J. Anat. (1970), **107**, 3, pp. 421–437
With 11 *figures*
Printed in Great Britain

Cytokinetics of neonatal brain cell development in rats as studied by the 'complete ^{3}H-thymidine labelling' method

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(*Received* 2 *October* 1969)

INTRODUCTION

Neural cells of the mantle layer of the cerebrum in the adult mammal are generally considered to be at rest cytokinetically and cannot be forced into regenerative activity after birth. Proliferative activity is assumed to be a prenatal phenomenon (Spielmeyer, 1922; Penfield, 1932). However, Messier, Leblond & Smart (1958) obtained evidence that a few nerve cells in the cortex of young rats can be labelled by means of tritiated thymidine (^{3}H-TdR) given as a single injection, and hence are in DNA-synthesis. This evidence for the existence of DNA synthesizing nerve cells after birth corresponds to the findings of Sugita (1918) and Smith (1934), who reported an increase in the number of neurons in the rat brain during the first 20 d of life. Occasional mitotic figures in the cerebral cortex were attributed mostly to glia cells (Hamilton, 1901; Allen, 1912; Noetzel & Siepman, 1965).

Altman recently published an extensive study of postnatal neurogenesis (Altman, 1962*a*, *b*, 1963, 1965, 1966; Altman & Das, 1965, 1966), in which cell proliferation in the brain of rats and cats was studied by means of autoradiography. He concluded from his findings that postnatal neurogenesis is restricted to a few short-axoned neurons in the brain cortex and that ^{3}H-TdR labelled neurons are also present on the dentate gyrus of the hippocampus, indicating the postnatal proliferative potential of the area of the cerebrum. In contrast, an appreciable fraction of neuroglial cells was found labelled throughout the brain. Similar results were obtained by other studies (Uzman, 1960; Angevine, 1965; Taber, 1966) by means of ^{3}H-TdR labelling methods.

In the present studies a new approach was used to investigate the cytokinetic aspects of the various cellular components of the rat brain after birth. It has been shown that *all* cells of a newborn animal can be labelled in their nuclear DNA if ^{3}H-TdR is available for all the DNA-synthetic phases which occur during the development of the foetus. This condition can be met by continuous intravenous infusion, or by sufficiently frequent injections of ^{3}H-TdR into pregnant rats during the entire period of gestation, or at least during the period of gestation when the organs of the foetus are being formed, i.e. from the 9th d of pregnancy to delivery. If there is no further ^{3}H-TdR administration after birth, the labelling intensity of cells is diluted as a function of their proliferative activity. 'Resting' cell populations retain their label throughout the life span of the cell or the organism (Haas *et al.* 1967; Fliedner, Haas, Stehle & Adams, 1968; Fliedner, Haas & Stehle, 1968). One

would expect that with this method all brain cells, regardless of their origin, would be ^{3}H-TdR labelled and that if they continued their DNA turnover postnatally their labelling intensity would decrease correspondingly.

MATERIALS AND METHODS

Animals

Four-month-old female Wistar rats with an average body weight of 180 g were used. Their stage in the ovulation cycle and time of fertilization, subsequent to copulation with male rats of the same strain, were determined by examination of vaginal smears (Preissecker, 1958).

Injection of ^{3}H-TdR

^{3}H-TdR was injected intraperitoneally into one rat every 6 h from the first day of pregnancy until the delivery of the newborn rats on the 22nd day. ^{3}H-TdR was injected every 6 h into six other rats from the 9th d of pregnancy until delivery. This regimen was based on the findings that organogenesis commences on the 9th d of pregnancy in the rat (Henneberg, 1937). Intervals of 6 h between injections of ^{3}H-TdR were chosen, because it has been found that the DNA synthesis time in tissues of the foetal rat is about 5–6 h (Fliedner, Kesse, Cronkite & Robertson, 1964; Wegener, Hollweg & Maurer, 1964).

Dosage of ^{3}H-TdR

^{3}H-TdR of a specific activity of 2 c/mM was injected every 6 h at a dose level of 0·4 μc/g body weight, giving a daily dose of 1·6 μc/g body weight. The total dose of the animal injected from the beginning of pregnancy to the day of delivery was 6·5 mc and in those injected from the 9th d of pregnancy to the day of delivery it was 4·2 mc.

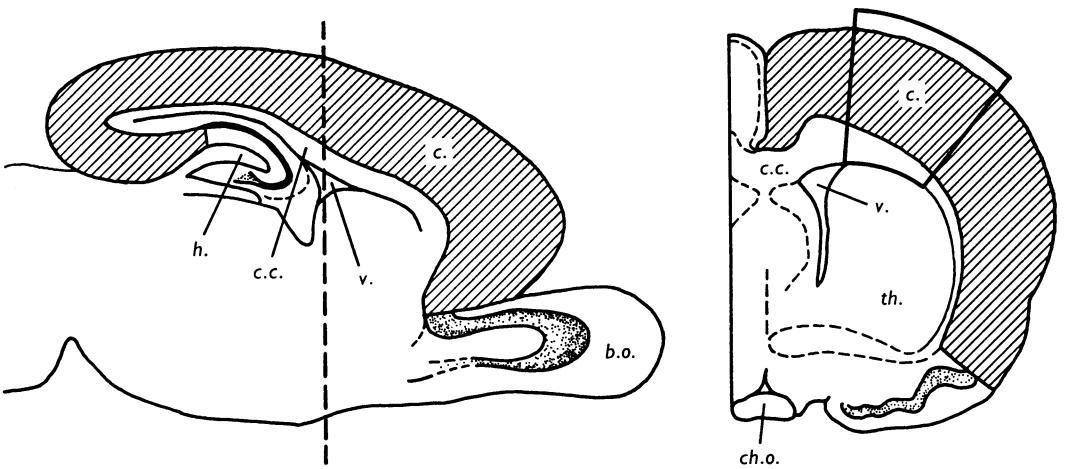

Fig. 1. Schematic representation of sagittal (a) and frontal (b) sections of the rat brain. The vertical line in (a) indicates the frontal plane from which tissue was taken for histological sections. In (b) is shown the sector of the brain cortex in which the labelling pattern was studied. *b.o.*, bulbus olfactorius; *c.*, cortex, *c.c.*; corpus callosum; *ch.o.*, chiasma opticum; *h.*, hippocampus; *th.*, thalamus; *v.*, ventriculus lateralis.

Sacrifice and examination of animals

Rats were killed at 1, 2, 6, 12, 24, 30 and 52 h, 3, 4, 6, 10, 14, 26 d and at 6, 18 and 20 weeks after birth. Frontal sections in the region of the chiasma opticum were taken and prepared for histological study (Fig. 1).

Autoradiography

Autoradiographs (3 μm thick sections) were made by the dipping technique, using Ilford L4 liquid emulsion and were exposed for 15 or 45 d. The approximate thickness of the emulsion was 3 μm. After processing of the autoradiographs, sections were stained with hematoxylin and eosin. For demonstrations of glia cells, one set of histological sections was stained with a Miquel–Calvo stain (Miquel & Calvo, 1958).

Evaluation of autoradiographs of brain sections

The final architecture of the brain cortex of the rat is not acquired until the 6th d of neonatal life. It was thus possible in animals sacrificed during the first 5 d to distinguish only between a superficial layer (layer A) consisting mostly of glia cells, an upper adjacent layer (layer B), a middle layer (layer C) and a deep nerve cell layer (layer D) (Fig. 3*a*).

From the 6th d of life onwards, the various layers of brain cortex could be identified and were numbered from I to VIb, according to the study of Sugita on the cortical cell lamination in the brain of the rat (Sugita, 1918). The following nomenclature was used and is illustrated in Fig. 2. The superficial layer of the cortex immediately below the pia was called layer I (lamina zonalis). The following layers II (lamina granularis externa) and III (laminar pyramidalis) were evaluated together. The next layers IV (lamina granularis interna) and V (lamina ganglionaris) as well as the layers VIa and VIb (lamina multiformis) were distinguished and evaluated separately. Layer VIb is adjacent to the corpus callosum. In all layers, neurons, astrocytes and oligodendrocytes were evaluated.

In the subependymal layer of the rat brain two cell types were distinguished (Smart, 1961). One type is a dark nucleated 'undifferentiated' cell and the other a light nucleated 'undifferentiated' cell.

Grain counts

For all time intervals studied after birth, 200 cells were evaluated in each of the layers distinguished and were classified according to their type (nerve cells, astrocytes, oligodendrocytes). The number of grains in the film emulsion overlying the nuclei was recorded and from this the labelling index and the mean grain count for each cell group were calculated.

Background correction

Autoradiographs of unlabelled sections showed that background was less than three grains per cell nucleus. Labelled cells were therefore considered to be cells with three or more grains.

Measurement of DNA content

From animals sacrificed at 6 h, 3, 5, 10 and 21 d after birth frontal sections of various thickness were prepared according to the nuclear diameters (Sandritter, 1966). In the Feulgen stained sections arbitrary units proportional to the DNA content of 250 cortical neuronal cells were measured by an integrating Microdensitometer (Barr & Stroud, Glasgow).

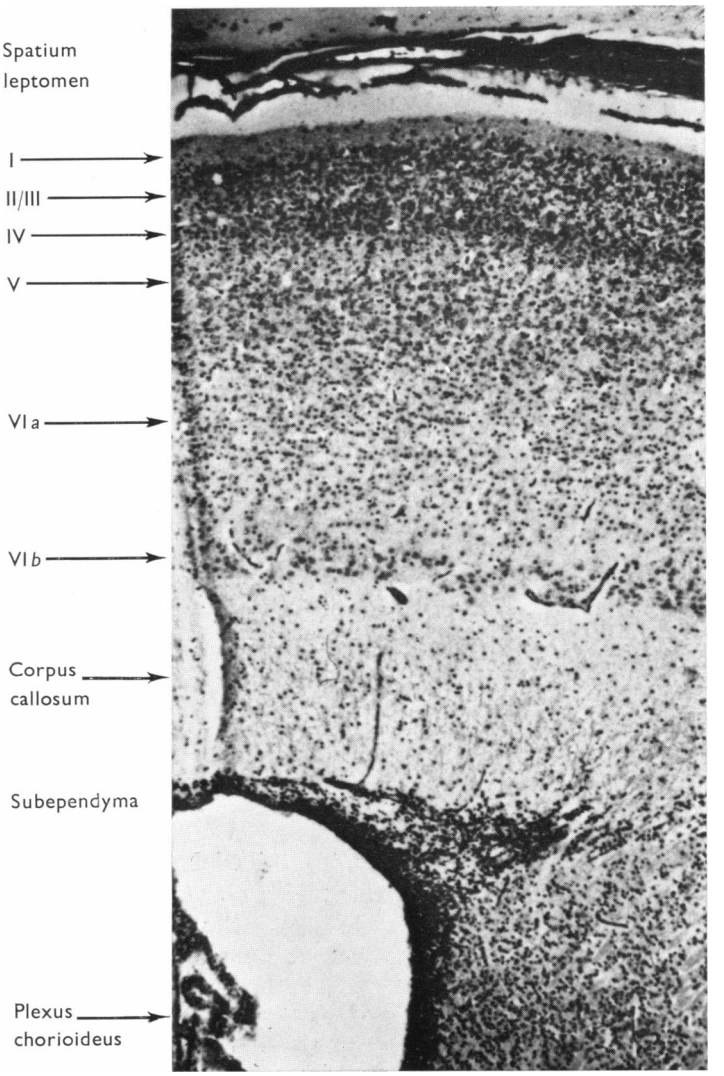

Fig. 2. Survey of the region of investigation showing the subdivision of the cortex into layers I to VI (brain of a 6 d old rat). I, lamina zonalis; II, lamina granularis externa; III, lamina pyramidalis; IV, lamina granularis interna; V, lamina ganglionaris; VI, lamina multiformis.

Measurement of mean nuclear diameters

The nuclear diameters of each 100 nerve cells in each of the different cerebral layers at 2 h, 3, 5, 6, 10 and 26 d in 3 animals at each point were measured in the same sections which were used for measurement of DNA content.

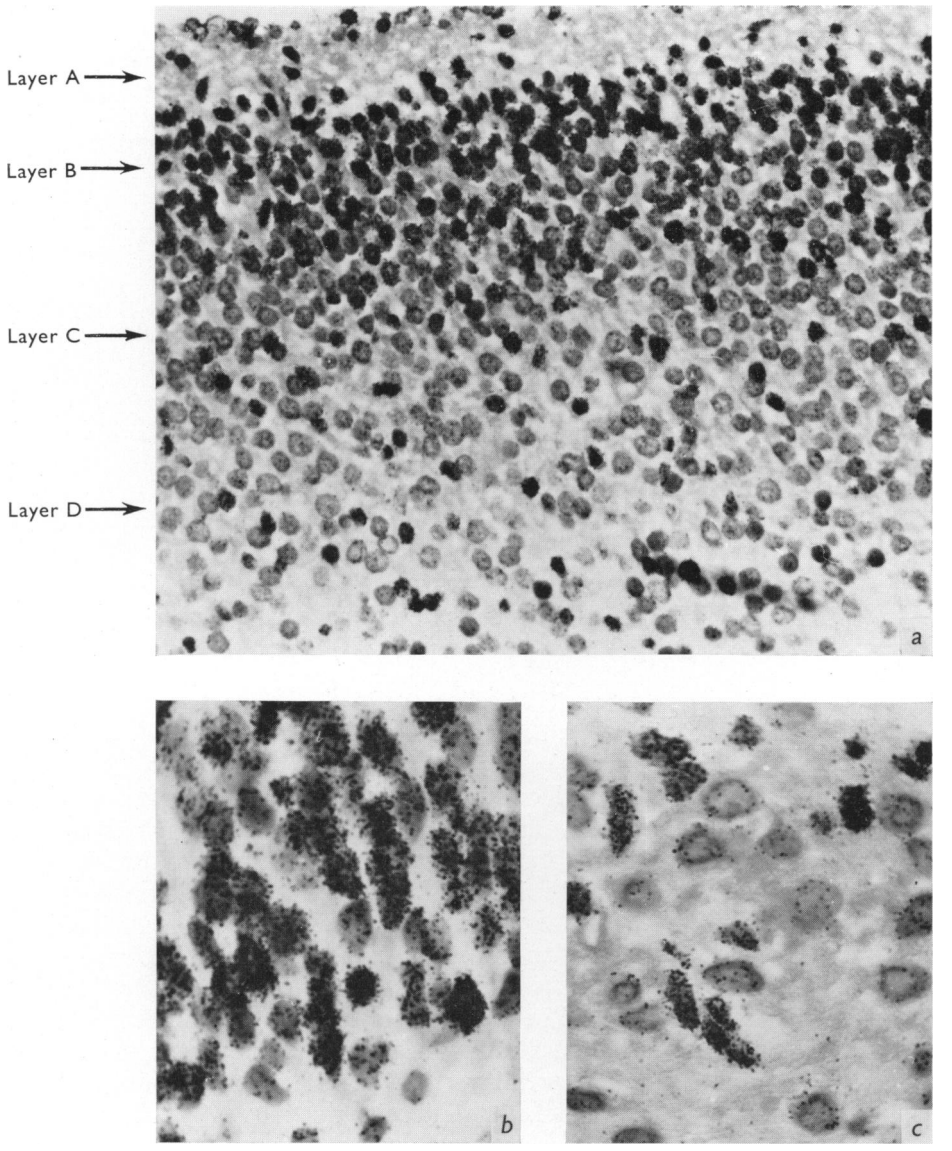

Fig. 3(*a*). Autoradiograph of a section from the cerebral cortex of a newborn animal (× 580). As indicated, four layers (A–D) were distinguished for evaluation. (b) Higher magnification of layer B of Fig. 3*a*. × 1160. (*c*) Higher magnification of layer C of Fig. 3*a* showing variation in labelling intensity (Ilford L4 emulsion, exposed 45 days. Haematoxylin–eosin). × 1160.

RESULTS

The labelling pattern of brain sections of ^{3}H-TdR labelled rats sacrificed within 2 h after birth

The evaluation of labelling index and labelling intensity in brain cells at birth resulted in no statistically significant difference, when animals whose mothers had received ^{3}H-TdR from day 9 of pregnancy until term were compared with animals from mothers which received ^{3}H-TdR from the first day of pregnancy onward.

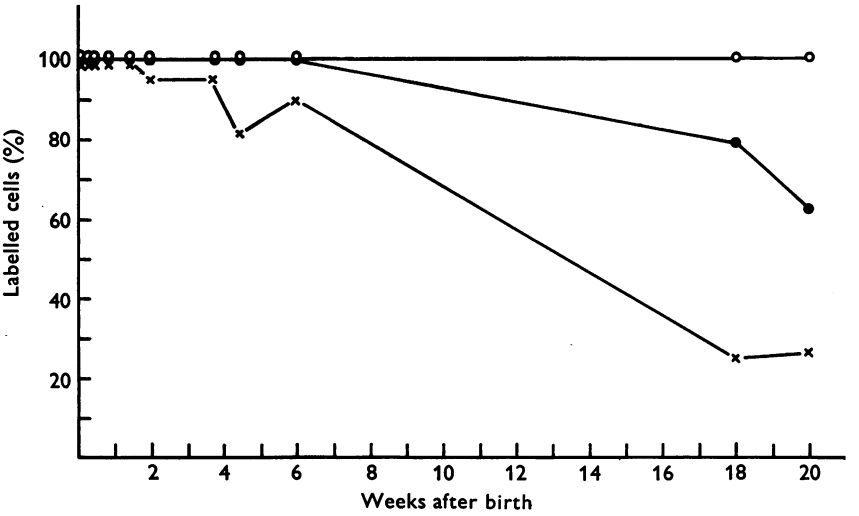

Fig. 4. Labelling index of nerve and glia cells as a function of time after birth. ○—○, neurons; ●—●, astrocytes; ×—×, oligodendrocytes.

All the nuclei of both nerve and glia cells were labelled after an autoradiographic exposure time of only 15 d. In Fig. 3*a* the pattern of labelling in the different layers A–D is compared. Although the labelling index of all cells was 100% their labelling intensity varied considerably in different layers of the cerebral cortex. In layer A nearly all cells were glia cells. These showed a uniform labelling intensity of about 30–40 grains per cell after an autoradiographic exposure time of 45 d. In layer B the population of neural cells at the time of birth had a mean grain count of about 70 grains' per cell (Fig. 3*b*). The mean nuclear diameter of these cells was $6{\cdot}0 \pm 0{\cdot}7$ μm. The glia cells (astrocytes and oligodendrocytes) had a labelling intensity of about 30–40 grains per cell.

The nerve cells in layer C could be divided into two classes of different labelling intensity. One cell group consisted of a few heavily labelled nerve cells with grain counts of about 70 grains per cell as in layer B, while the other group contained the majority of the nerve cells whose labelling intensity was markedly less (Fig. 3*a*). If these two groups of cells were considered as one population, then the mean grain count was found to be about 40 grains per cell. The mean nuclear diameter was $7{\cdot}3 \pm 0{\cdot}9$ μm. The few glia cells in the layer C had a similar labelling intensity to those in the layers so far described, i.e. 30–40 grains per cell.

In layer D a few nerve cells with a rounder, deeply stained, highly labelled nucleus could be detected, while the remaining neural cells showed a lower labelling intensity, similar to that seen in the majority of nerve cells in the middle layer (Fig. 3*c*). The mean nuclear diameter was $6{\cdot}2 \pm 0{\cdot}8$ μm.

The two major cell types of the subependymal layers on the vicinity of the lateral ventricle, i.e. the dark nucleated and light nucleated cells, had a high labelling intensity. The mean grain count over these cells was about 80–90 grains per cell.

Changes of the labelling pattern as a function of time after birth in brain sections of 'completely labelled' rats

The temporal changes of ^{3}H-TdR labelling of neurons, astrocytes and oligodendrocytes were studied over a period of 5 months after discontinuation of ^{3}H-TdR administration at birth. In Fig. 4 the labelling index of these cell types is plotted as

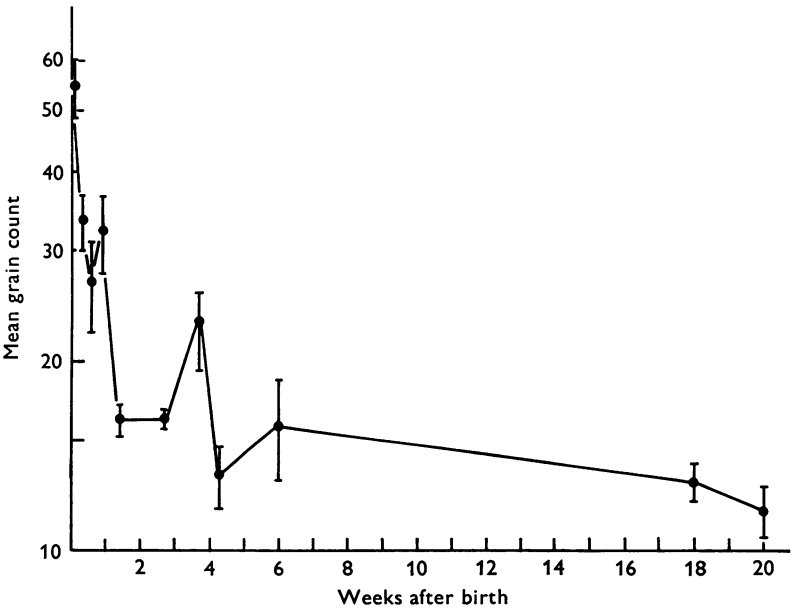

Fig. 5. Labelling intensity of nerve cells of different layers in the brain cortex as a function of time after birth. Vertical bars represent standard error.

a function of time. Two different types of cytokinetic behaviour could be distinguished. There is no doubt that the labelling index of all neuronal cells remained 100% for the entire observation period of 20 weeks. In contrast, the fraction of labelled astrocytes and oligodendrocytes decreased progressively, the labelling index of astrocytes reaching about 60–70% and that of the oligodendrocytes about 30% by 5 months after birth.

This observation was substantiated by the labelling intensity data. In Fig. 5 the labelling intensity of all neurons of the brain cortex is plotted as a function of time after birth. There was a marked decrease in mean grain count for a period of about 10–14 d after birth, but thereafter it appeared to remain approximately constant.

In Fig. 6 the diminution of the mean grain count of all astrocytes and oligodendrocytes is shown. During the first 10 d after birth, there was a reduction of the labelling intensity to about one quarter. From 2 weeks to 6 weeks after birth the mean grain count over astrocytes remained constant between 7 and 14 grains per cell, and over the oligodendrocytes it remained constant between 5 and 9 grains per cell. Between

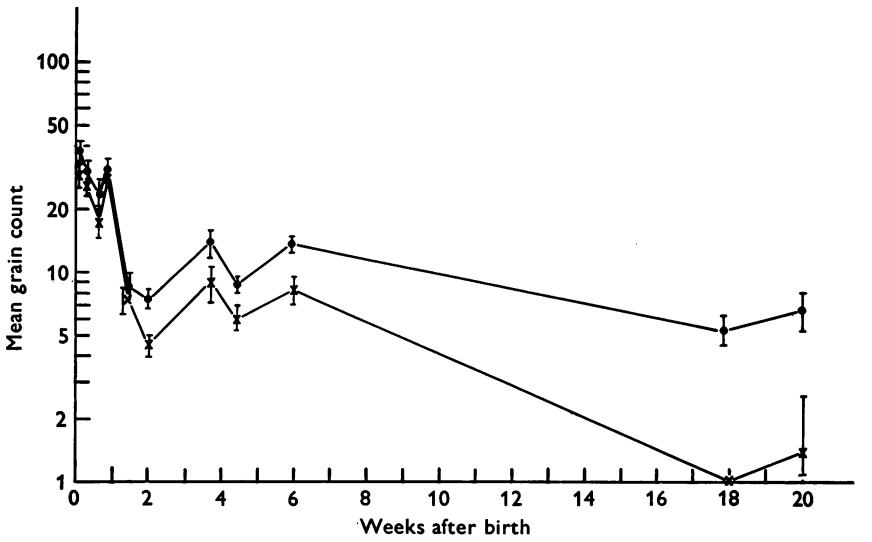

Fig. 6. Labelling intensity of glia cells as a function of time after birth. Vertical bars represent standard error; ●—●, astrocytes; ×—×, oligodendrocytes.

6 and 20 weeks after birth the difference between astrocytes and oligodendrocytes became increasingly marked. While there was no appreciable further dilution of the labelling intensity over astrocytes (in agreement with the labelling indices as shown in Fig. 4) the labelling intensity over the oligodendrocytes showed a further dilution, so that the mean grain count of *all* oligodendrocytes approached one grain per cell. However, about 20% of these cells showed three or more grains and hence were considered to be still labelled (Fig. 4), the mean grain count of the labelled population being 4–6 grains per cell.

In Fig. 7 the decrease of labelling intensity of neural cells of the different layers in the brain is shown as a function of time. Part A of the figure is drawn according to the theory of brain cortex development put forward by Berry & Rogers (1965) but for the first 5 d of life only three separate layers of nerve cells (layer B, layer C and layer D) were differentiated. Inset numbers indicate the mean grain count evaluated over neural cells in these layers. The labelling intensity decreased in all three parts of the cortex, although the initial degree of labelling at the time of birth was markedly different. The mean diameter of neuronal nuclei increased between birth and day 3 of newborn life by 8% in layer B by 16% in layer C and by 15% in layer D.

Part B of Fig. 7 shows that in the six layers identified from day 6 onwards the

labelling intensity was further diluted until day 10 after birth. A comparison of the mean grain count of layers II and III at day 6 with the degree of labelling of the layers in part A of Fig. 7 shows that layers II and III started with a higher labelling intensity than was found in any cortical layer before this time. The labelling intensity in layer II/III decreased between day 6 and day 10 of postnatal life from 55 to about 18 grains per cell. In contrast the diminution of the grain count of the cells in layer V and VI was markedly less. As can be noticed, there was only a reduction from 25 grains to about 15 during the same time interval.

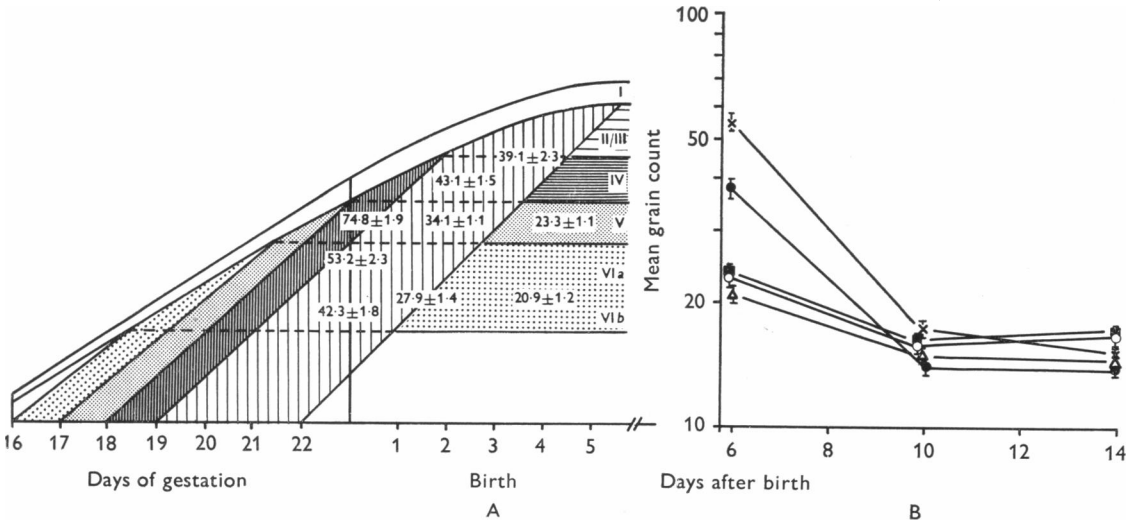

Fig. 7. Change in the labelling intensity of neuronal cells in different layers of the cerebral cortex as a function of time. A: Scheme of the development of cortical brain layers according to Berry & Rogers. The inset numbers indicate the mean grain count observed during the first 5 d after birth in the three layers evaluated. B: Change in labelling intensity of nerve cells from day 6 to day 10 after birth in the six layers evaluated. I, lamina zonalis; II, lamina granularis externa; III, lamina pyramidalis; IV, lamina granularis interna; V, lamina ganglionaris; VI, lamina multiformis. Vertical bars represent standard error. The decrease of mean grain count in all layers between day 6 and day 10 is highly significant ($P < 0{\cdot}001$) if one compares the data of day 6 with the data of day 10 and day 14 after birth. ×—×, N II/III; ●—●, N IV; △—△, N V; ○—○, N VI*a*; ■—■, N VI*b*.

The mean diameter of neuronal nuclei increased between day 5 and day 10 of postnatal life by 43 % in layer II/III, by 26 % in layer IV, by 5 % in layer V, by 3 % in layer VIa and by 14 % in layer VIb. Between day 10 and day 26 the mean diameter of neuronal nuclei was found to remain constant.

The labelling pattern of light and dark nucleated cells that are located in the subependymal zone of the lateral ventricle was also investigated. As shown in Fig. 8, the labelling index of the light nucleated cells remains constant at 100 %, or close to it, during 42 d of observation. In contrast, the labelling index of the dark nucleated cells decreases progressively from 100 % to about 30 % at 42 d after birth.

In Fig. 9 the changes of the labelling intensity as a function of time are demonstrated for these two cell types. Over the dark nucleated cells the mean grain count diminished

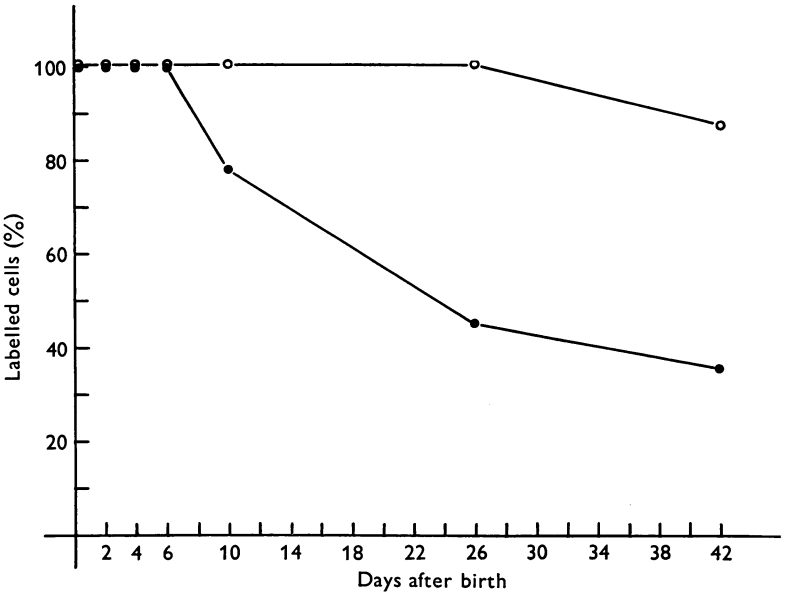

Fig. 8. Labelling index of the subependymal cells of the lateral ventrical as a function of time after birth. ○—○, light nucleated subependymal cells; ●—●, dark nucleated subependymal cells.

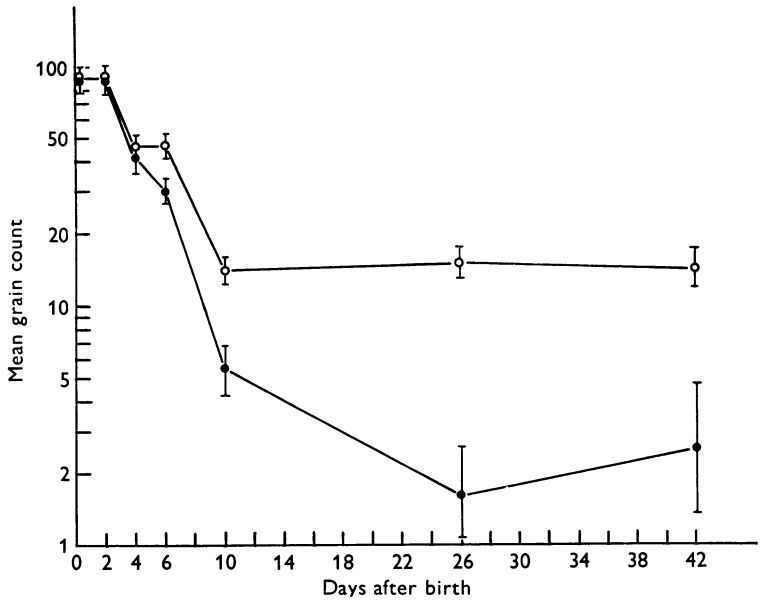

Fig. 9. Labelling intensity of the subependymal cells as a function of time after birth. Vertical bars represent standard error. ○—○, Light nucleated subependymal cells; ●—●, dark nucleated subependymal cells.

from 90 to about two grains per cell within 4 weeks. In contrast, the mean labelling intensity of the light nucleated cells decreased from 90 to about 15 grains per cell within 10 d and remained constant thereafter. Some mitotic figures in the subependymal layer could be observed, although it was difficult to say to which of the two cell types the mitoses belonged.

DISCUSSION

The findings presented serve to demonstrate a new approach to the study of the development and DNA turnover of cells of the central nervous system in the rat by means of tritiated thymidine (^{3}H-TdR) labelling. The complete labelling of all cell nuclei at birth makes it possible to study the development of the brain in the neonatal period by observation of the diminution in labelling index and labelling intensity of various cell types. The method serves not only to advance new arguments for or against the existing migration theories of central nervous system development, but also to compare the neonatal proliferative activities of nerve cells and glial cells.

The results have shown that all cell nuclei of the central nervous system of newborn rats are labelled after repeated injections of ^{3}H-TdR into pregnant rats from day one of pregnancy onward or at least from the onset of organogenesis (day 9 of pregnancy) until delivery. Previous communications (Fliedner *et al.* 1968) have described the usefulness of this method in the study of the cellular kinetics of slowly proliferating cell systems. These earlier studies failed to provide significant evidence for radiation toxicity to the offspring, when the daily dose administered to the mothers was 1·6 μc/g body weight.

The findings of the present study have indicated that at the time of birth the labelling intensity of nerve cells varies considerably throughout the cortex of the brain; two developmental processes could explain this pattern and must now be considered. One process is the migration of cells from the site of formation (ependymal layer) to their functional position, the labelling pattern at the moment of birth showing only one particular stage in a development process which commences several days before birth and ends several days afterwards. The other process is a possible further nerve cell DNA turnover after birth, which would result in a diminution of labelling intensity of single cells.

The present findings can be construed to support the hypothesis of nerve cell migration, put forward by Berry & Rogers (1965) from studies in the rat after a single injection of ^{3}H-TdR. The observations of Angevine & Sidman (1961) in the mouse after a single injection of ^{3}H-TdR also support this hypothesis. Berry and Rogers proposed that the six cell layers of the rat brain develop by a complex migration of nerve cells, originating from the ependymal and subependymal layers, to their final positions, the migration commencing at about the 16th d of gestation. The early formed cells which migrate on the 16th d later on build up layer VI. The cells which originate on the 17 d of gestation migrate through layer VI and form layer V, whereas those formed at the 18th d pass through both layers VI and V and end up in layer IV. The cells born at days 19, 20 and 21 of gestation cross all three layers and settle as layers II and III close to the surface.

If the concept is true, and if cells lose their proliferative capacity at the time they

leave the subependymal layer, they would at the same time cease to incorporate ^{3}H-TdR. Thus, these initially formed cells might show a lower labelling intensity as compared with those continuing to proliferate until birth or before they reach their final destination. In preliminary investigations (Werner, 1969) it has been found that there is an increase of labelling intensity of cells in the subependymal layer during late embryonic life, the labelling intensity at the 21st d of gestation being approximately four times that found at the 16th d. If cells cease to divide after leaving the subependymal layer and thereby retain the labelling intensity which they had acquired by the start of migration, it would be expected that those cells which migrate early, e.g. on the 16th d of gestation, would have a lower labelling intensity than those cells which do not migrate until the 20th or 21st d of gestation. The fact that we find the labelling intensity of nerve cells in layers V and VI to be lower than that in layers II and III is therefore consistent with the concept that layers V and VI are formed earlier than layers II and III. The occurrence of occasional heavily labelled nerve cells in the middle layer at birth amongst a majority population of less intensely labelled cells could then be explained as the migration of heavily labelled cells from the subependymal cells through the middle layer to form layers II and III. The labelling pattern seen at birth could then be understood as a composite picture of cells that have already reached their final position in the brain cortex and of cells that are still in their process of migration towards the surface.

Although the findings presented do not prove the model of Berry & Rogers, they argue strongly against the classical concept of brain development, advanced by Tilney in 1933. According to this latter theory, layers II and III are formed first while the inner layers are added consecutively one after the other from the subependymal layer. If this concept is correct, we would have expected to find the cells with the lowest labelling intensity in layers V and VI. The reverse, however, is true as shown and discussed above.

Besides the migration process the finding of a diminution of labelling intensity observed in the nerve cells remains to be discussed (Figs. 5, 7). Some decrease of labelling intensity in neural cells during the first days after birth would be in agreement with the findings obtained by flash-injections of ^{3}H-TdR at the time of birth into unlabelled newborn rats as performed by Altman (1962*a*, *b*, 1963, 1965, 1966; Altman & Das, 1965, 1966). These investigations showed the incorporation of ^{3}H-TdR into a few nerve cells of all nerve cell layers indicating continued DNA synthesis after birth.

For the interpretation of the diminution of the labelling intensity in the present study over the nerve cells in different layers it is necessary to allow for increase in cell volume and to correct the observed mean grain count over nuclei for variation in nuclear diameter as seen in the histological sections. For nuclei having the same total amount of radioactivity but different nuclear volume, the mean grain count per sectioned nucleus will be inversely proportional to the radius r (Pelc & Welton, 1967; Appleton, Pelc & Tarbit, 1969). The relation between the number (n) of atoms in a layer of nucleus of thickness t and volume v and the total number of atoms (N) in a nucleus of volume V can be expressed according to Appleton *et al.* (1969) by:

$$n = N\frac{v}{V}N\frac{3r^2\pi t}{4r^3\pi} = N\frac{3t}{4r}.$$

Since the grain count is proportional to the contained radioactivity, this relationship can be used to correct the grain counts for change in nuclear size. In the following table the observed grain counts over a sectioned nucleus of volume v have been used to calculate the expected grain counts from a nucleus of total volume V.

The calculation demonstrates that even after adjustment of the mean grain count per nucleus to the changes in nuclear diameters there is still a considerable decrease of labelling intensity. In particular the labelling pattern of the separate layers between day 6 and day 10 after birth indicates a continuation of DNA turnover mainly in layers II/III and IV.

Table 1.

Layer	Time after birth	2r in μm	n	N
B	2 h	6·0±0·7	74·8±1·9	99·7
	3 d	6·5±0·7	39·1±2·3	55·6
C	2 h	7·3±0·9	53·2±2·3	87·5
	3 d	8·5±1·0	23·3±1·1	43·5
D	2 h	6·2±0·9	42·3±1·8	58·3
	3 d	7·1±1·1	20·9±1·2	32·5
N II/III	5 d	7·2±0·9	55·2±2·1	88·3
	10 d	10·3±1·0	18·5±1·2	41·9
N IV	5 d	6·8±0·6	38·6±2·7	58·3
	10 d	8·6±0·6	14·5±0·9	27·7
N V	5 d	10·4±1·7	21·2±1·0	49·0
	10 d	10·9±1·0	15·3±0·5	37·4
N VI	5 d	8·4±0·8	22·4±1·0	38·8
	10 d	10·2±0·9	15·4±0·9	35·5

Further information can be obtained by the histogram, in which the distribution of grain counts in percentage is demonstrated for the different classes of labelling intensity (Fig. 10). As indicated, the decrease of mean grain count of neuronal cells is mainly due to a disappearance of heavily labelled cells. A possible explanation for these findings also would be that at least some of the neuronal cells continue to divide after day 6 p.p. In contrast to this assumption in the cerebral cortex after birth almost no mitotic figures could be observed in neuronal cells. Therefore the relative high rate of decrease in grain counts over neuronal cells may be at least partially due to other processes of DNA renewal.

Densitometric measurements of DNA-content of nerve cells in Feulgen stained brain sections (Sandritter, Pilney, Novakova & Kiefer, 1966; Lapham, 1967) from our animals have shown that there is no significant increase in DNA-content of cells, indicating that the ploidy level is not changing during the first 10 d of life, as shown in Fig. 11.

One of the possibilities to explain the observed renewal of DNA not leading to mitosis or polyploidy would be the assumption of the existence of metabolic DNA of neuronal cells. Recent data (Pelc, 1962, 1964, 1968) suggest that in 'non dividing tissues' there is metabolic DNA which is formed immediately after the last mitosis and connected with the differentiation of cells. If this is true for neuronal cells, a more

rapid DNA turnover in differentiating nerve cells than in mature neurons could then be expected. The loss of tritiated thymidine-labelled DNA would then be correlated with the development of differentiation of neuronal cells.

An alternative explanation for the decrease of labelling intensity would be the immigration of cells with progressively lower mean grain count from elsewhere. However, all published observations up to now point to the fact that the migration

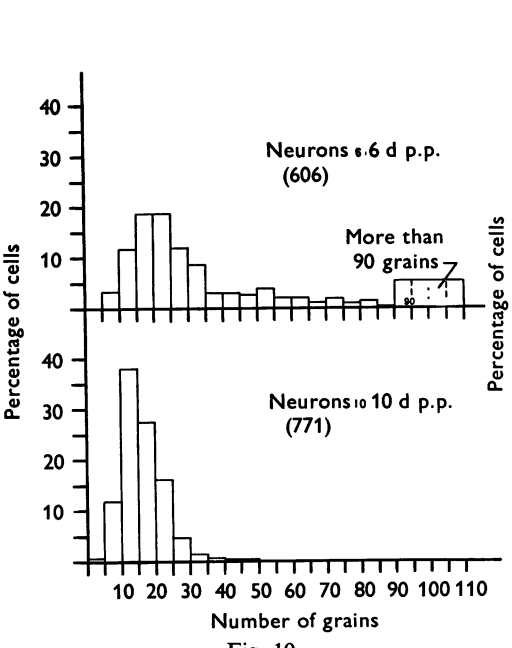

Fig. 10

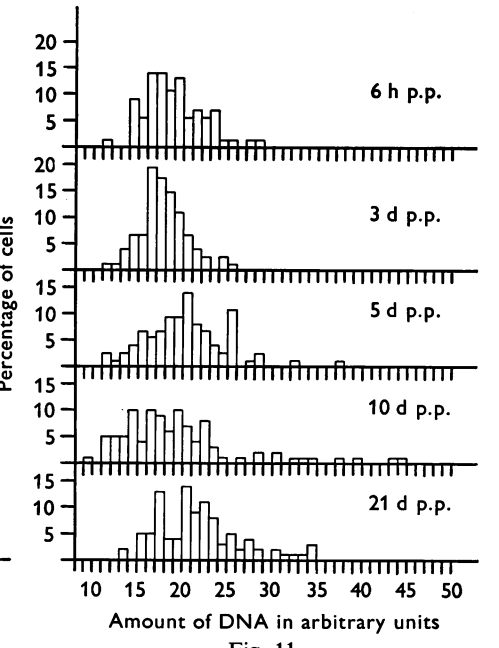

Fig. 11

Fig. 10. Histogram of grain counts of neural cells 6 and 10 days after birth. Figures in parentheses indicate the number of cells evaluated.

Fig. 11. Histogram showing DNA content per cell in arbitrary units for different times after birth. Between 6 and 10 d there is no appreciable increase in polyploid cells.

process ceases within the first 6 d after birth (Berry & Rogers, 1965). On the other hand, it is possible that the heavily labelled cells disappear because of cell death perhaps due to radiation toxicity, although the ^{3}H-TdR dosage used did not otherwise provide significant evidence for such damage (Fliedner *et al.* 1968). In addition, no morphological evidence of nerve cell death (as is seen after very high doses of external ionizing irradiation) was observed.

A final word should be said about the proliferative kinetics of the nerve and glial cell precursors that are supposed to be located in the subependymal zone. This zone is considered to be the prenatal 'stem-cell pool' of brain cells (Bryans, 1959; Smart, 1961). Morphologically, these cells were described above as the 'light' and the 'dark' nucleated subependymal cells. While the light nucleated cells appear to stop proliferation at about 10 d after birth—as indicated by the grain count diminution data presented in Fig. 9—the dark nucleated cells appear to go on dividing somewhat

longer. These findings correlate with observed mitotic figures in the subependymal area. The function of these cell types after birth is not known, although some other investigators feel that the dark nucleated cells are possibly precursors of corpus callosum glial cells during the postnatal period (Smart, 1961; Noetzel & Siepman, 1965). On the other hand, it has been suggested by Smart and others (Hamilton, 1901; Altman, 1965) that the light nucleated cells could postnatally serve as precursor cells for granularis cells of the hippocampus and other areas. Although our data cannot prove or disprove this theory, they may well support it, since both light nucleated cells and dark nucleated cells show a very active cell turnover during the first 10 d of life as evidenced by the grain reduction to about one tenth of the value at birth during this time.

SUMMARY

1. The complete ^{3}H-thymidine labelling method of all cell nuclei of newborn rats has been used to investigate the development of the nerve cell population of the cortex of the brain with respect to their postnatal DNA turnover and to obtain arguments for the nerve cell migration theory of Berry & Rogers.

2. The findings present evidence that some nerve cells cease to divide before birth and thus show a lower labelling intensity than those that continue DNA synthesis until birth. Furthermore, the dilution of labelling of some cells in all cell layers, especially in the superficial brain cell layers, indicates their continued DNA turnover. Beyond this, the data shown can be interpreted to support the theory of Berry & Rogers, according to which the layers of the cortex are formed by complex migration processes.

3. The findings provide evidence that glial cells, independent of their topographical position, may continue to divide until birth. After birth, some proliferative activity appears to be present throughout the observation period of 20 weeks.

4. In the subependymal layer of the lateral ventricle, there is evidence for a continuation of cell proliferation of light as well as of dark nucleated cells for at least 10 d after birth. After day 10 only dark nucleated cells appear to proliferate somewhat longer.

We are deeply indebted to Dr E. Harriss for helpful criticism and discussion.

Research supported by the European Atomic Energy Community (EURATOM contract Nos. 031-64-1-BIAD and 072-68-1-BIOD) and the Bundesministerium für Wissenschaftliche Forschung.

Parts of this paper will be submitted as a doctoral thesis to the Faculty of Theoretical Medicine of the University of Ulm by J.W.

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