the animal shown in Fig. 2, labeled for 31 hr. Thus the animal was 11 days old at the time of sacrifice. The cumulative labeling started at the same time as in the littermate in Fig. 2.

Labeled cells now occupy the entire thickness of the external granular layer, and most elongated nuclei in the molecular layer are heavily labeled. Labeled cells also increase in number in the internal granular layer. Note that most round nuclei in the molecular layer yet remain unlabeled. × 400.

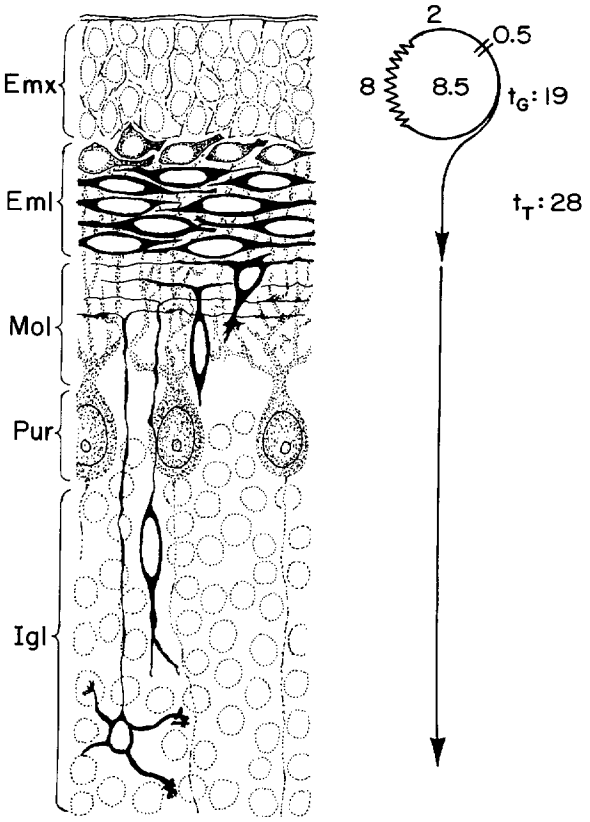

FIGURE 4 Schematic representation of relationship between morphology and kinetics of cells in the developing cerebellum. 10- to 11-day-old mouse. (Morphology of developing cells are drawn after Cajal). *Pmx* represents the external matrix layer; *Eml*, the external mantle layer; *Mol*, the molecular layer; *Pur*, Purkinje cell layer; *Igl*, the internal granular layer. Arabic numerals on the right represent hours of duration of corresponding fractions of generation cycle (t_G). t_T means transit time. Arrow indicates the direction of cell migration.

linearly as shown in Fig. 1, but the labeled cells were confined within the outer half of the external granular layer up to 10 hr of the cumulative labeling (Fig. 2).

The linear increase of the percentage of labeled cells as seen in Fig. 1 indicates that the rate of cell influx into the DNA synthetic phase is constant with time, and that all the cells in this population proceed in the generation cycle at the constant rate. Thus, it was concluded that this population is composed entirely of the same kind of proliferative cells, the *external matrix cells*, which are proliferating asynchronously. Their generation time (t_G) and DNA synthetic time (t_S) were estimated as follows. The labeling index or percentage of labeled cells at $t = 0$, i.e. 43%, is regarded as the fraction of cell cycle during which DNA synthesis occurs. Thus,

$$43 = 100 \times t_S/t_G$$

Under the conditions of cumulative labeling, all the cells that enter into the DNA synthetic phase become labeled, and the time at which the last cell enters into DNA synthetic compartment, i.e. the time at which 100% of the cells become labeled, corresponds to the sum of postsynthetic time, mitotic duration, and presynthetic time, namely, $t_2 + t_M + t_1 = t_G - t_S$. The time of 100% labeling, read from Fig. 1, was 11 hr. Thus,

$$11 = t_2 + t_M + t_1 = t_G - t_S$$

From the two equations above, estimates were given for t_G and t_S, 19 hr and 8 hr, respectively. Mitotic duration (t_M) was also estimated in a similar manner using equation

$$\text{Mitotic index} = 100 \times t_M/t_G$$

Thus,

$$t_M = 18 \times 2.6/100 \doteqdot 0.5 \text{ hr.}$$

Postsynthetic time (t_2) was determined as the time at which labeled metaphase first appears after injection of H^3-thymidine. In the present experiment, labeled metaphase first appears 2 hr after injection,

and within another hour all the mitotic cells become labeled. Thus, t_2 was estimated at 2 hr. Presynthetic time (t_1) was computed by subtracting $t_S + t_2 + t_M$ from t_G. Thus,

$$t_1 = t_G - (t_S + t_2 + t_M) = 8.5 \text{ hr.}$$

The result is shown schematically in Fig. 4. The estimation procedure applied above is valid only when the cell population is proliferating homogeneously and asynchronously. These conditions are fulfilled in the external matrix cells as stated above.

Migration of Granule Neurons from the External into the Internal Granular Layer

Up to 10 hr of cumulative labeling, labeled cells were restricted to the outer half of the external granular layer (Fig. 2). But, as the cumulative labeling proceeded beyond 10 hr, the region of the labeled cells spread inward row by row and, accordingly, the territory of the unlabeled cells in the external granular layer was gradually attenuated. By 28 hr, labeled cells eventually occupied the entire thickness of the external granular layer, and the unlabeled cells were left only occasionally in the innermost row adjacent to the molecular layer. In 11-day-old mice, the external granular layer itself was slightly reduced in thickness, now consisting of three to six rows of nuclei. Up to this time, labeled cells were still sparse in the molecular and the internal granular layers.

In the molecular layer of 10- to 11-day-old mice, several types of nuclei are recognizable. They behave differently in respect to their labeling pattern, so that separate description is necessary. They can be classified into four types (11): 1. Smaller round nuclei (presumably neuroglia); 2. Round nuclei of medium or larger size (presumably stellate neurons); 3. Spindle-shaped nuclei whose long axes are oriented perpendicular to the surface of the cerebellum (presumably migrating granule cells); and 4. Nuclei of blood vessels and blood cells. Although nuclei of vascular elements sometimes appear very similar in morphology to those of the third category, they can usually be discriminated from the latter by their larger size, by peculiar parallel and tandem arrangement, or by their characteristic spatial relationship to the vascular channel. Up to 28 hr of the cumulative labeling, labeled cells in the molecular layer were almost exclusively cells of either the first or the fourth category. At 28 hr, few cells of the third category were radioactive. In contrast, radioautographs taken at 31 hr of the cumulative labeling showed (Fig. 3) that the majority of elongated nuclei of the third category became heavily labeled, whereas numbers of labeled nuclei belonging to the other three categories still remained at very low levels as before. The same radioautographs also revealed a marked increase of labeled cells in the internal granular layer. These findings clearly indicate that neuroblasts having traversed the external mantle layer migrate into the molecular and the internal granular layer, 28–31 hr after they had carried out final DNA synthesis. Thus, the transit time of the neuroblasts in the external mantle layer is estimated at 28 hr (Fig. 4). These findings also render strong support to Cajal's observations (11) that the round or polygonal cells in the external granular layer assume an elongated shape when they migrate out and enter into the molecular layer (Fig. 4).

Quantitative Analysis of Inner Granule Cell Production during Postnatal Development

As is clear from the observations described above, neuroblasts that eventually migrate into the internal granular layer are produced in the external granular layer. When they are differentiated from the external matrix cells in the external granular layer, they cease DNA synthesis, escape from the external matrix layer, and accumulate immediately subjacent to it, forming the external mantle layer (see Figs. 2 and 4). This pattern of cytodifferentiation bears striking similarity to that of neuroblast differentiation as found in the ventricular matrix system (3). Therefore, neuron production in the system of the external granular layer was analyzed by the same technique of the cumulative labeling as has been applied for the ventricular matrix system. The principles underlying this analysis (3, 4) are as follows:

If one labels an animal with H^3-thymidine for more than one generation time of the matrix cell, the DNA of all the matrix cells becomes uniformly and heavily labeled. But, the neuroblasts which have been produced prior to the labeling remain permanently *unlabeled*, since they do not carry out further DNA synthesis. On the other hand, the neuroblasts that are produced later than the commencement of the cumulative labeling are labeled as homogeneously as the matrix cells, since they inherit labeled DNA from these progenitors. They

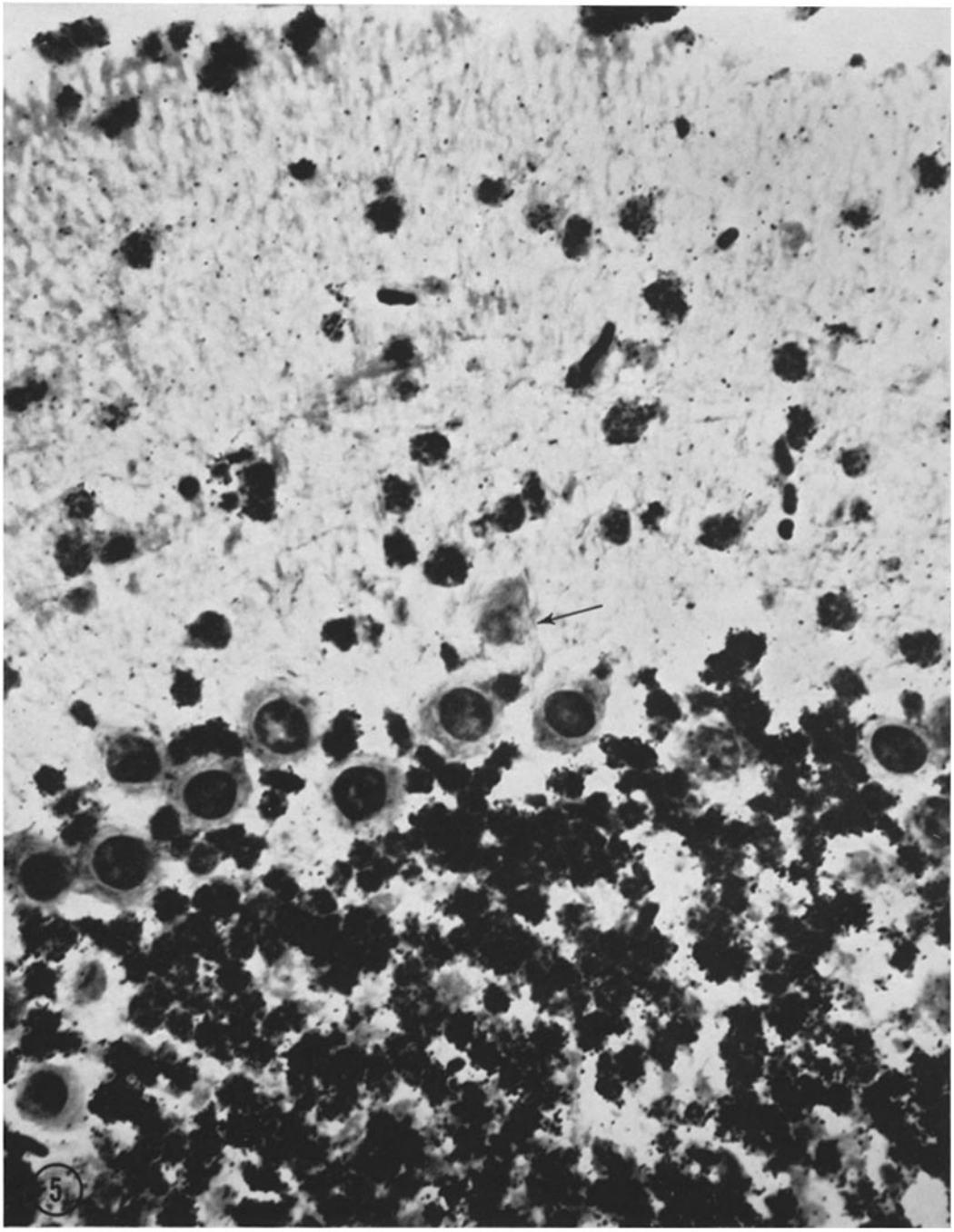

FIGURE 5 Radioautograph of the cerebellar cortex of 20-day-old mouse. Cumulative labeling started at 3 days of life. Section is 2 μ in thickness and stained by Bodian's method. Purkinje cells and presumable basket cell whose perikaryon is filled with neurofibrils (indicated by arrow) are unlabeled. Note homogeneous and heavy labeling of all the inner granule cells (lower half of picture). In the molecular layer, one round nucleus of a presumable stellate cell is found unlabeled (upper right), but the rest of the cells in this layer are totally labeled. Elongated elements in the molecular layer stained completely black and showing sharp contour are erythrocytes in blood vessels. × 700.

keep the label permanently without dilution. If H^3-thymidine is repeatedly injected until the time of the sacrifice of the animals, as it is in the present experiments, the heavy labeling of the matrix cells is maintained throughout the experiments, so that all the neuroblasts produced after the commencement of the cumulative labeling possess an equally dense label. Starting with cumulative labeling on the 1st day after birth and continuing each day thereafter, and examining the distribution of unlabeled and labeled neurons at later stages, one can map out the chronology of postnatal neuron production and, at the same time, one can measure the number of neurons produced at each day of postnatal life.

The cerebellum of a 20-day-old mouse whose cumulative labeling started at 0-day of life, i.e. approximately 2 hr after birth, showed that all the cells in the molecular and the internal granular layers except for Purkinje cells and Golgi II neurons were heavily labeled. This finding indicates that, in the cortex of mouse cerebellum only Purkinje cells and Golgi neurons are produced before birth. They originate from the ventricular matrix cell, but not from the external granular layer (3, 10). They always appeared unlabeled throughout present experiments. Therefore, they are excluded from the following description. When the cumulative labeling started at 1 or 2 days of life, a few unlabeled cells were found in the vermal region, but not yet in the hemispheres. Unlabeled cells appeared in the hemispheres in animals whose cumulative labeling started at age of 3 days or later (Fig. 5). In the cerebellar cortex with cumulative labeling from 3 or 4 days of life onward, there were 3 types of the negative cells. One type of cell possessed a larger round nucleus and was located here and there in the deeper one-third of the molecular layer (see arrow in Fig. 5). Many of them were found near the Purkinje cell layer. Their nuclear morphology and the presence of neurofibrils in the perikaryon which was occasionally revealed in Bodian-stained sections suggested that they are probably basket cells (Fig. 5). The basket cells seem to be restricted within the deeper one-third of the molecular layer (13). Another type of unlabeled cell was the granule cells in the internal granular layer. The percentages of negative inner granule cells counted in radioautographs of 20-day-old mice whose cumulative labeling started at various postnatal days are shown in Fig. 6. Within the same sector of the cerebellar cortex, frequently only presumable bas-

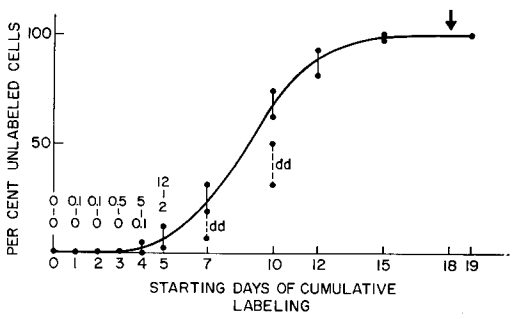

FIGURE 6 Percentage of unlabeled inner granule cells counted at 20 days of life. Abscissa, postnatal days at which the cumulative labeling started. Of the data between day 1 to day 10, the upper values represent counts in the vermal region, and the lower ones, those in the hemisphere. In the data between days 10 and 19, counts in lobulus ansiformis and nodulus are not included. Solid circles connected with dotted line and marked *dd* represent data of *dd* strain mice (8). Arrow at age of 18 days indicates the time of disappearance of the external granular layer in the Purdue mouse.

ket cells were unlabeled, whereas all the inner granule cells were uniformly labeled (Fig. 5). These findings suggested that the external matrix cells begin to produce basket cells already at postnatal day 1 in the vermal region or at day 3 in the hemisphere, usually preceding production of the first inner granule neurons. The third type of negative cell was rarely found in the outer half of the molecular layer. They possessed round nuclei of medium or larger size with lightly stained chromatin. They were tentatively identified as stellate cells of Smirnow type (13). In the molecular layer, the majority of nuclei of medium and larger size finished DNA synthesis before 7 days of life. Therefore, it was concluded that the stellate neurons, as a whole, were produced during the 1st postnatal week. The production of inner granule cells continued until much later. The cerebellum of mice which were exposed to H^3-thymidine later than 10 days of life showed marked regional differences in the pace of inner granule cell production within the hemisphere. In the posterior apical portion (Crus II) of the lobulus ansiformis (8), the majority of the inner granule cells were produced later than postnatal day 10; most inner granule cells in this region were labeled during 10–20 days of life. This tendency of late-differentiation of the apex of the lobulus ansiformis and, to the lesser degree, of the flocculo-nodulus was apparent in all cases in which cumulative labeling started later

than 10 days of life. Therefore, counts in the lobulus ansiformis and the flocculo-nodulus were not included in the data. Fig. 6 shows that, in most lobuli, except for those mentioned above, the majority (95%) of inner granule cells are produced between postnatal days 4 and 15.

Destiny of Cells which Remain in the External Granular Layer at 15–16 Days of Life

It has been suggested (7) that the external granular layer gives rise to some neuroglia cells at the terminal stage of its development. In Purdue-strain mice, the external granular layer disappears at 18 days of life. Therefore, postnatal days 15–21 were chosen as the experimental period since they are supposed to cover the terminal stage of the external granular layer. In an attempt to substantiate the neuroglia formation at this stage, a pair of animals of 15 days of age were labeled cumulatively during 24 hr (i.e. during postnatal days 15–16). When one animal was examined at the time of termination of the cumulative labeling, at 16 days of age, labeled cells were virtually restricted to the external granular layer which remained subjacent to pia as a layer of 1–3 cells in thickness (Fig. 7). Labeled cells were rare outside this layer (see Figs. 7 and 9). Another animal was allowed to survive for 5 days without receiving further injections of H^3-thymidine. During this period, cells which had taken up the label at postnatal days 15–16 migrated freely to their destination. As is well-known, H^3-thymidine, when injected into a postnatal animal, is rapidly cleared from the blood and com-

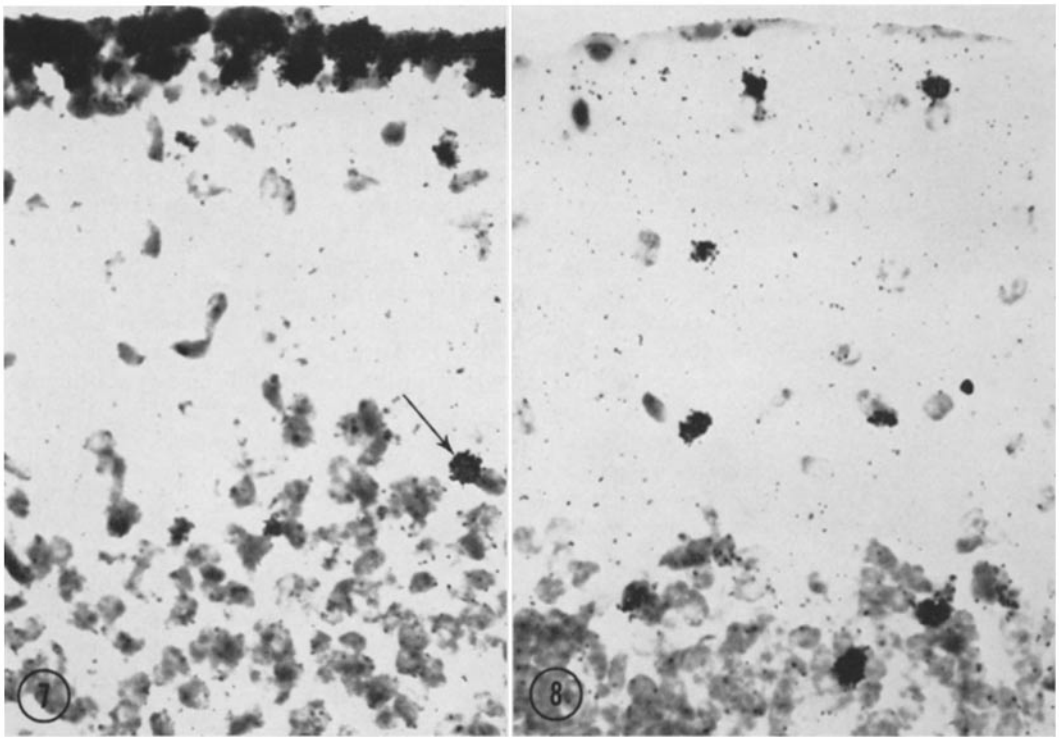

FIGURE 7 Radioautograph of 16-day-old mouse cerebellum, labeled for 24 hr prior to sacrifice. Labeled cells are prominent in external granular layer. Rare labeled cells are found in molecular and internal granular layers. Arrow indicates a labeled cell in the Purkinje cell layer. × 400.

FIGURE 8 Radioautograph of 21-day-old mouse cerebellum, labeled for 24 hr during 15–16 days of life, the same as the animal shown in Fig. 7, and killed after 5 days of survival. External granule layer disappears and labeled cells with small round nuclei increased in number in the molecular and internal granular layers. × 400.

pletely disappears in 2–3 hr. Therefore, during this period of the survival no incorporation of H^3-thymidine should take place, and the increase and decrease in the number of labeled cells in any parts of the cerebellar cortex can simply be interpreted as results of cell migration, provided no local proliferation at disproportionate rate occurred. Radioautographs taken from the animals killed before (Fig. 7) and after (Fig. 8) the 5 days of survival clearly showed that all the cells located in the external granular layer at 16 days of life migrated out into the internal granular layer and also into the molecular layer during those 5 days. Counts of labeled cells in the external granular, molecular, and internal granular layers were made before and after the migration (Fig. 9). Labeled cells in the molecular layer showed a ninefold increase in number when examined at 21 days of life (see Figs. 7 and 8) and most of them possessed small, round nuclei, thereby indicating their glioblastic nature (5). It is inconceivable that this ninefold increase in the number of labeled cells is produced by local proliferation of the labeled cells, since

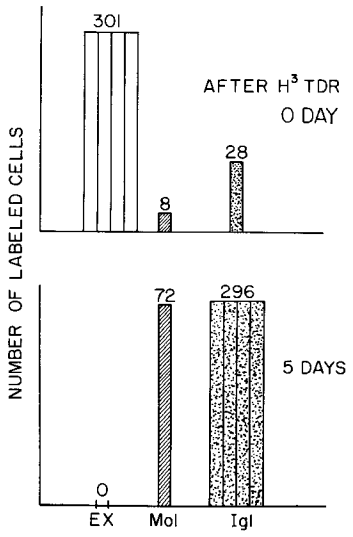

FIGURE 9 Histogram showing numbers of labeled cells in various layers of the cerebellar cortex. 15-day-old mice were labeled with H^3-thymidine for 24 hr; one was killed immediately after the labeling (top), and another 5 days later (bottom). All numbers are expressed as relative numbers per 10,000 inner granule cells. First white column (*Ex*) represents the numbers of labeled cells in the external granular layer; the second striated column (*Mol*), the molecular layer; and the third dotted column (*Igl*), the internal granular layer. Compare with Figs. 7 and 8.

the numbers of labeled cells in the molecular layer remained almost stationary during 24 hr of cumulative labeling, thereby indicating their low proliferative activity in situ. Since there are no small granule neurons in the molecular layer comparable in size to the glioblast, it is concluded that the external granular layer contributes some neuroglia cells (viz. glioblasts) to the molecular layer at the terminal stage of its development (later than 16 days of life).

DISCUSSION

In the present experiments, the generation time of the external matrix cell in the 10-day-old mouse cerebellum was estimated at 19 hr. Comparable values of 21 hr (7), and "within 24 hr" (10) have been found for the same type of cell in the mouse cerebellum. An extremely short generation time of 2 hr was reported (14) for the external matrix cells in the 9-day-old mouse. This estimate is, however, absolutely incompatible with observations of the present and previous (7, 10) investigators. As for the shortest generation cycle of cells in the central nervous system of the mouse, a generation time of 5 hr 20 min was found for the cerebral matrix cell of the mouse embryo of 10-day-post-conception (6). Generation times of 8.5 hr (9) and 11 hr (1) were reported, respectively, for the matrix cell in the spinal cord of mouse embryos at 10- and 11-day-postconception. In the central nervous system of the chick embryo, it has been found that the generation cycle of cells is prolonged as development proceeds (2). This appears also true for the mouse central nervous system.

The external matrix cells proliferate in the external matrix layer and give rise to neuroblasts which migrate out subsequently into the internal granular and the molecular layers. The first neuroblast in the vermal region arises at postnatal day 1, but the majority of neurons (95%) in the internal granular layer are produced between postnatal days 4 and 15 (Fig. 6). All the cells in the molecular layer are also produced postnatally. Among them, presumptive basket cells are the first neurons differentiated from the external matrix cell. Their differentiation usually starts slightly earlier than that of the inner granule neurons. Differentiation of other stellate neurons soon follows and the majority of them finish their DNA synthesis before 7 days of life. The production of stellate cells and that of inner granule neurons largely overlap. Both basket cells and other stellate cells in the molecular layer are doubtlessly de-

rived from the external matrix cells, since ventricular matrix cells, having disappeared already at the time of birth (7, 10), cannot give rise to these neurons of postnatal genesis. When the external granular layer approaches its terminal stage of development, the external matrix cells are greatly decreased in number; the balance between rate of proliferation and that of differentiation and migration shifts in favor of the latter. Finally, around 18 days of life a few cells that are left in the external granular layer change into glioblasts and migrate out into the molecular layer, leaving no trace of the external granular layer.

In our preliminary work with *dd* strain mice (7), it was found that the external mantle layer first appears at 3 days of life, and it was concluded that the production of the inner granule neurons in the *dd* mice should start at postnatal day 3. The present study using Purdue-strain mice revealed that the production of basket cells and inner granule neurons begins at postnatal day 1, i.e. 2 days earlier than the production in *dd* strain mice (Fig. 6). In the Purdue strain, the disappearance of the external granular layer takes place at 18 days of life, also 2 days earlier than that of *dd* strain mice. This tendency of prematurity of Purdue mice prevails in the entire course of development of the external granular layer, though the time pattern of cell differentiation within the developmental course of the external matrix system seems to be identical; as seen in Fig. 6, in Purdue mice, in comparison with *dd* mice, a corresponding percentage of cells is already produced at a date earlier by 2 days than the latter (Fig. 6). If one shifts the sigmoid curve in Fig. 6 horizontally toward the right by 2 days, it fits well into the neurogenetic pattern of *dd* strain mice.

Within the same cerebellum, there are marked regional differences in the pace of cell differentiation. In the vermal region, neuroblast differentiation invariably starts earlier than in the hemispheres. Among lobuli of the hemisphere, the posterio-apical portion of lobulus ansiformis is the last to differentiate. In this region, the majority of neuroblasts are produced later than 10 days. After 15 days of life, production of inner granule neurons markedly decreased in most lobuli, whereas in lobulus ansiformis and flocculo-nodulus many of them are still under active production. The presence of these late-differentiating matrix cells seems to be related to the delayed growth and phylogenetic hypertrophy of the corresponding lobuli in the mouse. Throughout postnatal development of the cerebellum, the external granular layer of the late-differentiating region was morphologically distinctly different; the layer was thinner and the cell composition was more uniform, with a poorly developed external mantle layer.

The potentiality of the external granule cells has been a subject of controversy (14). Present experiments revealed that they are multipotential germinative cells; they produce, in sequence, cells of their own type, basket and other stellate cells, neuroblasts of inner granule cells, and glioblasts. In this respect, they are comparable with the (ventricular) matrix cell (4) and deserve to be called the external matrix cells, which means externally dislocated matrix cells.

To interpret results of cumulative labeling experiments, one should take endogenous radiation effect into consideration, since the total dose of H^3-thymidine in the present experiments sometimes amounted to 60 μc/g of body weight. Radiation effects have been observed with much lower doses of H^3-thymidine (12). However, in the present experiments, little deleterious effects were apparent on the growth of animals and the morphology of cells, even in animals with the highest dose of 60 μc/g body weight. This conclusion, however, does not exclude the presence of chromosomal or other subcellular abnormalities but only means that, in the present experimental conditions, the endogenous radiation did not appear to interfere seriously with the developmental processes of the central nervous system.

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