Autoradiographic and Histological Studies of Postnatal Neurogenesis

I. A LONGITUDINAL INVESTIGATION OF THE KINETICS, MIGRATION AND TRANSFORMATION OF CELLS INCORPORATING TRITIATED THYMIDINE IN NEONATE RATS, WITH SPECIAL REFERENCE TO POSTNATAL NEUROGENESIS IN SOME BRAIN REGIONS

JOSEPH ALTMAN AND GOPAL D. DAS
Psychophysiological Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts

ABSTRACT Neonate rats were injected systemically with thymidine-H$^3$ and killed after different periods of survival. Cell proliferation, migration and transformation in the brain were studied autoradiographically. It was established that cells multiplying in the ependymal and subependymal walls of the olfactory ventricle migrate outward into the olfactory bulb, where they become differentiated into granule cells. These postnatally formed granule cells contribute to the formation of the granular and several other layers of the olfactory bulb. Cells multiplying at a high rate in the wall of the lateral ventricle migrate to the hippocampus and contribute to the formation of the granule cells in the granular layer of the dentate gyrus. Cells multiplying at a high rate in the external and internal granular layers of the cerebellum become differentiated into granule cells, and, to a lesser extent, other types of nerve cells of the cerebellar cortex. Evidence was also obtained of the postnatal origin of many of the granule cells of the cochlear nucleus. Postnatal neurogenesis is restricted to these short-axoned granule cells or microneurons; the long-axoned nerve cells or macroneurons of the brain are formed prenatally.

We have recently undertaken an extensive study of postnatal cell multiplication in the brain with the aid of thymidine-H$^3$ autoradiography. This paper, the first of a series, is concerned specifically with the proliferation, migration and transformation of those cells tagged in the brains of neonate rats which subsequently become differentiated into neurons.

Thymidine is a specific precursor of chromosomal DNA and it is incorporated into cell nuclei when new DNA molecules are formed by cells preparing for multiplication. When animals are injected with radioactively-labeled thymidine (such as thymidine-H$^3$), the cells proliferating at the time of injection tend to incorporate the administered radiochemical. The cells so tagged can be identified with fine-resolution autoradiography, a technique which provides histological sections with overlying photographic blackened grains to indicate the cellular sites of the incorporated radiochemical. Furthermore, by killing groups of animals at different survival periods after the injection, a time-lapse record can be obtained of the fate of the originally labeled cells, such as of the kinetics of their re-multiplication and of their migrations and transformations.

Thymidine-H$^3$ autoradiography is particularly well-suited to study the problem of postnatal neurogenesis. As a rule, differentiated neurons in the central nervous system of higher vertebrates do not divide, an observation which gave rise to the view that neurogenesis ceases after birth. However, the possibility cannot be excluded that undifferentiated cells divide postnatally in the brain and that these proliferating cells (which would become tagged

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with thymidine-H$^3$) subsequently differentiate into neurons. Such a process of neurogenesis, which characterizes the embryonic growth of the nervous system, could conceivably apply to the continued growth of the nervous system after birth. While it is customary to state that in higher vertebrates neurogenesis ceases after birth, direct or indirect evidence has been available for some time to indicate the occurrence of postnatal neurogenesis, not only as an exceptional or abnormal phenomenon but as the basis of the maturation of certain central nervous structures. It is well known, for instance, that the postnatal maturation of the cerebellum is dependent on the continued multiplication of "primitive" cells in a germinative zone forming the external granular layer (Obersteiner, 1883; Lahousse, 1888; Schaper, 1894; Popoff, 1895; Athias, 1897; Cajal, '11; and others). The external granular layer in the neonate or infant animal is composed of darkly-staining cells, many of them in various phases of mitosis, which apparently migrate into the interior layers of the cerebellar cortex (in the rat the external granular layer disappears at about three weeks of age). The majority of investigators studying the histogenesis of the cerebellum assumed that the cells of the external granular layer contribute not only to the newly forming glia cells of the cerebellum but also to its growing number of neurons, particularly those located in the molecular and (internal) granular layer. The validity of this conclusion was confirmed recently with thymidine-H$^3$ autoradiography (Uzman, '60; Miale and Sidman, '61) and will be further considered in detail in this paper.

In pilot studies we found recently that in adult rats with experimental lesions the nuclei of occasional neurons incorporate intracranially injected thymidine-H$^3$ (Altman, '62a, b). We obtained the same results subsequently with intraventricular injection of thymidine-H$^3$ into adult cats and systemic injection of this radiochemical into normal adult rats (Altman, '63). Our attention was drawn particularly to the labeling of a large number of granule cells in the dentate gyrus of the hippocampus, a region which is practically devoid of glial nuclei and where the presence of the radiochemical could be attributed only to uptake by the nuclei of these neurons. In a combined autoradiographic and histological study we were later able to establish (Altman and Das, '65) that a large proportion of the granule cells of the dentate gyrus of the hippocampus are formed postnatally in the rat. This study suggested that, in addition to local proliferation of cells, as indicated by the presence of occasional mitotic cells in this region, the increasing population of granule cells in the hippocampus of maturing animals was derived from cells multiplying at a high rate in the ependymal and subependymal walls of the lateral ventricles in the vicinity of the hippocampus. It was postulated that these undifferentiated cells migrate to the hippocampus and we identified them with the small, darkly-staining cells that are abundant in young animals in the internal border (or basal surface) of the dentate gyrus. These darkly staining cells, which are the ones that tend to be labeled when animals are killed within a few days after injection with thymidine-H$^3$, subsequently differentiate into granule cells and distribute themselves at the basal surface of the granular layer over the entire extent of the dentate gyrus. In animals that survived for longer periods after injection, labeled
granule cells were generally found in this portion of the granular layer.

The present series of studies represent a systematic attempt to investigate the problem of postnatal neurogenesis in the brains of rats from birth to maturity. Rats aged six hours to eight months were injected with comparable doses of tritiated thymidine, and the animals were permitted to survive after the injection for periods ranging from one hour to six months. In this first paper of the series we shall deal with data obtained from the groups of animals injected at the age of six hours or two days after birth (neonates). In this study we were able to confirm and extend our previous results regarding the postnatal origin of a large proportion of the granule cells of the dentate gyrus of the hippocampus. Moreover, we obtained conclusive evidence of the postnatal origin of the majority of granule cells in various other central nervous structures, such as the olfactory bulb, the cerebellum and the cochlear nucleus. Short-axoned neurons, or microneurons (McLardy, '63a), in other regions of the brain, as the stellate or Golgi type II neurons of the cerebral cortex, may also be formed postnatally.

MATERIALS AND METHODS

Laboratory-bred Long-Evans hooded rats were used in this study. A summary of the design of the experiment, with respect to the age of the animals at the time of injection and their survival time after injection, is presented in table 1. The numbers in italics refer to the animals in the neonate group specifically dealt with in this paper. All the animals were injected with thymidine-H3, 10 μc/gm body weight (the radiochemical was dissolved in isotonic saline, 1.0 mc/ml; specific activity 6.7 C/mM). At the end of the designated survival periods the younger animals were killed by decapitation followed immediately by immersion in 10% neutral formalin; the older animals were killed by cardiac perfusion with formalin. The removed brains were further fixed in formalin and were then embedded in Paraplast. Serial sections were cut at 6 μ and, depending on the age of the animals and the sizes of their brains, three consecutive sections (or set of sections) were preserved out of every 10 to 30. Of the preserved sections, one set was stained with a modified Bodian stain for fibers (2 hour Protargol-s method) and two sets were stained with gallocyanin chromalum. Of the latter, one set was coated in the dark with Kodak NTB-3 nuclear emulsion by the dipping technique, exposed at 5°C for 91 days and developed in the usual manner (Altman, '64).

<table>
<thead>
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<th>TABLE 1</th>
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<tr>
<td><strong>Survival after injection</strong></td>
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<td>Age at injection</td>
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<tr>
<td>Neonates</td>
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<td>6 hours</td>
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<td>Adolescents</td>
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<td>30 days</td>
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<td>Adults</td>
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In addition, rats of different ages were injected with the mitotic poison, colchicine, to arrest cell division and thus obtain collateral information of mitotic activity in the brain. All the animals were killed four hours after the injection and the brain sections obtained were stained with cresyl violet. A summary of the design of this experiment is given in table 2.

<table>
<thead>
<tr>
<th>Age at injection (days)</th>
<th>Dose of colchicine (mg/kg)</th>
<th>No. of animals</th>
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<td>6 days</td>
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<td>16 days</td>
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<td>30 days</td>
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<td>105 days</td>
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Evaluation of cell multiplication

The stained autoradiograms were scanned at 640 × magnification and the proportion of labeled to unlabeled cells was determined. In all structures to be described below a minimum of 1,000 cells were counted and classified. The attempt was always made to scan homologous sections, and homologous regions within sections, for all the animals compared. In general, a dichotomy was merely made between labeled and unlabeled cells, in other instances the labeled cells were classified in terms of degree of labeling.

Quantitative autoradiographic studies using thymidine-H³ and aimed at estimating rates of cell multiplication are predicated on the assumption that thymidine-H³ injected in a single dose remains available for a limited period and that the cells that are preparing to multiply at the time of injection are the only ones that will utilize and retain the radiochemical (Cronkite et al., '59; Hughes et al., '58; Messier and Leblond, '60). The other assumption generally made (Hughes, '59) is that DNA is metabolically stable and thymidine-H³, therefore, is lost only when the cell multiplies and some of its DNA is transferred to its daughter cells. Hence, continued multiplication of already labeled cells will lead to an initial increase in the number of labeled cells, combined with the dilution of the radiochemical and a reduction in the number of blackened grains within cell nuclei. In a rapidly and continually multiplying population of cells the descendants of originally labeled cells may lose their labeling altogether.

Degree of labeling and changes over time in the number of labeled cells will be used, accordingly, as criteria of the rate of cell multiplication and of the cessation or continuation of cell proliferation over the survival period of the injected animal. The interrelationship between the kinetics of cell multiplication, on the one hand, and of cell labeling and dilution, on the other, are presented in diagrammatic form in figure 1. Figure 1A represents a cell population in which cell multiplication is both rapid and continuous. As a consequence, there is an initial rapid rise in the number of labeled cells together with a rapid fall in the mean number of radiation-induced blackened grains within the cells. As the dilution of the radiochemical continues with repeated multiplication, more and more cells lose their tagging altogether until finally none of the descendants of originally labeled cells appear label-
beled. Thus, rapidly and repeatedly multiplying cell populations will tend to give an inverted U curve when the number of labeled cells are plotted against survival time. Figure 1B represents a cell population characterized by rapid initial cell multiplication that tends to slow down over time as the animal matures. In such a population the initial rapid increase in labeled cells and label dilution within cells is slowed down, and the flattening of the curves can serve as indicators as to when the deceleration of cell multiplication has occurred. Figure 1C represents a cell population which displays sluggish initial proliferation and in which multiplication comes to a halt after a brief period. In such a cell population there will be an early phase of label dilution in at least a small proportion of the cells, though many other cells, those that do not multiply, may remain intensely labeled over prolonged periods of survival. Finally, figure 1D illustrates the situation where reduction in the number of labeled cells at a proliferative site is due not to continued cell multiplication but to the migration of the labeled cells to another site. As we shall see, these four types of cell populations may all be identified in certain proliferative regions of the central nervous system.

RESULTS

Olfactory bulb

The germinal olfactory ventricle. In the neonate and infant rat the olfactory ventricle (the rostralmost extension of the lateral ventricle) occupies a considerable part of the entire mass of the olfactory bulb. The wall of the olfactory ventricle consists at this stage of development of a 1-2-cell-thick ependymal layer and a large subependymal surround (fig. 2). The ma-

Fig. 2 Olfactory bulb. Low-power photomicrograph of a galloxyanin-chromalum-stained autoradiogram from a rat injected with thymidine-H$^3$ at the age of six hours and killed six days later. G, glomerulus; M, mitral layer; V, olfactory ventricle. Magnification × 47.
Majority of the subependymal cells are of small size with darkly staining nuclei. In colchicine-treated animals, and occasionally in untreated ones, mitotic cells can be observed in this region. With increasing age during development, the size of the subependymal matrix surrounding the olfactory ventricle decreases, with a concomitant growth in and differentiation of the various layers of the olfactory bulb, particularly of the internal and external granular layers. In the adult rat the olfactory ventricle becomes very small, represented by a small aggregation of darkly staining cells without clear delineation of the lumen of the ventricle.

In the two animals injected at the age of six hours and killed six hours later, 22 and 23% of the ependymal and subependymal cells of the olfactory ventricle were found labeled. In the same group, the number of labeled cells in this region has risen to 42 and 43% in the two rats killed 24 hours after the injection, and to 79 and 81% in the two animals killed three days after the injection. In a single animal with six days' survival period (the olfactory bulb was not available in the other member of this group) the percentage of labeled cells has not appreciably changed (83%), but there was a drop to 70 and 72% in the animals surviving for 12 days after the injection (fig. 3).

The labeled cells in the olfactory ventricle (figs. 19-20) were classified into four categories in terms of grain concentration or intensity of labeling over the cell nuclei. We classified as solid black cells those most intensely labeled cells over which the blackened grains formed a continuous opaque surface. Predominantly black cells were those less intensely labeled cells in which grain-free areas were present to some extent, but the blackened grains predominated. We called predominantly light cells those over which there were larger patches of grain-free than grain-filled areas. Finally, we classified as very light cells those over which only a few blackened grains were visible, about 2-3 times above the noise level in comparable cell-free areas of the section. In figure 4 we plotted the percentage distribution of these four cell types in the olfactory ventricle as a function of survival.

Fig. 3 Percentage of labeled cells in the olfactory ventricle, and in the mitral and granular layers of the olfactory bulb, in rats injected with thymidine-H3 at six hours of age and surviving for different periods after the injection. Each point on the graphs represents 2,000 cells counted and classified in a single animal.

Fig. 4 Degree of labeling (as defined in the text) of cells of the olfactory ventricle, as a function of length of survival after injection.
after injection. The most intensely labeled cells showed already a decrease after one day of survival and they were altogether absent (an occasional cell excepted) by the sixth day. The number of very lightly labeled cells increased rapidly over the entire period with indications of deceleration from 6 to 12 days, while the two intermediate types of labeled cells showed an initial increase, followed by a decline to zero by the twelfth day after injection.

In the animals injected at two days of age the percentage of labeled cells after different periods of survival showed the same initial trend as in the previous group (fig. 5) though generally the percentages were of somewhat lower values. The major difference in the two groups was the more rapid drop in the percentage of labeled cells after 12 days survival. In this series the two animals surviving for 20 days showed a drop in percentage of labeled cells to the low value of 12 and 13%. In summary, the olfactory ventricle may be classified as a type A proliferative zone in which there is rapid and continued cell multiplication up to at least 22 days of age

Fig. 5 Percentage of labeled cells in the olfactory ventricle, and in the mitral and granular layers of the olfactory bulb, in rats injected with thymidine-3H at two days of age and surviving for different periods. The abrupt decline in the number of labeled cells the sixth day after injection is attributable to a large extent to the migration of these cells from the shrinking olfactory ventricle to the various layers of the olfactory bulb.

Lamination of the olfactory bulb. In the olfactory bulb of mature animals six layers may be distinguished (Cajal, '11): (1) the external layer of olfactory nerve fibers; (2) the layer of glomeruli with periglomerular granule cells; (3) the external plexiform layer, composed of fibers and small mitral and tufted cells; (4) the layer of large mitral cells and associated granule cells; (5) the inner plexiform layer of fibers; and (6) the inner granular layer, below which is a layer of white matter. The terminal arborization of the olfactory nerve fibers synapse in the glomeruli with the dense dendritic processes of the tufted and mitral cells, while the axons of the large mitral cells compose the olfactory tract and contribute to the anterior commissure. The granule cells of the olfactory bulb were described as small neurons with dendritic processes but no axons (similar to the amacrine cells of the retina). They are found aggregated around the glomeruli, where they enter into synaptic relationship with the terminal branches of the olfactory nerve and the dendrites of the mitral cells, and are also present in the lower layers, forming dense rows of cells below the mitral cells and in the internal granular layer. Scattered in all these layers are also small neurons with short axons.

While several of these layers are clearly recognizable in the olfactory bulb of neonate rats, the differentiation of the olfactory laminae takes place during the infancy of the animal. The external layer of olfactory nerve fibers, and below it the layer of glomeruli, are present in the neonate, but periglomerular cells are scarce. The external plexiform layer and the mitral cell layer are also evident. The mitral cells are of comparatively small size and the packing density or number of cells across the layer greatly exceeds at this stage what is seen in the mature animal. This may be attributed to the subsequent distribution of these cells over a larger surface as the size of the olfactory bulb increases during development. Another difference between the mitral cell layer of the neonate and the maturing animal is the virtual absence in the neonate of granular cells in this layer.
The inner plexiform layer is clearly recognizable below the mitral cells, and underneath it are seen the undifferentiated cells of the inner granular layer. The latter are in intimate contact with the cells of the extensive, proliferating olfactory ventricle.

In both groups of animals with six hours survival after injection with thymidine-\(^{3}H\), intensely labeled cells were found in large number in the olfactory ventricle, as described earlier, and also near the surface of the bulb in the external fibrous layer. Labeled cells were also seen, though in smaller number, below this layer around or near the glomeruli. Labeled cells, except for occasional ones, were absent in the mitral cell layer and in the other layers of the olfactory bulb. Little change in the pattern of cell labeling was observed in the animals surviving for one day, except for a slight increase in the number of labeled cells and for a trend indicating dilution of label concentration within cells (fig. 23). In the animals surviving for three days after the injection the most obvious change was the appearance of labeled cells in the internal granular layer in the basal regions adjacent to the ventricle (figs. 21, 24). This indicates that the undifferentiated proliferating cells of the ventricle migrate outward into the internal granular layer or that with the shrinkage of the ventricular subependymal layer, cells are left behind which become differentiated into granule cells. In the animals surviving for six days after the injection (fig. 5) labeled cells were found throughout the entire extent of the enlarged internal granular layer and labeled granule cells were also seen in the mitral cell layer. By this time (the animals were six and eight days old, respectively) the number of labeled cells increased greatly also in the first and second layers of the bulb, combined with a decrease in grain density. However, few labeled cells were seen in the third layer, suggesting that the increase in labeled cells in the first and second layers was due to the multiplication of the originally labeled cells found immediately after the injection in this region rather than to outward migration from the ventricle. In the animals surviving for 12 days there was a considerable increase in the number of labeled cells in the internal granular layer and in the mitral cell layer (figs. 22, 25, 26). In these animals the laminar organization of the olfactory bulb has essentially assumed the adult pattern. There was no evidence from this date onward of any further increase in the number of labeled cells in the various layers of the bulb, though decrease in labeled cells and increase in label dilution is evident in the olfactory ventricle up till the twentieth day after injection. The changes in the percentage of labeled cells in the mitral cell layer and internal granular cell layer for the two groups of animals are summarized in figures 3 and 5. In the group injected at two days of age about 40% of the granule cells in the mitral cell layer and 50% of the granule cells in the internal granular cell layer were found labeled (a small number of these cells may be glia cells). The mitral cells and other large types of neurons were never labeled.

The foregoing findings indicate that a large proportion of the granule cells of the olfactory bulb are formed postnatally. The granule cells of the glomerular layer are derived from local multiplication of cells near the subarachnoid surface, while the granule cells of the mitral and internal granule cell layers originate from the highly proliferative subependymal matrix of the olfactory ventricle with the possibility of added local proliferation. The olfactory ventricle is a type A proliferative zone which shows rapid and continuing cell multiplication up till at least 20–22 days postnatally in the rat, whereas the population of granular cells in the various layers represent type B or type C proliferative regions.

**Dentate gyrus of hippocampus**

The large hippocampus of the rat is interposed in an oblique position between the midbrain and thalamus on the one hand and the cortex on the other. There is a diversity of classifications with regard to what structures constitute the hippocampus and also about its detailed cytological subdivisions (e.g., Cajal, '11; Lorente de Nó, '34; Krieg, '46; Blackstad, '56; McLardy, '63b). For the purposes of our discussion it will be satisfactory to distinguish two major components of the hippocampus, the dentate gyrus (fascia dentata,
area dentata) and Ammon's horn. Here we shall be primarily concerned with the dentate gyrus.

The dentate gyrus is a trough-shaped structure and we shall distinguish in the course of our discussion between its internal and external arm (fig. 6). We shall define as the internal arm that portion of the three-layered dentate gyrus which faces the mesencephalon or diencephalon. The internal arm of the dentate gyrus is situated ventrally in the dorsal hippocampus, medially in the lateral part of the hippocampus, and dorsally in the ventral hippocampus. The external arm of the dentate gyrus faces the cortex, and it represents the dorsal, lateral and ventral components of the dentate gyrus in the corresponding parts of the hippocampus. The internal and external arms join one another, forming a hilus, both in the dorsal hippocampus (anteriorly) and the ventral hippocampus (posteriorly).

The three layers of each arm of the dentate gyrus are the superficial plexiform layer, intermediate granular layer and basal polymorph cell layer. In the superficial plexiform layer terminate the dendrites of the granule cells of the granular layer and the dendrites of the polymorph cells of the basal polymorph cell layer. The granular layer is composed largely of the densely packed cell bodies of the granule cells; this layer is practically devoid of nuclei of glia cells. We shall refer to the rows of granule cells which are closer to the superficial plexiform layer as the superficial rows of granule cells, while those which are closer to the basal polymorph cell layer as the basal row of granule cells.

![Fig. 6 Subdivisions of the dorsal hippocampus of the adult rat, as used in this study.](image)

A, Ammon's horn; BP, basal polymorph cell layer of dentate gyrus; EA, external arm of the dentate gyrus; G, granular layer; H, hilus of dentate gyrus; IA, internal arm of dentate gyrus; SP, superficial plexiform layer of dentate gyrus; T, thalamus. Arrows point to the margin of the dentate gyrus. Gallocyanin-chromalum, magnification, × 59.
The axons of the granule cells, the mossy fibers, are located in the basal polymorph cell layer and they establish synaptic contact with the pyramidal cells of Ammon's horn.

The dentate gyrus is poorly differentiated in the neonate rat (fig. 7). In the external arm of the dentate gyrus a 2-3-cell-thick row of granule cells is at least partially differentiated, in the sense that their cell bodies begin to assume the appearance of granule cells. Such partially differentiated granule cells may also be seen at the hilus or juncture of the external and internal arms. However, only a few differentiating granule cells are seen in the internal arm, and these are located near the hilus. In the region of the presumptive internal arm a large number of small, darkly-staining cells are present. This type of cells is also seen in large number scattered throughout the superficial and basal dentate layers. From the latter regions these small dark cells may be followed along the fimbria to the internal wall of the lateral ventricle and, in the dorsalmost region of the hippocampus, along the fornix to the region of the interventricular foramen.

At this stage of development the lateral ventricle is still enlarged, being surrounded by a clearly recognizable ependymal and subependymal wall that varies in thickness in different regions along the ventricular lumen. In the animals treated with colchicine, but also in untreated ones, mitotic cells are seen in an appreciable number in the ependymal and subependymal layers of the lateral ventricle (fig. 8). During the infancy of the animals the size of the lateral ventricle and its cell population de-

Fig. 7 Dorsal hippocampus in a one-day-old rat. A, Ammon's horn; GEA, differentiating granular layer of the external arm of the dentate gyrus; GIA, darkly-staining, undifferentiated cells in the presumptive granular layer of the internal arm of the dentate gyrus. Gallocyanin-chromalum stain; magnification, about ×292.
Fig. 8 Ependymal and subependymal germinal matrix surrounding the lateral ventricle in a four-day-old rat injected with 5 mg/kg colchicine and killed four hours after the injection. Arrows point to some of the cells with arrested mitosis. Cresyl violet stain; magnification, $\times$ 410.
creases. Paralleling this change there is an increase in the dentate gyrus in the number of small, darkly-staining cells, and also in the number of differentiating granule cells. The dentate gyrus grows in size, and new rows of differentiating granule cells are added to the external and internal arms. The small, dark cells are eventually reduced in number with a concomitant increase in the number of granule cells. A quantitative analysis of these changes with respect to the age of the animals was presented by us in a previous paper (Altman and Das, '65).

In the animals injected with thymidine-$H^3$ at six hours and two days of age a large number of labeled cells were seen in the ependymal and subependymal walls of the lateral ventricle (fig. 9). Of these cells the majority was intensely labeled. The total number of labeled cells (fig. 10) increased rapidly up to the third day after

![Fig. 9 Low-power photomicrograph of a gallo-cyanin-chromalum-stained autoradiogram of the hippocampus and surrounding regions in a rat injected with thymidine-$H^3$ at six hours of age and killed 24 hours later. C, cortex; D, dentate gyrus of the hippocampus; F, fimbria; H, Ammon's horn; P, choroid plexus; T, thalamus; V, lateral ventricle. Magnification, x 59.](image-url)
injection, and then, with the progressive dilution of labeling within cells, the number of labeled cells declined to a low level (type A germinative zone).

In the dentate gyrus of the hippocampus (fig. 11) from 7 to 10% of the cells were labeled in the two groups of animals after six hours' survival. In both groups the number of labeled cells rose rapidly for at least six days after the injection and, with some variability from animal to animal, the population of labeled cells remained essentially unchanged from 12 to 20 days after injection (type B germinative region).

The type and distribution of labeled cells in the dentate gyrus changed radically over the survival periods tested (figs. 27–31). After six hours and one day of survival the labeled cells that were found were commonly small dark cells. These primitive cells were aggregated in largest number in the fibrous tracts of the hippocampus (fornix and fimbria) in the vicinity of the ventricles. This indicates that during this short period of survival the cells multiplying near the ventricle or in the fibrous tracts have not had sufficient time to invade the entire dentate gyrus. Curiously, the total number of labeled cells, and the cell-thickness of the subependymal matrix, is lower in the internal wall of the lateral ventricle facing the hippocampus than in the external wall facing the cortex (fig. 9). The labeled cells do not appear to migrate through the lumen of the ventricle, hence they either move around the ventricle from the external wall or, else, the smaller number of cells in the internal ependymal wall of the ventricle is due to the faster migration of the newly formed cells from this region and, therefore, the more rapid depletion of these cells. Labeled cells were also present in considerable number in the dentate gyrus in the basal and superficial layers up to the region of the hilus. Accordingly, the proliferation of at least some cells locally within the dentate gyrus cannot be excluded. Indeed, in our colchicine material, cells with arrested mitosis were seen in moderate number in the dentate gyrus.

By the third day after injection the labeled cells were distributed over the entire extent of the superficial and basal layers of the dentate gyrus but the majority of these were still small, darkly-staining cells. By the sixth day (fig. 29) many of the labeled cells have migrated into the granular layer and have assumed the appearance of small, but characteristic granule cells. At this time, however, the majority of the labeled cells were undifferentiated and were still located in the surrounding layers (being more abundant in the basal polymorph layer). By the twelfth day (fig. 30) after injection the great majority of labeled cells were in the granular
layer and many of these appeared as differentiated cells of varying sizes.

In the partially-differentiated granular layer four types of granule cells could be distinguished in our autoradiographic material. First, there was a 2-3-cell-thick row of granule cells which were characteristically unlabeled. These cells represent the largest and best differentiated granule cells at this age (12–14 days). Since they were unlabeled we may assume that they were formed either prenatally or early after birth before the administration of the tritiated thymidine. Scattered in this region were other large and well-differentiated cells with intense or intermediate labeling. The high label concentration in these cells indicates that these cells or their precursors have undergone few or no cell divisions after the original incorporation of the administered thymidine. That is, they represent the “oldest” of the postnatally-formed cells and the first among these to become differentiated. In the 12-day survival group virtually all the cells forming the basal rows of the granular layer were labeled but the label concentration in these tended to be low. This large group of cells represents postnatally-formed neuroblasts which have undergone many divisions before their subsequent differentiation. The basal row of cells, accordingly, are late-formed neurons of the granular layer and 12 days after the injection of neonates these are still small and only partially differentiated. In the animals killed 20 days after the injection (their ages being 20 and 22 days) the lightly-labeled cells to be found were well differentiated but the basal row of cells was formed of smaller, partially differentiated cells the majority of which were unlabeled (fig. 12). These cells represent the “youngest” of the granule cells which presumably were formed of cells which no longer retained detectable amounts of the originally administered tritiated thymidine. This mode of addition of new

Fig. 12 Pattern of labeling of granule cells in the dentate gyrus in a rat injected with thymidine-
H\(^1\) at two days of age and killed 20 days later. Gallocyanin-chromalum-stained autoradiogram; magnification, \(\times 746\).
cells from the “inside” to the growing granular layer of the dentate gyrus is characteristic over the entire expanse of the hippocampus.

Labeled small cells were also found in Ammon’s horn in the animals with short survival after injection. However, in the animals that survived for longer periods we did not observe any labeled pyramidal cells, only occasional granule cells, and smaller cell types which may be either neuroglia cells or undifferentiated neuroblasts. We may conclude, therefore, that unlike the large proportion of the granule cells of the dentate gyrus, the pyramidal cells of Ammon’s horn are formed prenatally.

Cerebellar cortex

The cerebellar cortex consists of three layers, (1) a superficial molecular or plexiform layer, (2) an internal granular layer, and interposed between the two is (3) a single-cell-thick layer of Purkinje cells. The molecular or plexiform layer is composed largely of fiber processes projecting into this region from the cerebellar white matter, from the granule cells of the granular layer and the Purkinje cells. The afferents of the cerebellum, the mossy fibers, synapse with the granule cells. The granule cells, which form the dense and thick granular layer, are small neurons, each with a small number of dendrites and a short axon. The Purkinje cells are the efferents of the cerebellar cortex whose axons project to the deep cerebellar nuclei. In addition to these cell types, stellate cells and basket cells are scattered in the molecular layer, while larger Golgi cells may be seen in the granular layer, usually in the vicinity of the Purkinje cells.

In the adult, the cerebellum is a highly convoluted structure with numerous folia. In the neonate the cerebellum is inadequately developed and few of the folia are formed (figs. 34, 35). Microscopically, the lamination of the cerebellar cortex of the neonate differs from that seen in the adult. The most obvious difference is the presence of an external granular layer, a superficial band composed of small, darkly staining cell nuclei, many of which show mitotic activity (figs. 32, 33). Underneath the external granular layer is a very thin cell-free layer, the undeveloped molecular layer. Below this layer is a cellular lamina composed of several rows of lightly stained cells (these are undifferentiated Purkinje cells) which merge with the internal granular layer.

The first cells to differentiate in the course of postnatal development are the Purkinje cells. Though not yet of adult size, the Purkinje cells may be identified by the sixth day as a 2-3-cell-thick row of differentiating cells underneath the still very thin molecular layer. As the volume of the cerebellum and its surface increases with continuing maturation, the cell-thickness of the Purkinje layer decreases with a concomitant growth in the size of single Purkinje cells. By about the twentieth day the Purkinje cells assume their normal appearance and the Purkinje layer is reduced to a row made up of single cells. The external granular layer, which is obviously a subpial germinative zone (figs. 32, 33), initially increases in thickness and it continues in an active mitotic state up till the fifteenth to eighteenth day after birth. At this period the dissolution of the external granular layer begins and only vestiges of it remain in a few scattered regions by the twentieth day. By this age the superficial molecular layer and the internal granular layer have essentially assumed their adult appearance.

In the animals injected with thymidine-H3 at six hours and two days of age, and killed six hours after the injection (fig. 36), labeled cells were present in high number in the external granular layer and in lesser but not inconsequential number also in the internal granular layer and in the medullary layer below the cerebellar cortex. In the animals injected at six hours of age 41–42% of the cells sampled were labeled in the external granular layer (fig. 14). In the animals injected at two days the number of labeled cells after the same period of survival was 55% for both animals (fig. 15). In both groups of animals the number of labeled cells greatly increased after one day survival: to 57–62% in the younger group, to 84–86% in the somewhat older group, combined with considerable dilution of labeling within cells (fig. 37). Both findings indicate a somewhat higher rate of cell proliferation.
in the second group of animals. In the animals injected at six hours there was a further increase in the number of labeled cells in the external granular layer after three days' survival (fig. 38), but a fall occurred by that time in the second group. The latter finding may be attributed to the fact that the relatively higher rate of cell proliferation in the animals injected at two days of age led to a dilution of the label below recognition threshold in a larger proportion of the cells. In both groups the number of labeled cells fell to a low level by the twelfth day after the injection: to the original value of about 40% in the younger group, and to the much lower level of less than one-fifth of the original percentage in the older group. (In the latter group the percentage of labeled cells is indicated as zero by the twelfth day after injection, but this is merely due to the fact that by this time the cells of the external granular layer have disappeared.)

In terms of these findings we may conclude that the subpial external granular layer represents a type A proliferative zone, characterized by rapid and continuous cell multiplication.

A small number of labeled cells was also seen in the region between the external and internal molecular layer in the animals with shorter survival (and hence of less advanced age). However, by studying the autoradiograms of the animals with longer survival it became evident that the differentiating Purkinje cells situated in this region were never labeled, indicating that the Purkinje cells are all formed prenatally (figs. 39, 40, 41). In contrast, an appreciable rate of local cell proliferation is evident in the internal granular layer. The concentration of labeled cells was 18-20% in the animals injected at six hours of age, and it ranged between 14 and 24% in the animals injected at two days of age. In both groups there was a rapid rise in the relative number of labeled cells, combined with concomitant label dilution which was initially faster in the older group but which reached an asymptote level of about 77% in both groups by the sixth day after injection. In both groups there was a decline by the twelfth day to 48-66% in the younger group and to 18-32% in the older group. In the latter group of animals the percentage of labeled cells fell to 10-15% by the twentieth day after injection. These results indicate that the internal granular layer represents also a type A proliferative zone.

The curves (figs. 14, 15) summarizing our data on cell labeling in the external and internal granular layers indicate that the initial rate of cell multiplication is much lower in the internal granular layer. In spite of this the percentage of labeled cells in the internal granular layer eventually approximates that of the external granular layer, though this happens with a considerable time lag. Since the internal granular layer continually grows in size and cell thickness, while the external granular layer disappears, it is conceivable that at least some of the cells formed in the external granular layer migrate and make a considerable contribution to the cell population of the internal molecular layer. Another site where the rapidly proliferating cells of the external granular layer may be unloaded is the fast growing adjacent molecular layer.

Few labeled granule cells could be seen in the cerebellar cortex by the twentieth day after injection and those that were labeled had very few overlying grains (very light labeling). However, in these animals some other types of cells were commonly found labeled and these were associated with a moderate number of grains. Many such labeled cells presumably Bergmann glia cells were seen in the vicinity of the Purkinje cells (fig. 40). These were characterized as pale cells of intermediate size and they were particularly numerous at the interfolial confluence of the Purkinje layer. Occasionally a larger neuron, presumably a Golgi cell (figs. 41, 42) was also seen labeled in the granular layer. Some intermediate and larger size neurons, presumably stellate and basket cells were often seen with label in the molecular layer. In contrast, no labeled Purkinje cells...
Labeled cells in external granular layer

Labeled cells in internal granular layer

Fig. 14 Percentage of labeled cells in the external and internal granular layers of the cerebellar cortex, as a function of survival time, in rats injected with thymidine-H$^3$ at six hours of age.

Neocortex

The neocortex of the newborn rat is made up almost exclusively of a large population of very densely packed cell bodies of neurons. The arrangement of these cells suggests the sparseness of neural and glial processes in the cortex at this stage of the animal's development. Though the majority of cortical cells are immature, the laminar architecture of the cortex is already evident, and in some regions certain characteristic cell types, such as pyramidal cells, are already recognizable. In addition to the larger type of differentiating neurons, which are ovoid in shape and stain relatively lightly, scattered in the cortex are also smaller, darkly staining round cells. In the autoradiographic sections many of these smaller cell types were labeled.

In contrast to the large proportion of labeled cells in the brain regions previously discussed, labeled cells in the cortex were not numerous (fig. 16). In the animals injected at six hours or two days of age only 1–2% of the cells were labeled after six hours survival. The number of labeled cells rose slowly with prolonged survival, reaching 14% by the twelfth day after injection in the animals injected at six hours of age and 22–25% in the animals injected at two days. For all survival periods tested a larger proportion of labeled cells was obtained for the animals injected at two days than in the animals injected at six hours of age. In terms of our earlier classification the cortex may be designated a type C proliferative region, showing a rela-
Fig. 16 Percentage of labeled cells in the neocortex, as a function of survival time, in rats injected with thymidine-H\(^{3}\) at six hours and two days of age.

A relatively slow rate of cell multiplication that comes to a halt relatively early during postnatal development.

Labeled cells appeared to be randomly distributed in the cortex. However, they showed high concentration just underneath the pia and within the white matter, and the possibility of inward migration of cells from these two regions requires further consideration. The most common type of labeled cells in the oldest group of animals (22 days) were intermediate-sized pale cells which we identified as astrocytes. At this age oligodendrocytes in the cerebral cortex were still uncommon and very few of these were labeled. A large proportion of the cells of the pial membrane were labeled, so were also some endothelial cells and occasional microglia cells. In addition to these cell types, larger-sized, round and pale cells with a thin surround of cytoplasm and a prominent nucleolus were often found labeled, sometimes in clusters, in various regions of the cortex (figs. 44–50). We postulate that these cells represent undeveloped short-axoned neurons (stellate or Golgi type II cells). Since in the rat these granule cells do not form a distinct layer, the identification of these cells with our staining technique must remain hypothetical. It will be necessary to use animals with longer survival period in conjunction with different staining techniques to test this hypothesis.

Lateral geniculate nucleus

As an example of a thalamic nucleus, we have examined cell labeling in the lateral geniculate nucleus. The curves obtained for the two groups of animals with different survivals after thymidine-H\(^{3}\) injection (fig. 17) resemble the curves obtained for the neocortex (representing a type C proliferative region). However, a slightly higher rate of cell multiplication is indicated for the lateral geniculate nucleus, reaching by the twelfth day 25–27% in the six hour injection group, and 29–30% in the two-day injection group. As in all other structures considered, the initial and subsequent rate of cell multiplication is somewhat higher in the two day than six hours injection group. It is interesting to note that cell multiplication in the lateral geniculate nucleus ceases by about 12–14 days of age, a period associated in the rat with the opening of the eyes.

Fig. 17 Percentage of labeled cells in the lateral geniculate nucleus, as a function of survival time, in rats injected with thymidine-H\(^{3}\) at six hours and two days of age.

Unlike in the cortex, oligodendroglia cells were quite common in the lateral geniculate body of three-week-old animals. Very few of these, however, were labeled. The labeling of astrocytes was more common. In general, labeled glia cells occurred in higher number in the wall of the lateral geniculate body than in its interior, presumably because these cells are associated with the fibers of the optic tract and optic radiation.

Medulla

To obtain an estimate of cell labeling in the medulla, homologous strips at the level of the superior olivary nucleus were scanned across the medulla in all the animals and the percentage of labeled cells was determined. In both groups of animals an initial rapid rise in the number of labeled
cells was obtained from six hours to one day after injection of tritiated thymidine (fig. 18), a somewhat slowed down increase by the third day with little evidence of further increase in the groups of animals with longer survival. Of all the brain regions, the medulla appears to show the earliest signs in cessation of postnatal cell multiplication. Examination of the labeled cells in the medulla of the 22-day-old animals suggested that the most commonly labeled cells were astrocytes (fig. 51). In contrast, the majority of oligodendrocytes were not labeled. In the various fibrous tracts, larger types of unidentified cells were often lightly labeled.

Ventricular germinative pools

The foregoing results indicate that the ependymal and subependymal layers surrounding the ventricles represent the major source of newly formed cells in the course of the continued postnatal neurogenesis and gliogenesis of the central nervous system. We obtained clear evidence that the bulk of the postnatally formed cells of the olfactory bulb are derived from the highly proliferative olfactory ventricles. Good evidence was obtained that the same holds for the genesis of the granule cells of the dentate gyrus, which are to a large extent formed in the ependymal and subependymal layers of the lateral ventricles, whence they migrate by way of the fimbria and fornix to the hippocampus. Similarly the granule cells of the cochlear nucleus could be traced with some certainty to the lateral recess of the fourth ventricle. An exception to this ventricular origin of the bulk of newly forming cells is the postnatal genesis of the granule cells and other cell types of the cerebellar cortex, which are either formed locally or migrate from the subpial external granular layer into the deeper layers of the cerebellar cortex. In all instances, the major sites of postnatal cell multiplication (as during embryony) are in the vicinity of the cerebrospinal fluid system. In general, rate of cell labeling, as determined with thymidine-H\textsuperscript{3} autoradiography, is positively correlated with the thickness of the subependymal layer surrounding the ependymal wall of a ventricular site and with the presence of darkly staining cell nuclei in these regions. Mitotic activity, as demonstrated with the colchicine technique, is also high in such regions. Rate of cell multiplication at any given site of the ventricular system, furthermore, appeared to be correlated positively with the size and fold-density of the choroid plexus in that region.

Rate of cell labeling and label dilution in the various components of the ventricular system may be taken as reliable indicators of continuing neurogenesis and gliogenesis in surrounding structures. The highest rate of initial and continuing cell multiplication is indicated for the walls of the lateral ventricles (figs. 9, 53) and ol-
factory ventricles (figs. 19, 20). High rate of continuing cell multiplication was also indicated for the roof and floor of the third ventricle (figs. 54, 56). (We have obtained evidence, which will be considered in detail elsewhere, that the cells multiplying in these two regions contribute neuro-endocrine cells to the pineal and pituitary glands. Both of these latter structures show a large proportion of labeled cells and a growth in size during postnatal development.) Cell multiplication is low in the thalamic portion of the third ventricle (fig. 55) and in the aqueduct. Cell labeling and label dilution is low in the fourth ventricle (fig. 13), with the exception of the lateral invagination of the fourth ventricle, as mentioned before. Lowest rate of initial cell multiplication, with virtual cessation after birth, was found in the ependymal wall of the spinal canal. In this region the cell thickness of the ventricular wall is reduced to a single cell and these tend to stain lightly.

In general terms, then, there is a caudo-rostral gradient in rate of cell multiplication, with the ventricular regions associated with the forebrain showing higher proliferative activity after birth than the regions associated with the spinal cord, medulla and brain stem.

DISCUSSION

Our results indicate clearly that cells multiplying in the brains of six-hour and two-day-old rats make a significant contribution to the formation of granular neurons in several brain structures. We have paid particular attention to the genesis of granule cells in regions where their identification does not pose a serious problem, namely, where these short-axoned neurons form distinct granular layers. We have within a few days after systemic injection of thymidine-H³ a good proportion of the cells of the granular layers in the olfactory bulb, hippocampal dentate gyrus, cerebellar cortex and ventral cochlear nucleus were labeled. In all these instances the major sites of cell multiplication could be identified as ventricular or subpial germinal zones. From these proliferative zones the migration of apparently undifferentiated cells to their regional destination could be easily traced. As judged by our colchicine material, regional or local proliferative activity is quite low; it is at these sites that the differentiation of the migratory primitive cells into recognizable granule cells begins.

In the case of the olfactory bulb the labeled cells could be traced migrating from the olfactory ventricles into the adjacent granular layer and up to the mitral cell layer. In terms of proportion of labeled cells an asymptotic level was reached in both groups of animals on the twelfth day after injection. Presumably by this date the bulk of the migrating primitive cells have arrived and local cell multiplication has come to an end. We have no information whether by this date the granule cells are entirely differentiated.

In this study we could confirm our previous conclusion (Altman and Das, '65) that the postnatal neurogenesis of the granule cells of the hippocampal dentate gyrus is primarily dependent on cells multiplying in the ependymal and subependymal wall of the lateral ventricle. We could trace the migration of these labeled primitive cells along the fimbria (and also the fornix) to the gradually growing and differentiating dentate gyrus. In the dentate gyrus the deposition of new cells occurred in a very regular fashion. In animals injected with thymidine-H³ in the first two postnatal days, the top row of granule cells, those in intimate contact with the superficial plexiform layer, were not labeled. These are the "oldest" or earliest-differentiating cells of the dentate gyrus, derived from cells which were formed pre- or postnatally before the administered radioactive thymidine became available. Intensely labeled cells occurred most commonly below the unlabeled cells. Absence of appreciable label dilution indicated that these were among the labeled cells which ceased to proliferate soon after the injection; that is, they were among the "oldest" of the labeled cells. Moving from the superficial to the basal rows of granule cells intensity of cell labeling tended to decrease, and many unlabeled cells were found in the basal rows of cells. Cell size and cellular differentiation in the younger animals showed the reverse of this; the superficial cells tended to be large and differentiated, whereas the basal cells were
smaller and often apparently undifferentiated. We may point out here that in animals injected at later ages the intensely labeled cells tend to be displaced basally; when adult animals are injected with thymidine-H\(^3\) the labeled cells are found almost exclusively in the basal rows. Pattern of cell labeling in the dentate gyrus may thus serve for "dating" the cells or the time of injection.

The high rate of cellular proliferation in the cerebellar cortex has been known for some time and reference to this earlier work was made above. The transient external granular layer of the cerebellar cortex represents a germinal bed which disappears altogether in the rat at about three weeks of age. Our results, as those of others using thymidine-H\(^3\) autoradiography (Uzman, '60; Miale and Sidman, '61), confirm the hypothesis that the proliferating cells of this germinal zone migrate inward past the Purkinje cell layer and contribute cells to the growing and differentiating internal granular layer. Our results clearly suggest that cell proliferation is quite high also in the internal granular layer where label dilution with increasing survival is considerable. Less label dilution is seen in the occasionally tagged basket cells in the molecular layer and the Golgi neurons in the granular layer, suggesting that the neurogenesis of the latter cell types ceases earlier than that of the granule cells.

In addition to these structures, the cells of the granular layer of the ventral cochlear nucleus were also found labeled. Accordingly, wherever granular nerve cells form a discrete layer in the brain, their postnatal origin is easy to demonstrate. More difficult is the identification of granule cells with our staining technique in regions where they are scattered among larger types of neurons and astrocytes, as in the case of the cerebral cortex of the rat. The cortex, cells of intermediate size, with pale nuclei, a nucleolus and a thin surround of cytoplasm, were often seen labeled in animals which survived to about 20 days of age. Are these cell types with labeled nuclei, which were also seen in other brain structures, astrocytes or granule cells? Only if we can establish with certainty that these are also granule cells will we be justified in concluding that a large proportion of the granule cells over the entire neuraxis are formed postnatally.

In the same structures in which the bulk of granule cells were labeled in neonate rats injected with thymidine-H\(^3\), the nuclei of neighboring larger types of neurons remained typically unlabeled. Thus, in the olfactory bulb the nuclei of the large mitral cells never incorporated the tagged thymidine. We have never observed labeled polymorph cells in the dentate gyrus or labeled pyramidal cells in Ammon's horn. Similarly, labeled Purkinje cells were never seen in the cerebellar cortex. In general, large neurons, perhaps the cortex excepted, are never labeled. Accordingly, these findings suggest that, from the developmental point of view, we may make a distinction between the small or short-axoned granule cells (microneurons) and the large or long-axoned nerve cells (macroneurons). Macroneurons are typically formed prenatally, whereas the bulk of microneurons are formed postnatally.

We shall not, in this context, speculate about the possible significance of the largely postnatal origin of microneurons. We have elsewhere entertained the hypothesis (Altman, '66) that the postnatally-formed microneurons, which are interposed among the prenatally-formed input, relay and output elements, are the modulatory and plastic elements of the central nervous system whose structural and functional maturation may be dependent on the animal's interaction with its external environment.

**CONCLUSIONS**

Tritiated thymidine was injected systematically into six-hour and two-day-old rats and the animals were killed after periods ranging from six hours to 20 days. Cell proliferation, migration and transformation was studied autoradiographically, with special reference to postnatal neurogenesis in some selected brain structures. In an ancillary investigation, rats of different ages were injected with colchicine and the occurrence and distribution of mitotic cells was determined histologically.

1. Changes in the number of labeled cells, and label dilution within cells, with different periods of survival indicated a rapid and continuing process of cell multi-
plication in the ependymal and subependymal walls of the olfactory ventricle. From the olfactory ventricle the labeled cells could be traced migrating into the olfactory bulb. In the olfactory bulb the labeled cells became differentiated into granule cells, forming its granular layer and contributing granule cells to the internal plexiform and mitral cell layers.

2. Cells multiplying at a high rate in the wall of the lateral ventricle could be traced migrating along the fimbria to the hippocampus. These cells migrated into the granular layer and became differentiated into granule cells forming the granular layer of the dentate gyrus. Those cells which ceased to multiply soon after the injection, that is, the most intensely labeled or "oldest" granule cells, were typically distributed in the superficial aspect of the granular layer, whereas the lightly labeled, late-differentiating "younger" granule cells commonly occurred in its basal aspect.

3. A high rate of cell multiplication was observed in the external granular layer of the cerebellum. An increase in labeled cells and decrease in label dilution was also seen with a time delay in the internal granular layer. These proliferating cells are the source of the growing granule cell population of the internal granular layer and, to a lesser extent, of the Golgi, stellate and basket cells, of the cerebellar cortex.

4. A large proportion of the granule cells of the granular layer of the ventral cochlear nucleus was labeled. The proliferation of these cells could be traced to the lateral recess of the fourth ventricle.

5. Occasional small neurons with labeled nuclei were encountered over the entire neuraxis, the spinal cord included. Cells that resemble granular neurons were seen with considerable frequency in the neocortex, but the identification of these cells was ambiguous.

6. In the same brain regions in which a large proportion of the granule cells were labeled, the nuclei of adjacent large neurons were virtually never labeled. These observations justify our conclusion that the long-axonated neurons or macroneurons of the brain are formed prenatally, whereas a large proportion of the short-axonated neurons or microneurons are formed postnatally.

7. The significance of the postnatal origin of microneurons in the brains of rats remains to be elucidated.

LITERATURE CITED


Hughes, W. L., V. P. Bond, G. Brecher, E. P. Cronkite, R. B. Painter, H. Quastler and F. G. Sherman 1958. Cellular proliferation in the mouse as revealed by autoradiography with


Note added in proof: After submitting this manuscript, Angevine published a monograph (Angevine, J. B. 1965 Time of neuron origin in the hippocampal region. An autoradiographic study in the mouse. Exp. Neurol., Suppl. 2: 1–70) dealing extensively with hippocampal neurogenesis before and after birth in mice. The results of Angevine are largely in agreement with ours and will be discussed in detail in the second paper of this series.

PLATE 1

EXPLANATION OF FIGURES

Labeled cells in olfactory ventricle. Gallocyanin-chromalum-stained autoradiograms; magnification, × 312.

19 Intensely-labeled cells in the wall of the olfactory ventricle of a rat injected with thymidine-H3 at six hours of age and killed six hours later. L, lumen of ventricle.

20 Increased number of labeled cells around the olfactory ventricle (with decrease in label concentration within cells) in a rat injected at six hours of age and killed three days later.
POSTNATAL NEUROGENESIS

Joseph Altman and Gopal D. Das

PLATE 1

19

20
Olfactory bulb. Migration of labeled cells from olfactory ventricle to the differentiating granular and mitral cell layers. Gallucyanin-chromalum-stained autoradiograms; magnification, ×122.

21 Rat injected with thymidine-H\(^3\) at six hours of age and killed three days later. Olfactory ventricle large and the majority of cells are labeled. A small proportion of the cells of the granular layer (G) are labeled, but very few in the mitral cell layer (M).

22 Rat injected with thymidine-H\(^3\) at six hours of age and killed 12 days later. The olfactory ventricle has receded and labeled cells have invaded the granular layer (G) as well as the mitral cell layer (M).
Olfactory bulb; higher magnification. Migration of labeled cells from olfactory ventricle to the granular and mitral cell layers. G, granular layer; M, mitral cell layer; V, subependymal matrix of the olfactory ventricle. Galloxyanin-chromalum-stained autoradiograms, magnification, $\times 312$.

23 Rat injected with thymidine-H$^3$ at six hours of age and killed 24 hours later. Few labeled cells are seen in the thin granular layer or in the mitral cell layer.

24 Rat injected at six hours of age and killed three days later. A few labeled cells have reached the widening granular layer.

25 Rat injected at six hours of age and killed 12 days later. A large proportion of the cells of the granular layer are labeled and labeled cells have reached the mitral cell layer.
PLATE 4

EXPLANATION OF FIGURE

26 The mitral cell layer, with mitral cells (M), and upper part of the granular layer (G), in a rat injected with thymidine-11C at six hours of age and killed 12 days later. Unlike the granular cells, the mitral cells are not labeled. Gallocyanin-chromalum-stained autoradiogram; magnification, × 883.
Dorsal hippocampus. Sequence of events in the postnatal neurogenesis of the granular layer of the dentate gyrus. Gallocyanin-chromalum-stained autoradiograms; magnification, $\times 122$.

27 Rat injected with thymidine-H$^1$ at six hour of age and killed six hours later. Intensely-labeled cells abound near the margin of the dentate gyrus, and near the presumptive granular layer and the basal polymorph cell layer of its internal arm. V, lateral ventricle.

28 Rat injected at six hours of age and killed 24 hours later. Apart from an increase in the number of labeled cells (combined with label dilution within cells), there are no major changes in the distribution of labeled cells.
Dorsal hippocampus. Sequence of events in the neurogenesis of the granular layer of the dentate gyrus (continuation of plate 5). Galloycyanin-chromalum-stained autoradiograms; magnification, × 122.

29 Rat injected with thymidine-H\(^{3}\) at six hours of age and killed six days later. The number of labeled cells in the basal polymorph cell layer of the dentate gyrus has greatly increased and many labeled cells are seen in the granular layer, particularly at its base.

30 Rat injected at six hours of age and killed 12 days later. The labeled cells have migrated from the basal polymorph cell layer into the granular layer, where their number has greatly increased. Many of the labeled cells in the granular layer are already differentiated granule cells.
Differentiated granule cells, with different degrees of labeling, in the dentate gyrus of a rat injected with thymidine-H\(^3\) at two days of age and killed 20 days later. Gallocyanin-chromalum-stained autoradiogram. Oil immersion; magnification, \(\times 1,312\).
Cells with arrested mitosis in the external granular layer of the cerebellar cortex in a rat injected with colchicine at two days of age and killed four hours later. EG, external granular layer; IG, internal granular layer; P, Purkinje cell layer.

32 Cresyl violet; magnification, × 312.
33 Cresyl violet; magnification, × 781.
PLATE 9
EXPLANATION OF FIGURES

Low-power photomicrographs of coronal sections of the brain through the midbrain, cerebellum and medulla. A, aqueduct; C, cerebellum; IC, inferior colliculus; IG, internal granular layer of cerebellar cortex; EG, external granular layer of cerebellar cortex; M, medulla; V, fourth ventricle. Gallocyanin-chromalum-stained autoradiograms; magnification, × 20.

34 Rat injected with thymidine-H\(^\text{3}\) at the age of six hours and killed 24 hours later. Concentration of labeled cells very high in the external granular layer of cerebellum. At this age few of the cerebellar folia are formed.

35 Rat injected at six hours of age and killed six days later. Concentration of labeled cells high in both the external and internal granular layer. The folia of the cerebellar cortex have increased in number with concomitant enlargement of the cerebellar cortex.
PLATE 10
EXPLANATION OF FIGURES

Sequence of events in the postnatal development of the cerebellum. 
EG, external granular layer; IG, internal granular layer; M, presumptive 
molecular layer; P, presumptive Purkinje cell layer. Gallo-cyanin-chroma-
malum-stained autoradiograms; magnification, ×312.

36 Rat injected at six hours of age with thymidine-H³ and killed six 
hours later. Concentration of intensely-labeled cells is high in the 
external granular layer, and there are also many labeled cells in the 
internal granular layer (particularly at the base of the layer).

37 Rat injected at six hours of age and killed 24 hours later. The pro-
portion of labeled cells has increased in the internal granular layer, 
and a reduction in label concentration within cells is already evident.
Sequence of events in the postnatal development of the cerebellum (continuation of plate 10). Gallocyanin-chromalum-stained autoradiograms; magnification, × 312.

38 Rat injected with thymidine-H\(^3\) at six hours of age and killed six days later. Label concentration is greatly reduced in cells of the external granular layer. A large proportion of the cells of the internal granular layer, particularly in its upper aspect, are now labeled. Purkinje cells, now recognizable as such, are beginning to form a separate layer.

39 Rat injected at six hours of age and killed 12 days later. Label dilution continues in cells of the external granular layer. The molecular layer has greatly increased in width and the Purkinje cells are approaching their adult size. Label dilution is also evident in the internal granular layer.
PLATE 12
EXPLANATION OF FIGURES

High-power photomicrographs, illustrating types of labeled cells in the cerebellar cortex of a rat injected at two days of age and killed 20 days later. A, astrocytes (Bergmann cells); GR, granule cells; GO, Golgi cells; P, Purkinje cells. Granule cells are darkly stained, but most of them are unlabeled.

40–41 Gallo cyanin-chromalum-stained autoradiograms; magnification, × 781.

42–43 Pale, labeled cell in figure 43 is not identified. Gallo cyanin-chromalum-stained autoradiograms. Oil immersion; magnification, × 1,952.
High-power photomicrographs of the various types of cells found with labeled nuclei in the cortex. From a single section from a rat injected with thymidine-H\(^3\) at the age of two days and killed 20 days later. Gallocyanin-chromalum-stained autoradiogram. Oil immersion; magnification, \(\times 1,952\).

44–47 Less frequently occurring types of labeled cells.

48–50 Commonly seen types of labeled cells in the cortex. It remains to be determined whether these are glia cells (astrocytes?) or neurons (granule cells).
51 Labeled cells in the ventral horn of the cervical spinal cord in a rat injected at two days of age and killed 20 days later. A, astrocytes; M, motor neuron; O, oligodendroglia. A large proportion of the astrocyte nuclei are labeled, whereas oligodendroglia are seldom labeled. Labeled cell in upper left corner may be a small neuron. The nuclei of large neurons are never labeled. Gallocyanin-chromalum-stained autoradiogram; magnification, × 781.

52 Labeled cells in the granular layer (GL) of the ventral cochlear nucleus in a rat injected with thymidine-H³ at two days of age and killed 20 days later. Gallocyanin-chromalum-stained autoradiogram; magnification, × 781.
Pattern of cell labeling around the third and lateral ventricles in a rat injected with thymidine-H$^+$ at the age of six hours and killed six hours later. Galloxyanin-chromalum-stained autoradiograms; magnification, $\times 133$.

53 Anterior portion of the lateral ventricle. CC, corpus callosum; CN, caudate nucleus; CP, choroid plexus in lateral ventricle; TH, thalamus. Concentration of labeled cells, and of cells in general, is highest in the subependymal matrix in the dorsolateral corner of the ventricle.

54 Concentration of labeled cells is high around the pineal recess in the roof of the third ventricle. The cells of the subcommissural organ (SC) are not labeled.

55 Concentration of labeled cells is low in the middle or thalamic portion of the third ventricle.

56 Concentration of labeled cells is high around the pituitary recess of the floor of the third ventricle.