STABILITY OF DNA IN PURKINJE CELL NUCLEI OF THE MOUSE

An Autoradiographic Study

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ABSTRACT

Neurons of the mouse were labeled with [^{3}H]thymidine during their prenatal period of proliferation. The ^{3}H activity of the Purkinje cell nuclei was then studied autoradiographically 8, 25, 55, and 90 days after birth. The measured grain number per nucleus decreased by about 14% between the 8th and 25th postnatal days and then remained constant up to 90 days. There was no significant decrease of the ^{3}H activity of the Purkinje cell nuclei after correction of the measured grain number per nucleus for increasing nuclear volume of the growing Purkinje cells and for the influence of [^{3}H]β self-absorption in the material of the sections.

Injection of a high dose of [^{3}H]thymidine into young adult mice did not result in ^{3}H labeling of either Purkinje or other neurons in other brain regions.

The results agree with the concept of metabolic stability of nuclear DNA. "Metabolic" DNA could not be observed in these experiments.

INTRODUCTION

During recent years the concept of metabolic stability of the nuclear DNA of a cell has been extended by the assumption that in addition to the stable "genetic" DNA, an unstable "metabolic" DNA exists. However, conclusive experimental proof of one or the other concept is still lacking.

Cytophotometric determinations of the nuclear DNA content have shown that the amount of DNA possibly might change depending upon the corresponding physiological conditions. However, quite a number of authors have not been able to confirm those results (see Roels, 1966; Mareš and Lodin, 1971; Pelc, 1972). Even constancy in the nuclear DNA content does not prove metabolic stability of DNA, since extensive turnover might occur without change in the total amount of DNA.

However, it is also possible in this case to study the metabolic stability of DNA by investigating DNA prelabeled with [^{3}H]thymidine. If the DNA is stable, the ^{3}H labeling should be constant with increasing time after [^{3}H]thymidine application. Neurons are an ideal subject for those studies, since they no longer divide in the adult animal.

The advantage of the autoradiographic method used in the present study lies in the possibility of investigating the labeling of single neurons. Purkinje cells are of special interest with respect to metabolic DNA because of discrepancies in the data obtained in previous autoradiographic and Feulgen studies. Former determinations of the Feulgen DNA in Purkinje cells have shown that the amount of DNA increases by about 60%

between the 2nd and 150th postnatal days (Mareš et al., 1973). On the other hand, no incorporation of even high doses of [^{3}H]thymidine into Purkinje cells of young adult mice was observed within this time interval. In the meantime, Cohen et al. (1973) were able to show by chemical determination of the DNA in mechanically isolated Purkinje cells that the Purkinje cells remain diploid after birth. This constancy in ploidy also makes these cells a preferred subject for studying the metabolic stability of DNA.

MATERIALS AND METHODS

Animals

Mice of the NMRI and CBA strain (Zentralinstitut für Versuchstierzucht, Hannover, W. Germany) were used. 33 offspring of five mother animals (NMRI) and four young adult mice (CBA) were studied. Mice were mated overnight for 12 h. Midnight was taken as time zero of pregnancy. The animals were kept in air-conditioned animal rooms at 23°C, with artificial light from 6 am to 6 pm. The adult animals were fed with Altromin (Altrogge, Lage/Lippe, W. Germany) standard diet and water ad libitum, and the offspring were suckled up to 28 days postnatally.

Labeled Thymidine

[*methyl*-^{3}H]Thymidine (6.7 Ci/mmol) from New England Nuclear, Boston, Mass., and [6-^{3}H]thymidine (20.0 Ci/mmol, from the Institute for Isotope Research and Utilisation, Prague), were used.

Administration of Labeled Thymidine

Pregnant mice received the labeled thymidine as daily intraperitoneal injections. For dosage and time of administration see Table I. In other experiments, young adult mice (18–20 g, CBA strain) received intraperitoneal injections of 60 μCi/g body wt [^{3}H]thymidine.

Preparation of Sections and Autoradiographs

The animals were killed by decapitation without anesthesia. Brains were immediately removed and fixed by immersion in Carnoy solution (absolute alcohol, chloroform, glacial acetic acid, 6:3:1) for 12 h at 4°C and embedded in Paraplast. 5 μm sections were cut from the central sagittal plane of the brain. Autoradiographs were prepared using AR 10 stripping film (stripped off in a 0.5% solution of KBr) and were exposed for 14 days–3 mo at +4°C or −20°C, respectively. The autoradiographs were stained through the emulsion with Mayer's haematoxylin and eosin (1%).

Evaluation of the Autoradiographs

The number of grains per nucleus was counted using an oil-immersion objective. The background, evaluated by counting grains in a corresponding area of the neuropile surrounding the labeled nuclei, ranged from zero to two grains for 100 μm^2.

DNAse Treatment

Sections were incubated with DNAse I (Sigma Chemical Corp., St. Louis, Mo., 0.05 mg/ml of Tris-maleic buffer), or with buffer only, for 24 h at 37°C, and autoradiographs were then prepared. After DNAse treatment no grains were observed over the nuclei; buffer only had no effect.

Nuclear Size Measurement

Minimal and maximal diameters (a, b) of Purkinje cell nuclei in the 5-μm thick sections were measured by an ocular microscale. The area of the nucleus in the sections was determined according to the formula,

$$\frac{a \cdot b}{4} \cdot \pi$$

Mass Density Measurements

Deparaffinized sections (5 μm) were mounted in distilled water. The optical-path difference (Γ cm) was determined using a double-beam microinterferometer (E. Leitz, Inc., Wetzlar, West Germany), in the "homogeneous light field" with monochromatic light (Hg green, λ = 5461 ÅU). The mass density (grams per square centimeter) was calculated according to the formula,

$$\text{mass density} \frac{g}{cm^2} = \frac{\Gamma}{\alpha},$$

where α = specific increment. For protein and DNA, α = 0.170 (cm^3/g) (Vöhringer and Maurer, 1971).

Statistical Evaluation

The results are given as means ± SEM. Statistical significance was estimated by using student's t test.

RESULTS

Labeling of Neuronal DNA by Prenatal Injection of [^{3}H]Thymidine

Labeling of neurons with [^{3}H]thymidine is only possible during embryonic proliferation of the neuroepithelial precursor cells. These neuroepithelial cells then differentiate into labeled neuroblasts. The neuroblasts do not divide but further differentiate into labeled neurons. The formation of neuroblasts of Purkinje cells in the mouse is completed on the 13th day of gestation (Miale and Sidman, 1961).

If labeling of neuroepithelial cells occurs long before the end of proliferation, the labeling de-

creases at repeated mitotic divisions. Labeling of cell nuclei in neurons is most intense if [^{3}H]thymidine is injected shortly before the final division preceding differentiation into neuroblasts.

"Light" and "Dark" Purkinje Cells

Examination of the cerebellar cortex of mice prenatally injected with [^{3}H]thymidine did not reveal any apparent difference from the normal structure described in previous studies (Fujita et al., 1966; Mareš and Lodin, 1971). The cerebellum is still immature in 8-day old mice. Purkinje cells are relatively small and are clustered between the transient external granular layer and the developing internal granular layer. In older animals (21–90 days) Purkinje cells are already typically located between the granular and molecular layers. Most of them have large and "lightly" stained (vesicular) nuclei containing prominent nucleoli and abundant cytoplasm. Chromatin is accumulated predominantly near the nuclear membrane and the nucleolus.

In addition to these predominantly lightly stained Purkinje cells there were also cells with hyperchromatic nuclei of shrunken appearance ("darkly" stained Purkinje cells). In adult animals the number of dark Purkinje cells ranged from approximately one-fifth to one-third of the total cell population. It is of interest that these cells were not present in 8-day old mice. The dark Purkinje cells are considered to be artefacts due to fixation, since they are less frequent after perfusion fixation procedures (Cammermeyer 1962; Lodin et al., 1969). In the present work, attention has been centered mainly on the light Purkinje cells. The cell structure is well preserved and accurate grain counts can be obtained.

Grain Density in Purkinje Cells Labeled Prenatally

Mice received daily injections of 10 or 5 μCi [^{3}H]thymidine per gram body weight on the 10–15th or 11–14th day of pregnancy (Table I, columns I and II). The offspring were delivered after 21–22 days of pregnancy. The young animals were sacrificed in groups (Table I, column III) at 8, 25, 55, and 90 days after birth (column IV).

Autoradiographs were prepared from 5-μm thick sections of the brain as described in Materials and Methods. In order to obtain the highest possible comparability of grain number per nucleus between animals, all sections of one series of experiments were covered with stripping film of the same batch and were exposed, developed, and fixed together (Table I, column V). The stripping film was checked for low background before use.

Three series of experiments were carried out (Table I). The purpose of the first two pilot experiments was to show whether the grain number per nucleus changes with the age of the animals and how far the animals of the same litter were comparable. Table I presents the experimental conditions. After prenatal labeling with [^{3}H]thymidine the offspring of one litter were sacrificed in groups at three postnatal time intervals in both experiments. Most of the Purkinje cells (99%) were labeled.

Experiment 1 and 2

In Experiment 1 the grain number per nucleus of the lightly stained Purkinje cells was 38.2 ± 0.9 (417 nuclei), 39.5 ± 0.7 (285 nuclei) and 36.7 ± 0.8 (230 nuclei) on the 21st, 51st, and 91st postnatal day, respectively. The three values are very similar.

In Experiment 2 the number of daily injections of [^{3}H]thymidine was reduced from six to four and the daily [^{3}H]thymidine dose by a factor of two. Thus, the total dose of [^{3}H]thymidine and the possible radiation damage were three times less than in Experiment 1. In the seven young animals of the one litter, the values for the mean grain number, per nucleus of the lightly stained Purkinje cells were 11.9 ± 0.5 (two animals, 611 nuclei), 14.2 ± 0.7 (two animals, 270 nuclei) and 11.9 ± 0.5 (three animals, 515 nuclei) on the 25th, 55th, and 90th postnatal days.

Experiment 3

Since Experiments 1 and 2 showed that the spread of the grain number per nucleus within one and the same animal and among different animals is small, an experiment was carried out using 22 offspring of three litters and four time intervals of sacrifice, at 8, 25, 55, and 90 days (Table I). The animals were distributed into four groups so that each litter was possibly equally represented in each age group. Table II shows the mean grain number per nucleus of the light Purkinje cells for individual animals and for all animals in the same age group. The grain number per nucleus is quite similar for the different animals within the same age group. A total of 7,149 Purkinje cell nuclei were examined.

The grain number per nucleus decreases by about 14% between the 8th and 25th postnatal days

TABLE I

Conditions for Experiments 1, 2, and 3

	I	II	III		IV	V
Experiment no.	[^{3}H]TdR dose per day	Daily injection during gestation period	Number of mice		Killed on postnatal day no.	Exposure time of autoradiographs
			mothers	offspring		
	μCi/g body wt	*days*				*days*
1	10	10–15	1	4	21, 51, 91	24
2	5	11–14	1	7	25, 55, 90	21
3	10	11–14	3	22	8, 25, 55, 90	14

and then remains practically constant between the 25th and 90th days (Fig. 2, dashed line). For each of the four age groups, the frequency distribution of the grain number per nucleus for all nuclei was determined (Fig. 1). The two diagrams in Fig. 1 pertain to 751 and 2,158 single nuclei, respectively (Table II). The same distribution pattern was found in all four cases. This indicates that there is no loss of strongly labeled nuclei because of radiation damage, especially in older animals. In general, a loss of Purkinje cells by cell death should not be expected in these relatively young animals. In the normal rat, a decrease in the number of Purkinje cells was observed only in very old animals (Inukai, 1928).

The measured mean grain number per nucleus was corrected (*a*) for the increase in nuclear volume of the Purkinje cells during developmental growth, and (*b*) for the influence of [^{3}H]β-self-absorption in the sections of the nuclei.

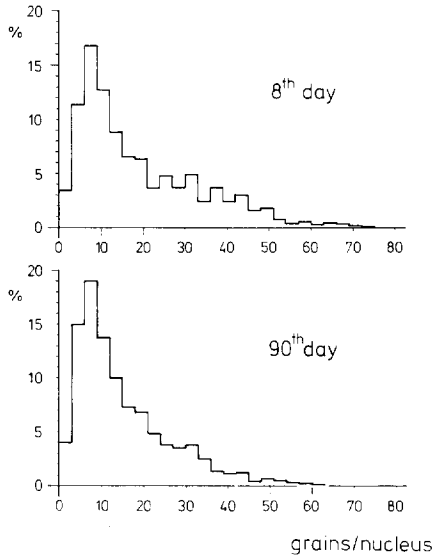

FIGURE 1 Frequency distribution of the grain number per nucleus for the 8th (751 nuclei) and 90th (2,158 nuclei) postnatal days. (The columns represent the percentages of nuclei with 0, 1, 2, grains, with 3, 4, 5 grains, etc.)

Correction of the Mean Grain Number per Nucleus for Increasing Nuclear Volume of Purkinje Cells in Experiment 3

The relationship between the volume n of a section through the center of a sphere having the thickness t and the total volume N of the sphere with the radius r is equal to (Appleton et al., 1969):

$$\frac{n}{N} = \frac{3}{4} \cdot \frac{t}{r}.$$

With a constant thickness t of the nuclear section, the volume of the section related to the total volume of the sphere is smaller the greater the radius r. The same is true for the mean ^{3}H activity of the nuclear section and of the total nucleus. Thus, the measured mean grain number per nucleus does not represent the total ^{3}H activity of a nucleus.

The diameters of centrally cut light Purkinje cell nuclei were measured in sagittal brain sections parallel to those used for the autoradiographs in Fig. 2. For details, see Materials and Methods. The values found for the mean area of the nuclear sections on days 8, 25, and 90 are listed in Table IV, column I. The correction factor applied for 25 days was taken to be equal to 1.00 in Table IV, column II. Consequently, the correction factor for 8 days would be, for instance,

$$\sqrt{\frac{\text{nuclear area 8 days}}{\text{nuclear area 25 days}}}.$$

After multiplication by these factors, the corrected grain number per nucleus is independent of the nuclear volume.

Correction for the Influence of [^3H]β-Self-Absorption on the Developing Purkinje Cell Nuclei in Experiment 3

Chromatin stained with haematoxylin is not equally distributed within the Purkinje cell nucleus. There are clumps of chromatin of different size and next to them "empty" spaces containing only karyoplasm. The autoradiographs demonstrate that most of the grains are located above the chromatin (Fig. 3). Only a few of the total grains

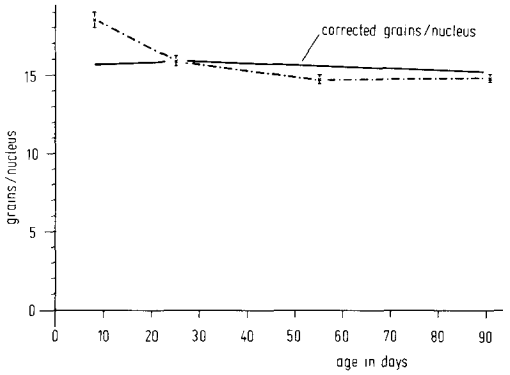

FIGURE 2 Grain number per nucleus of light Purkinje cells between days 8-90 postnatal (Experiment 3). Dashed line:measured grain number per nucleus; Solid line: after correction for increasing nuclear volume and changes in ^3H β selfabsorption in the nuclear sections.

are located above the nucleolus and the nucleolus-associated chromatin. Therefore, the [^3H]β-self-absorption depends mainly on the mass density of the chromatin clumps. This mass density was measured interferometrically as described in Materials and Methods. Table III presents the results. Because of the clumped consistency of the chromatin, its mass density is similar to that of the nucleolus.

Maurer and Primbsch (1964) determined experimentally the [^3H] β-self-absorption coefficient as a function of dry mass (milligrams per square centimeter) of histological sections with special respect to the geometry of autoradiographs. This coefficient provides the factor by which the counted grain number is smaller than without [^3H]β-self-absorption. It can be derived from this study, for instance, that for a dry mass of 0.1 mg/cm^2 (Table III) the grain number is 0.22 times smaller than without [^3H]β-self-absorption. Corresponding factors were derived for the other values in Table II. In this way the correction factors of Table IV were obtained. The value for 25 days was normalized to 1.00.

Corrected Grain Number per Nucleus for 8, 25, and 90 Days

Correction of the measured mean grain number per nucleus both for change in nuclear volume of the Purkinje cell nuclei (Table IV, column II) and for [^3H]β-self-absorption (Table IV, column IV) leads to the values in Table IV, column VI. These values are represented by the solid line in Fig. 2.

TABLE II

Mean Grain Number Per Nucleus of (a) Light and (b) Dark Purkinje Cell Nuclei in Experiment 3

	8-day old mice		25-day old mice		55-day old mice		90-day old mice	
	mean ± SEM	*n*	mean ± SEM	*n*	mean ± SEM	*n*	mean ± SEM	*n*
(*a*) Light Purkinje nuclei								
Liter A	16.8 ± 1.73	81	15.4 ± 0.53	538	14.9 ± 0.57	433	15.2 ± 0.95	225
	17.9 ± 1.52	105	14.7 ± 0.62	482	12.5 ± 0.46	362	13.2 ± 0.68	245
			19.5 ± 0.91	342	16.9 ± 0.86	270	13.4 ± 0.50	386
Litter B	18.6 ± 0.84	284	14.8 ± 0.57	525	16.1 ± 1.15	103	16.7 ± 0.67	359
			16.1 ± 0.55	504	14.5 ± 0.77	257	17.9 ± 0.97	231
							14.1 ± 0.47	334
Litter C	19.1 ± 0.84	281	15.3 ± 0.56	422			14.5 ± 0.51	378
	Σ = 18.5 ± 0.53	751	Σ = 15.9 ± 0.25	2815	Σ = 14.7 ± 0.31	1425	Σ = 14.8 ± 0.24	2158
(*b*) Dark Purkinje nuclei			Σ = 10.2 ± 0.60	290	Σ = 10.3 ± 0.80	171	Σ = 8.9 ± 0.57	314

Σ, mean grain number per nucleus.
SEM, standard error of mean.
n, number of measured cells.

TABLE III

Density of Dry Mass of Light Purkinje Cell Nuclei and Nucleoli in Deparaffinized Histological 5-μm Thick Sections

Age of mice	Nucleus		Nucleolus	
	density of dry mass (mean ± SEM)	number of samples	density of dry mass (mean ± SEM)	number of samples
days	*mg/cm²*		*mg/cm²*	
8	0.103 ± 0.0019	50	0.163 ± 0.0047	44
25	0.100 ± 0.0024	50	0.144 ± 0.0037	47
90	0.115 ± 0.0021	62	0.208 ± 0.0047	60

TABLE IV

Correction of Measured Grain Number Per Nucleus for Increasing Volume of Light Purkinje Cell Nuclei (I, II), and for Changes in [³H]β-Self-Absorption in the Nuclear Sections (III, IV)

	Correction for change of					
	Nuclear volume		[³H]β-self-absorption		Grain number per nucleus	
	I	II	III	IV	V	VI
Postnatal age	mean nuclear area	correction factor	dry mass	correction dactor	measured (Table II)	corrected
days	*μm²*		*mg/cm²*			
8	41.1 ± 0.47 (255)*	×0.85	0.103 (0.22)‡	×1.0	18.5	15.7
25	56.9 ± 0.49 (356)*	×1.00	0.100 (0.22)‡	×1.0	15.9	15.9
90	48.4 ± 0.33 (583)*	×0.92	0.115 (0.20)‡	×1.1	14.8	15.2

*, Number of nuclei scored.

‡, Coefficient of [³H]β-self-absorption.

The curve demonstrates that the labeled nuclear DNA in Purkinje cells does not change between 8 and 90 days.

Grain Density in Dark Purkinje Cell Nuclei

The values for grain number per nucleus in dark Purkinje cells were always about one-third lower than the values for light cells (Table II, *b*). Similarly, with light cells the grain density did not change significantly between the 25th and the 90th days of postnatal life. Dark Purkinje cells cannot be seen in 8-day old animals.

Grain Density in Pyramidal Cells of the Hippocampus Labeled Prenatally

The values for the mean grain number per nucleus for the pyramidal cells of the hippocampus were also determined on the autoradiographs of Experiment 3. The mean grain numbers per nucleus were 6.8 ± 0.26 (five animals, 528 nuclei) on the 25th day after birth and 6.0 ± 0.22 (six animals, 627 nuclei) on the 90th day after birth. No correction was applied to these values.

Incorporation of [³H]Thymidine into Brains of Adult Mice

Four young adult mice (18–20 g, CBA strain) received six injections of 10 μC [³H]thymidine per g body wt at 0, 6, 12, 18, 36, and 48 h, and were killed 3 h after the last injection. Autoradiographs were prepared from sagittal sections (exposure: 21 days at +4°C and 4 mo at −20°C). Glial cells were heavily labeled, with more than 60 grains per nucleus. In none of the Purkinje cells or other well-differentiated nerve cell nuclei (e.g., pyramidal cells) was tritium labeling observed. A total of about 530,000 cells in all brain regions was evaluated. Among these, only one nucleus could be considered doubtfully a labeled nerve cell nucleus.

DISCUSSION

It is generally accepted that the nuclear DNA of the cell is metabolically stable and that DNA synthesis occurs only during chromosome doubling, i.e. during the DNA synthesis phase (S phase) before mitosis or polyploidization. Apart from the S-phase nuclei, the nuclear DNA content

should be constant. However, an unobjectionable experimental confirmation of the metabolic stability of nuclear DNA is lacking up to now.

On the other hand, some authors argue that in addition to the metabolic stable "genetic" DNA, an unstable "metabolic" DNA exists, the amount of which varies within the cell according to the physiological functional conditions (Pelc, 1968; 1972).

A great number of cytophotometric studies (Feulgen-DNA) are concerned with this question of stability or partial instability of nuclear DNA. However, information in the literature concerning the amount of Feulgen-DNA is often contradictory. For the same research objective, one author reports stability of DNA, and another finds changes in the amount of DNA depending on the experimental conditions (for references, see Roels, 1966). All determinations of Feulgen-DNA imply the assumption that after the Feulgen staining the ratio of the amount of stain to the amount of DNA is constant. However, this is not necessarily the case (for stoichiometry of Feulgen staining and problems of cytophotometric measurements, see Pearse, 1968; Kasten, 1964; Wied, 1966; Mann and Yates, 1973). For instance, Mareš et al. (1973) found that the Feulgen DNA content of mouse Purkinje cells increases by about 60% between the 2nd and 150th postnatal days, starting from diploid values. Repeating these experiments, with chemically isolated DNA from mechanically isolated Purkinje cells, Cohen et al. (1973) found a constant diploid DNA content independent of time.

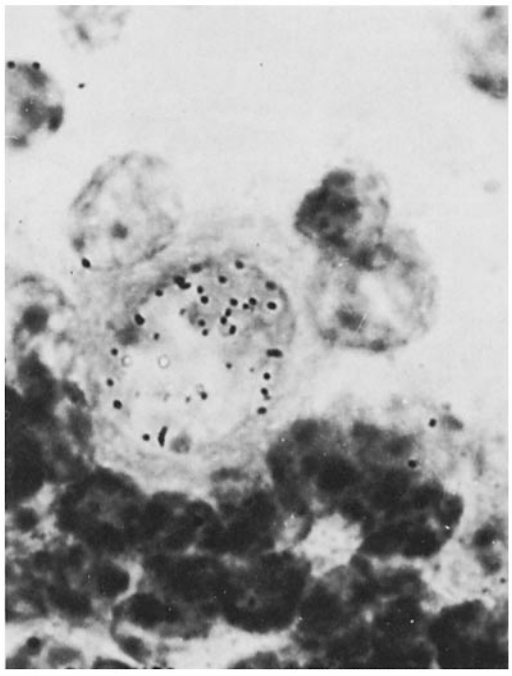

FIGURE 3 Labeled Purkinje cell nuclei in 25-day old mouse. (Stained with haematoxylin and eosin). × 1,500.

The disadvantages of DNA Feulgen staining and its cytophotometric determination can be avoided when DNA is isolated and measured chemically. There are a number of reports in which DNA was labeled with radioactive nucleosides and the retention of the label was measured. If the DNA is metabolically stable, the radioactivity of the prelabeled DNA must remain constant with increasing time. Hecht and Potter (1956) injected [6-^{14}C]orotic acid into rats after partial hepatectomy and measured the ^{14}C activity of the chemically isolated liver DNA. The specific ^{14}C activity of the DNA increased up to 24 h after injection and then remained constant up to the end of the experiment at 100 days. Similar experiments were carried out by Fresco and Bendich (1960) using [^{15}N]adenine and [2-^{14}C]glycine in rats after a two-thirds hepatectomy. The specific activity of the nucleosides in the liver DNA was constant between 26 and 121 days after hepatectomy. The values were corrected for the increase in liver DNA due to liver growth. Different results were obtained by Gerber et al. (1960) for the DNA of the normal liver and other organs of the rat. After application of [^{3}H]thymidine, the specific ^{3}H activity of the chemically isolated DNA was measured for a number of organs. In all organs studied, there was a varying but distinct decrease in the specific ^{3}H activity of DNA, with half-lives of the label between 2 and 40 days. In the liver the half-life of the ^{3}H activity was about 15 days. Thus, in the case of normal liver and after partial hepatectomy, the results are contradictory.

Devik and Halvorsen (1963) repeated these experiments, injecting [^{3}H]thymidine into mice and studying the normal as well as the hepatectomized liver. Unexpectedly, the ^{3}H activity of the normal liver significantly decreased between 1 and 116 days. These results are in good agreement with those of Gerber et al. (1960). However, the ^{3}H activity of the liver DNA after a one-quarter hepatectomy remained about constant up to 116 days. The same results were found by Hecht and Potter (1956) as well as Fresco and Bendich (1960) for a two-thirds hepatectomy in rats. Obviously,

those experiments in normal liver and after hepatectomy differ in one fundamental point.

The contradictory results may be explained by the fact that different cell types are labeled in these two cases. In the normal liver, about 60% of the cells are hepatocytes and about 40% are mesenchymal cells. Experiments using [^{3}H]thymidine have shown that the labeling index of cells of the reticulated endothelial system (RES) is about 10 times higher than that of the hepatocytes. Thus, the total labeling of the RES cells in the liver should be 10 times that of the hepatocytes. On the other hand, the labeling index of hepatocytes after hepatectomy is about 400 times higher than that of the hepatocytes in the normal liver, while the labeling index of the RES cells increases much less after hepatectomy. This results in a much more intense ^{3}H labeling of hepatocytes after hepatectomy than of RES cells. In the normal liver, the observed decrease of the ^{3}H activity as a function of time is mainly due to the labeled RES cells, while the constancy of the ^{3}H activity with increasing time after hepatectomy mainly represents the ^{3}H activity of the hepatocytes. Thus, these cases are not comparable. According to the literature, migration and loss of RES cells might occur, whereas hepatocytes are stationary.

Bennet et al. (1960) studied the stability of DNA in neurons. Neurons are an adequate subject for these studies, since they do not divide. Pregnant mice received [^{14}C]adenine on the 15th day of pregnancy. The specific activities of [^{14}C]DNA-adenine and [^{14}C]DNA-guanine of the total brain in the offspring were then measured at different time intervals. The specific activities of both nucleosides decreased between the 9th and 40th postnatal days and then remained constant up to 365 days. Corrections for brain growth during 9 and 40 days and the increase of DNA associated with it resulted in constant, specific [^{14}C]DNA activity for the entire duration of the experiment, between 9 and 365 days. The authors concluded that there is no turnover of DNA in neurons.

In chemical studies, the specific activity of the DNA of the brain inevitably is related to all cells of the brain. The measured ^{3}H activity consists to a small extent of the ^{3}H labeling of glial cells, on the one hand, and on the other, it is related to the total amount of DNA of neurons *and* glial cells. Since glial cells proliferate in the adult animal to a small extent, but persistently, the specific activity of the DNA of the total brain does not necessarily represent the DNA of neurons.

The final step out of this methodological difficulty is the determination of the labeling of isolated neurons by the autoradiographic method. In the present work the number of grains per nucleus of Purkinje cells measured first decreases by about 14% between 8 and 25 postnatal days. The application of corrections for the change of the nuclear volume and for the influence of β-self-absorption has shown that the decrease is due to a corresponding increase in nuclear volume; the [^{3}H]β-self-absorption is unchanged at 8 and 25 days. According to Fig. 2, the corrected grain number per nucleus is practically constant between 8 and 90 days. During the entire experiment, the corrected curve decreases by only about 3%. The ^{3}H activity present on the 8th postnatal day does not change within the following 82 days, suggesting metabolic stability of the labeled DNA of Purkinje cell nuclei. As is to be expected in such a case, the frequency distribution of the grain number per nucleus does not change at 8, 25, 55, or 90 days (Fig. 1). The application of the DNAse treatment described in Materials and Methods proves that the ^{3}H labeling observed in the Purkinje cell nuclei is indeed ^{3}H labeled DNA.

Similar results were found for the pyramidal cells of the hippocampus. The uncorrected mean grain number per nucleus did not decrease between 25 and 90 days after birth.

Haas et al. (1970) found a considerable decrease in the grain number per nucleus in different cortical neurons of the rat during the first 2 postnatal wk (in spite of correcting for nuclear volumes changes) after continuous labeling of pregnant rats with [^{3}H]thymidine. However, the grain count was not corrected for changes in dry mass and [^{3}H]β-self-absorption of tritium. The latter correction could play an important role in explaining the decrease in grain counts, as the cortical neurons are very immature in newborn rats and a substantial increase in nuclear dry mass can be reasonably assumed in early postnatal life. Furthermore, in contrast to our experiments, the frequency distribution of the grain number per nucleus showed that strongly labeled nuclei disappeared with increasing age, although the total dose of [^{3}H]thymidine injected was similar to that used in the present experiments. This might be due to the different sensitivities of cortical and Purkinje

cell nuclei to eventual radiation damage. The disappearance of heavily labeled cortical neurons could also be due to persisting mitotic activity of some neurons in early postnatal life (Smart and Leblond, 1961).

The results of the present experiments do not completely exclude the possibility that Purkinje cell nuclei contain unstable metabolic DNA in addition to the stable DNA. This unstable DNA-fraction might have lost its label up to the 8th postnatal day because of metabolic turnover. However, it should be possible to label this unstable DNA with [^{3}H]thymidine in the adult animal. Corresponding experiments in mice with high doses of [^{3}H]thymidine (60 μCi/g), with long exposure times of the autoradiographs and careful evaluation of the background did not show any indication of labeling of neurons exceeding ca. 0.01 of the grain number of glial cells. Throughout the whole brain, glial cells had an equal grain number per nucleus within statistical variations. This is in good agreement with the results of other authors (Smart and Leblond, 1961; Noetzel and Rox, 1964: Rakic and Sidman, 1968; Mareš et al., 1968; Kraus-Ruppert et al., 1973; Korr et al., 1973). Among more than 500,000 neurons scored in the present work, no labeled neuron was found. In the meantime about 5×10^6 neurons of adult mice have been scored in this institute and a labeled one has not been found.[1] This indicates either that neurons contain no unstable DNA or an extremely small amount of it, or that this unstable DNA cannot be labeled with [^{3}H]thymidine.

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