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Changes in Nuclear Ribonucleoprotein Constituents and Chromatin Disposition during Neuronal Differentiation and Maturation

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Morphological and quantitative changes of ribonucleoproteic (RNP) structures and chromatin are studied in the nuclei of undifferentiated cells, neuroblasts and neurons in several degrees of maturation, in order to relate them to the drastic modifications in transcription and/or RNA processing taking place during cell differentiation. Undifferentiated (matrix) cells of 2-day embryos differ from that of 4-day embryos in their nucleolar volume and in the amount of compact chromatin. These differences are interpreted as the earliest signs of neuroblast differentiation. All over the process of matrix cell-bipolar-multipolar neuroblast differentiation there is a large spreading of compact chromatin well before any important change in RNP structures or in nuclear volume. The most remarkable increase in nuclear volume and in the amount of RNP particles occurs during the differentiation of multipolar neuroblasts to immature neurons, which is characterized by large synaptogenic activity. The interpretation of these changes is discussed in connection, on one hand with the metabolic effects of synapses, and on the other hand with the variations of gene expression taking place in cell differentiation and under other natural and experimental situations.

Key-words: *Nucleus - Differentiation - Ribonucleoproteins - Chromatin.*

INTRODUCTION

The publication of the first practical method for preferential staining of ribonucleoprotein (RNP) particles by Bernhard (2) was followed by numerous studies of the nucleolus, interchromatin granules, perichromatin fibrils and granules. It has been repeatedly demonstrated that the alterations in transcription and/or nuclear processing of RNA produce changes in number, morphology, cytochemical or autoradiographic features of these particles (16, 17, 22, 25, 27, 28, 35, 36). Cell differentiation is a series of progressive changes in gene expression mainly due to modifications of transcription and/or nuclear processing of RNA (5). Studies on RNP particles and chromatin during development have been carried out in mouse blastomeres (7). In these very early stages of development the changes of nuclear structures are related with the storage of RNA of maternal origin and with the onset of transcriptional activity in the embryos.

The present work was undertaken to know more about the changes in RNP particles and chromatin disposition taking place during the drastic modifications of transcription and/or processing of RNA accompanying cell differentiation. To fulfill this end the modifications of nuclear components are studied during the transformation of undifferentiated matrix cells into non-divising neuroblasts, the differentiation of neuroblasts to neurons and neuronal maturation. The variation of nuclear structures in successive stages of cell differentiation are related to well known landmarks of neurogenesis. Chick embryo spinal cord is an advantageous material to make this correlation because several aspects of neural development have been extensively studied in this subject: neuroblast differentiation (4, 10, 14, 38); synaptogenesis (19,31); myelination (6); ontogeny of bioelectric activity (24); embryonic motility (18); and embryonic spontaneous cell death (11).

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MATERIAL AND METHODS

The ventral half of the cervical region of the spinal cord of chick embryos of 2, 4, 9, 13 and 21 days of incubation, corresponding to developmental stages 12, 23, 35, 39, 45 and newborn chicken (12), constitute the material of this study. Fixation was carried out in 2.5 % glutaraldehyde in 0.15 M cacodylate buffer at pH 7.3. The fixative was dropped for 20 min on the 2- and 4 days embryos. Older embryos were perfused through the left ventricle with the same solution for 30 min. A few samples were postfixed in 2 % osmium tetroxide, but most of them were dehydrated and Epon-embedded without postfixation. General staining was performed with uranyl acetate and lead citrate. Preferential contrasting of RNP particles was carried out with uranyl acetate-EDTA-lead citrate regressive stain according to Bernhard (2). In order to study chromatin, glutaraldehyde-fixed material was embedded in glycol-methacrylate, sectioned and contrasted with phosphotungstic acid as described by Vázquez-Nin *et al.* (34).

Sampling

As contrasting methods used for electron microscopy in this study do not reveal cytoplasmic details, in order to know to which type of cell belongs each nucleus studied, systematic sampling was carried out in standarized regions of the spinal cord of embryos in well known stages of development:

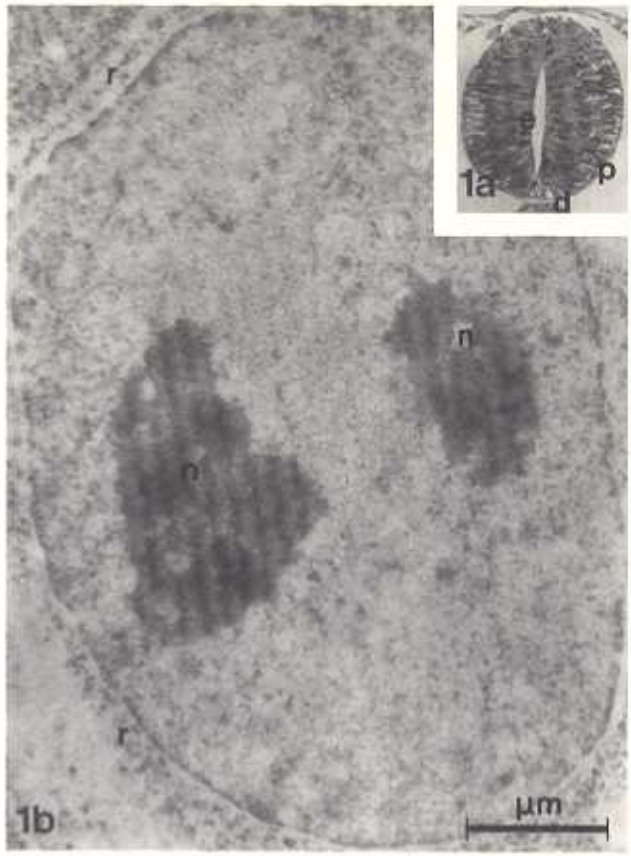
— In 2-day embryos interphasic nuclei of the ependymal layer were studied in order to sample undifferentiated cells (4).

— In 4-day embryos three regions were analyzed: ependymal layer, intermediate layer and the anlage of the anterior horn, called in this study peripheral layer. Ependymal layer is solely or mainly formed by undifferentiated cells (9). Intermediate layer corresponds to the mantle layer, it is situated between the ependymal layer and the non-cellular marginal layer. It is composed by bipolar neuroblasts (4). The developing anterior horn is constituted by multipolar neuroblasts (4).

— From 9 days forward only motoneurons were studied.

Morphometry

Quantitative analysis were carried out on systematically taken pictures of all nuclei of a predetermined area. Electron optical magnification was accurately fixed (8400 = 100) in an EM 9S (Carl Zeiss) electron microscope. No less than three non-consecutive sections of each nucleus were studied in order to estimate the variations due to non-homogeneous distribution of the structures inside the nucleus. Stereological evaluations were carried out using well known methods for calculating the fraction of the volume of the container occupied by the element in study (32). Nuclear and nucleolar volume were estimated under light microscope (Axiomat, Carl Zeiss) using 2 µm thick sections of the same embryos employed for electron microscope determinations. The following parameters were measured in nuclei and nucleoli: largest diameter, the diameter perpendicular to the middle point of the largest one, and the position of the central point of the nucleolus refered to a Cartesian system of coordinates with their zero point in the intersection of nuclear diameters. The relative frequency of total RNP particles was estimated by the point fraction method (32). The condensed chromatin density was calculated by the areal fraction procedure (32). Comparative measurements of the DNA content per nucleus were carried out in Feulgen stained preparations (20) illuminated with green light (530 nm) under strictly standarized conditions. A HP 9825T (Hewlett Packard)



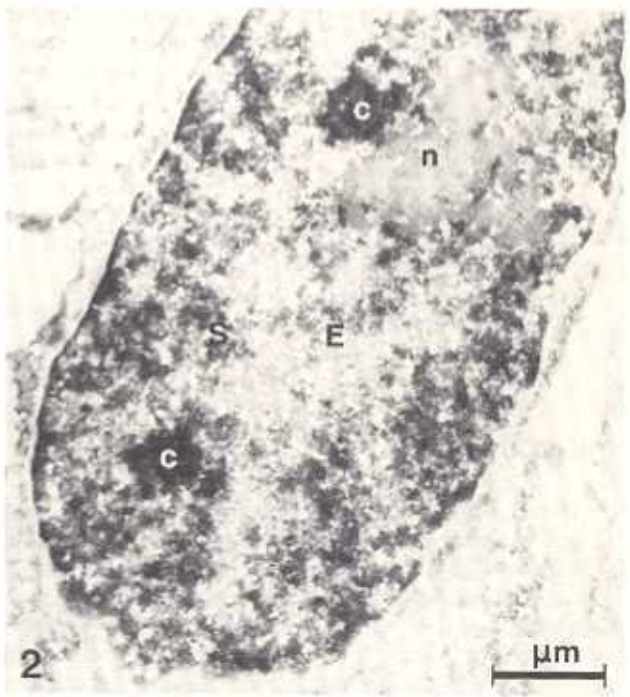
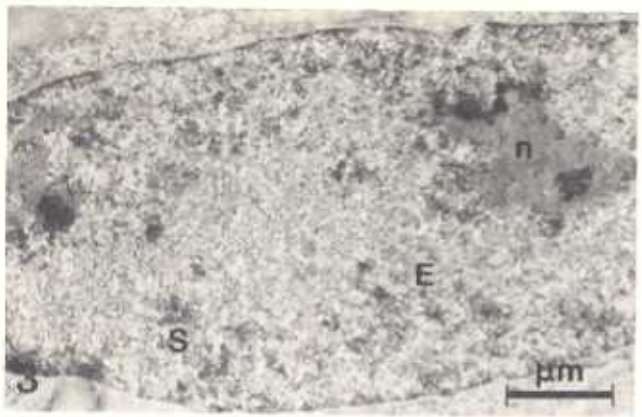


FIGURE 1. — Embryos of 2 days of incubation. *a*) Light micrograph of a transverse section of the cervical spinal cord. d-dorsal cord. e-ependymal layer, p-peripheral layer. × 290. *b*) Electron micrograph of matrix cells in the ependymal layer contrasted by uranyl-EDTA-lead method for RNP particles. Nucleoli (n) are large and compact. Other RNP structures are evenly distributed in nuclear area. r-ribosomes. × 19,000.

FIGURE 2. — Matrix cell in the peripheral layer of a 2-day embryo contrasted with phosphotungstic acid. Clumps of compact chromatin (c) are darkly stained. Semicompact chromatin (S) occupies most of the nuclear area. RNP structures including nucleolus (n) are not stained. E-extended chromatin. × 15,000.



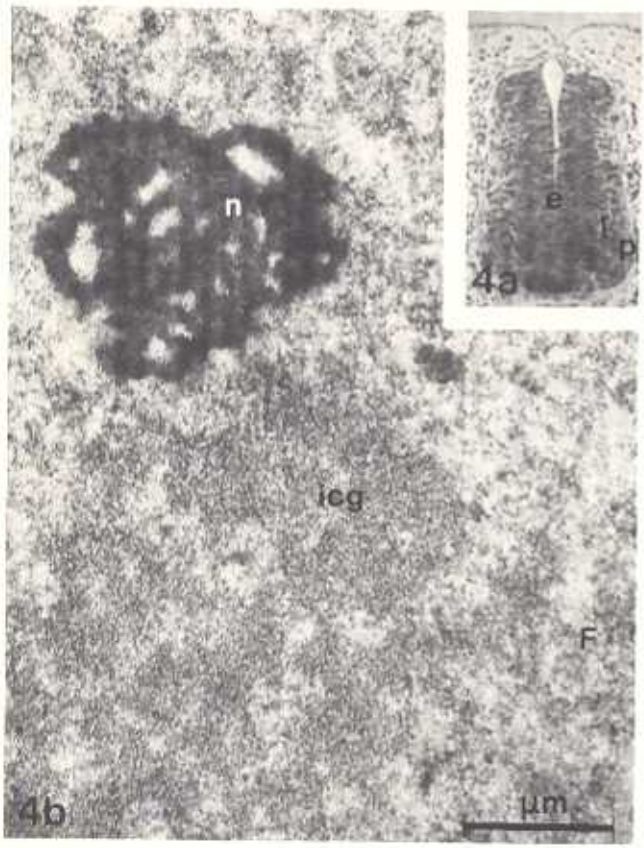
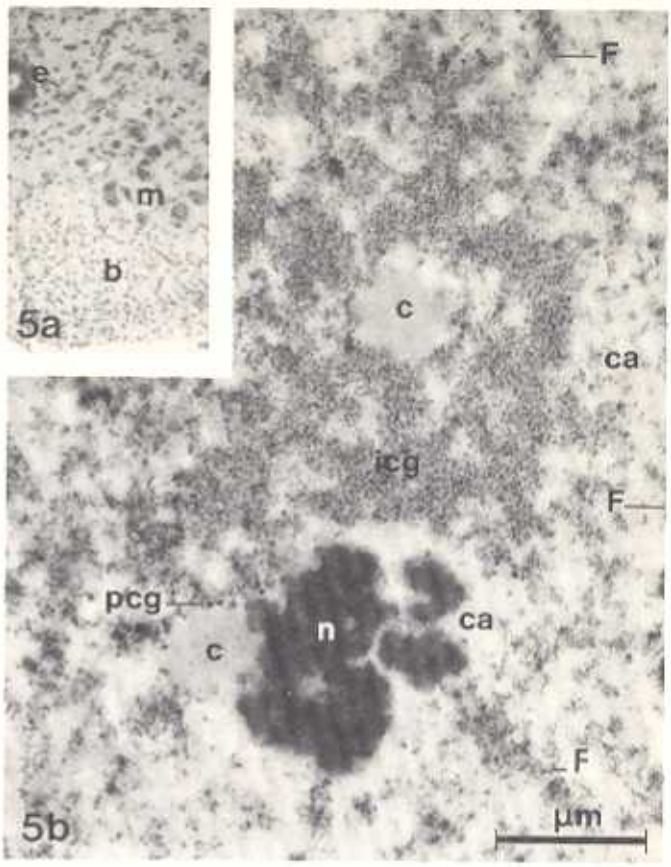


FIGURE 3. — Bipolar neuroblast in the intermediate layer (i, in figure 4*a*) of a 4-day embryo contrasted with phosphotungstic acid. Semicompact chromatin (S) is less abundant than in the matrix cell nucleus in figure 2. On the contrary, there is more extended chromatin (E). × 14,000.

FIGURE 4. — Embryos of 4 days of incubation. *a*) Light micrograph of a transverse section of the cervical spinal cord. The peripheral layer (p) is composed by the cells forming the anlage of the ventral horn. i-intermediate layer. e-ependymal layer. × 315. *b*) Electron micrograph of the nucleus of a multipolar neuroblast in the peripheral layer contrasted with uranyl-EDTA-lead method. The nucleolus (n) shows a nucleolonemal structure. Interchromatin granules (icg) and fibrils (F) are abundant. × 20,000.



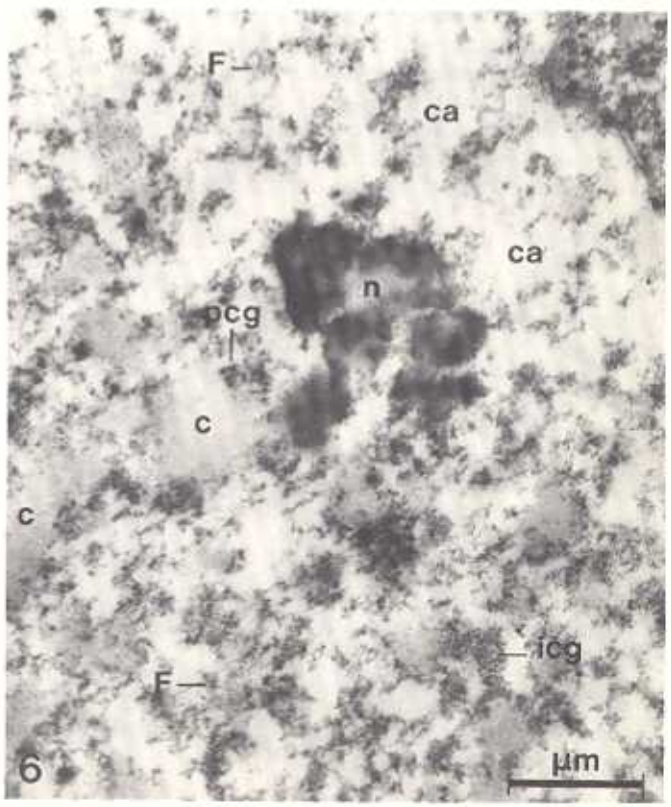


FIGURE 5. — Embryos of 9 days of incubation. *a*) Light micrograph of a transverse section of the cervical spinal cord showing: e-ependyma; m-immature motoneurons; b-non-myelinated white matter. × 160. *b*) Electron micrograph of a nucleus of an immature motoneuron stained by uranyl-EDTA-lead method. The tangential section of the nucleolus (n) shows its nucleolonema. Interchromatin granules (icg) form a large group. Clear areas (ca) without RNP particles are numerous. c-clumps of compact chromatin. pcg-perichromatin granules. F-RNP fibrils. × 20,000.

FIGURE 6. — Nucleus of a motoneuron of a newborn chicken stained by uranyl-EDTA-lead method. (n)-tangetial section of the nucleolus showing the nucleolonema. (c)-clumps of compact chromatin. (ca)-clear areas without RNP particles. (icg)-interchromatin granules. (pcg)-perichromatin granules. F-RNP fibrils. × 18,000.

programable calculator provided with digitizer, plotter and a floppy disk unit, was used in measurements of areas, statistical analysis of data and drawing of histograms.

RESULTS

Morphology

The nuclei in the ependymal layer of embryos of 2 days of incubation are elliptical in shape, they have a very large elongated nucleolus or several smaller spherical ones (Fig. 1). These nucleoli are constituted by a compact mass of RNP fibrils and granules seldom organized in a nucleolonemal structure. No fibrillar centers are found. Perichromatin fibrils and interchromatin granules are densely packed throughout the nucleoplasm (Fig. 1). A few clumps of compact chromatin are in contact with the nuclear envelope, isolated in the nuclear sap or adjacent to the nucleolus (Fig. 2). Semicompact chromatin is formed by a network of filaments densely stained by phosphotungstic acid. It can be seen occupying a large part of the nucleus in Figure 2.

Nuclei in the ependymal and intermediate layers of 4-day embryos resemble those of 2-day embryos except for the loosening of meshwork of semicompact chromatin (Fig. 3) and the smaller size of the nucleoli. Neuroblasts in the development anterior horn of 4-day embryos have spherical nuclei with multiple nucleoli. Perichromatin fibrils and interchromatin granules are densely packed leaving few clear spaces (Fig. 4). The disposition of the chromatin is similar to that of the neuroblasts situated in the intermediate layer.

Immature neurons of the anterior horn of 9-day and 13-day embryos have a large spheroidal nucleus with one or two nucleoli with nucleolonemal structure. Interchromatin granules are grouped in large ill-defined clumps. RNP containing fibrils are unevenly distributed in the nuclear sap, but they seldom surround the few clumps of compact chromatin present in these nuclei (Fig. 5).

The nucleus of the neurons of the anterior horn of the spinal cord of newborn chicken are large, spheroidal in shape, with frequent invaginations of the nuclear membrane. They have one or two large nucleoli with a loose nucleolonemal structure (Fig. 6). Interchromatin granules form very large irregular groups.

Clear areas without RNP particles appear between 4 and 9 days of incubation, as can be seen comparing figure 4 with figure 5. This clear areas increase in size and number as development proceeds (Fig. 6).

Stereology

The nuclear volume of matrix cells and neuroblasts does not change in embryos of 2 and 4 days of incubation (Fig. 7). Immature neurons of 9-day embryos have significantly larger nuclei than those of younger embryos ($p < 0.001$). From 9 days of incubation to hatchnig the nuclear volume increases less steeply (Fig. 7).

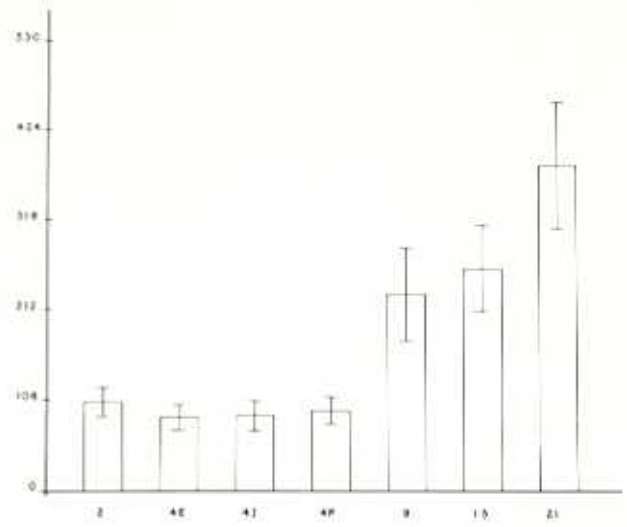


FIGURE 7. — Changes of nuclear volume during development. The time of incubation is represented in abscissa. E-ependymal layer cells. I-intermediate layer cells. P-peripheral layer cells. Ordinate: nuclear volume in cubic micrometers. Vertical lines represent the standard deviation.

The nucleolar volume shows two maxima; one corresponding to the matrix cells of 2-day embryos, and the other to the motoneurons of newborn chicken (Fig. 8).

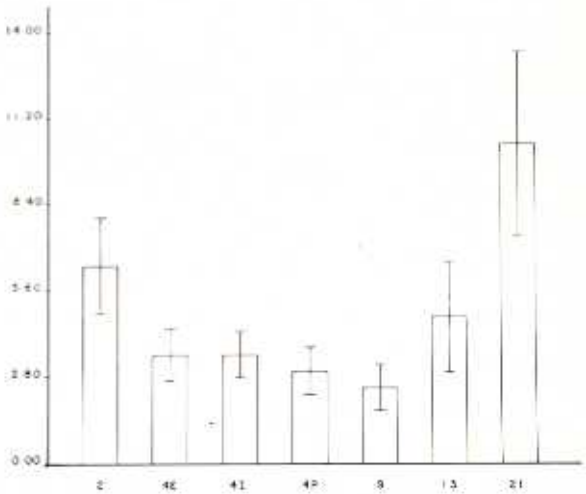


FIGURE 8. — Changes of nucleolar volume. Coordinates as in the preceeding figure.

The position of the nucleolus was studied in nuclei with only one nucleolus using main diameters as a system of coordinates. The location of the nucleolar central point is expressed as a fraction of nuclear diameters. Bimodal distributions along the longest nuclear diameter are observed in all types of cells studied from matrix cells in 2-day embryos to large motoneurons at hatching (Fig. 9). On the contrary, when the distribution of nucleoli is studied along the transverse diameter, a strong central tendency is evidenced

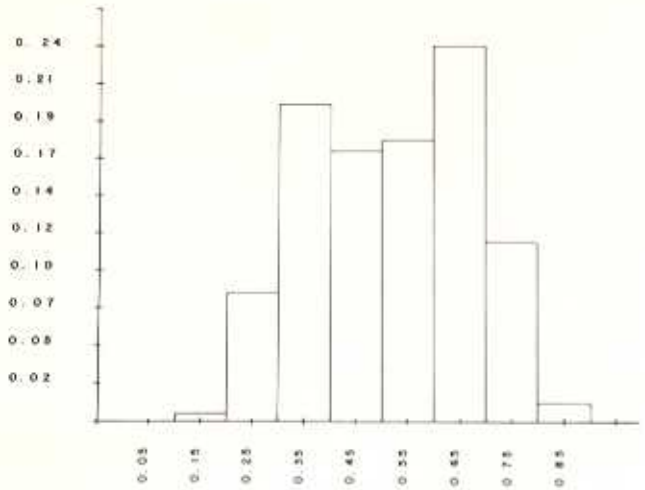


FIGURE 9. — Distribution of positions of nucleolar central points measured along the longest nuclear diameter of 433 neuroblasts. Ordinate, relative frequency. In the abscissa are shown the midpoints of the 10 classes in which the total length of the nuclear diameter is divided.

(Fig. 10). This demonstrates the existence of two sites of maximal possibility of finding the nucleolus, both are situated along the longest diameter of the nucleus. They are separated by a central zone of 20% of the nuclear diameter in length, in which the possibility of encountering the nucleolus is lower.

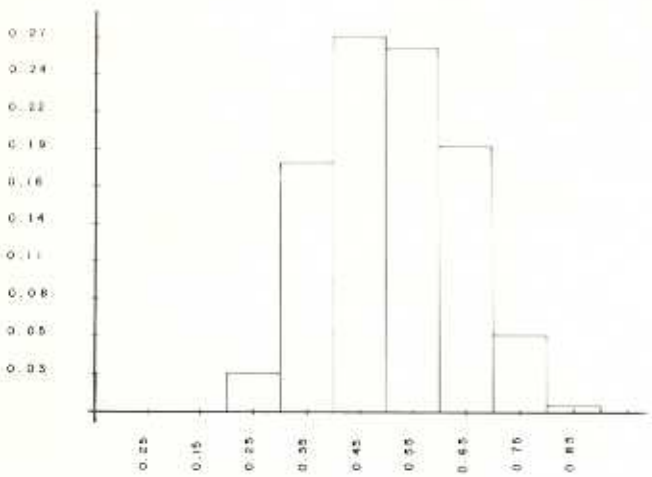


FIGURE 10. — Distribution of nucleolar central points measured along the shortest nuclear diameter in 434 neuroblasts. Coordinates as in figure 9.

The absolute volume occupied by RNA-containing fibrils increases sharply during the maturation of motoneurons between 9 and 21 days of incubation (Fig. 11).

The point fraction occupied by RNP-containing structures shows a marked decrease between 4-day and 9-day embryos (Fig. 12, hachured bars). This diminution is coincident with the development of intranuclear clear areas without RNP particles described in the preceding section. However, the absolute

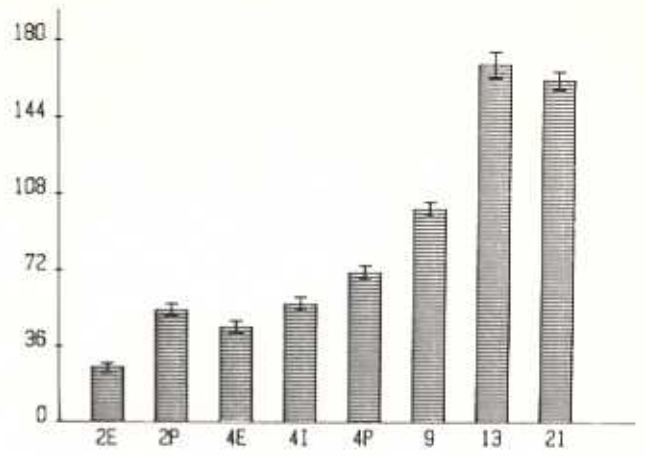


FIGURE 11. — Changes in the amount of RNA containing fibrils. Ordinate, cubic micrometers occupied by fibrils. Abscissa, as in figure 7.

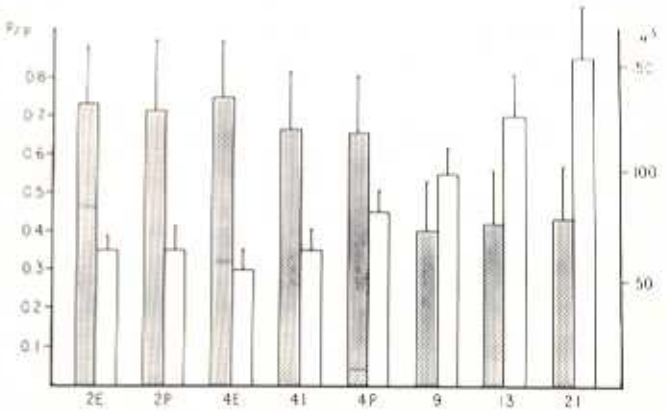


FIGURE 12. — Hachured bars represent the fraction of nuclear volume occupied by RNP particles expressed as point fraction as shown in ordinate on the left scale. White bars represent the absolute volume occupied by these structures in cubic micrometers, as shown in the right scale. Abscissa, as in figure 7.

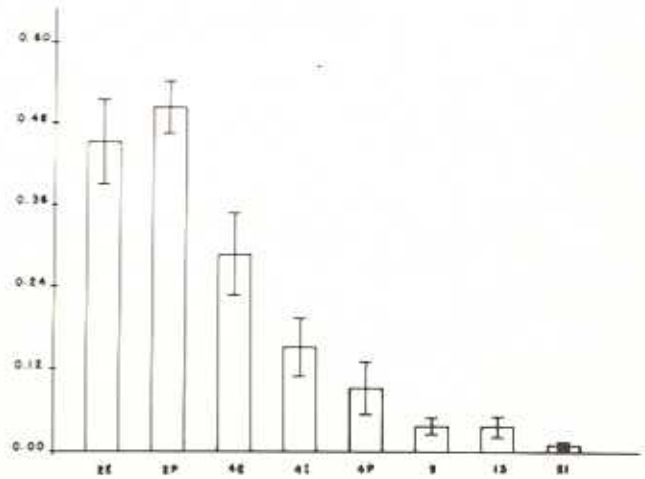


FIGURE 13. — Fraction of the nuclear space occupied by compact chromatin. Ordinate: areal fraction; total nuclear area = 1. Abscissa, as in figure 7.

volume occupied by RNP structures increases steadly between 4 days of incubation and hatching, as shown in figure 12 by white bars. In 4-day embryos there is a significant increase in RNP particles between peri-ependymal matrix cells and peripheral neuroblasts. These estimations of the absolute volume occupied by RNP structures are valid only for comparison purposes between observations performed with the same preparative techniques and analyzed with the same stereological methods as those reported here.

The fraction of nuclear area occupied by compact chromatin decreases continuously from 2 to 21 days of incubation (Fig. 13).

The quantity of DNA per nucleus in matrix cells of 2-day embryos, in ependymal- and intermediate-layer cells of 4-day embryos, is significantly higher than the amount 2n represented by anaphase plates (Fig. 14). Multipolar neuroblasts in the peripheral layer of 4-day embryos and the motoneurons in different degrees of maturation of 9-, 13-, and 21-day embryos have essentially the same amount of DNA as anaphase plates.

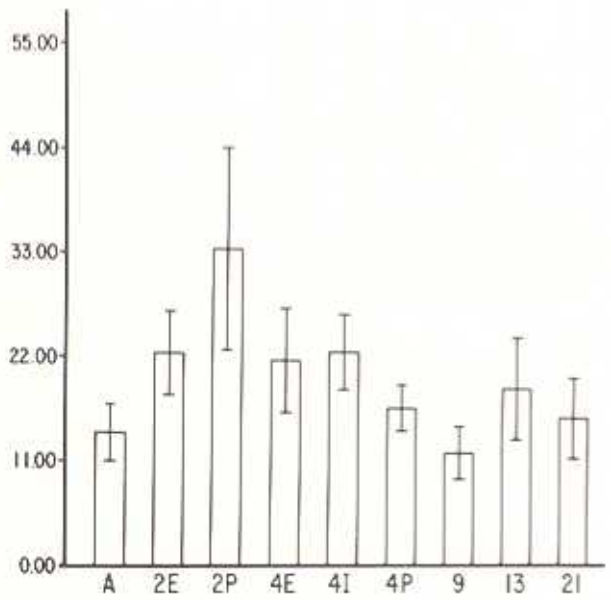


FIGURE 14. — Changes of the amount of DNA per nucleus. The ordinate is calibrated in arbitrary units. Abscissa, as shown in figure 7; A-anaphase plates.

DISCUSSION

Several studies indicate that the primitive ependymal layer is exclusively formed by germinal or matrix cells in mitotic cycle (9, 10, 29). The cervical spinal cord of 2-day embryos is composed mainly by these matrix cells (9). The innermost and the outermost nuclear layers of 2-day embryos were studied separately in order to test if measurable nuclear changes exist before any other sign of differentiation. However, no clear cut differences between them were found in the nuclear parameters studied except for the amount of DNA per nucleus which is much higher in peripheral layer cells. On the contrary, there are differences between matrix cells of 2-day embryos and ependymal-layer cells of 4-day embryos. The main changes are: a significantly diminution of nucleolar volume and a reduction to one fourth of its value of the abundance of compact chromatin ($p<0.001$ in both cases). Both types of cells are indistinguishable by means of silver impregnations (4) or by their ability to incorporate [^{3}H]-thymidine (12, 13). Smaller nucleoli and unraveling of the chromatin characterize also bipolar neuroblasts in the intermediate layer of 4-day embryos. Thus, these changes may be the earliest signs of differentiation of post-mitotic matrix cells transforming to neuroblasts in the ependymal layer of 4-day embryos.

Peripheral neuroblasts in the anterior half of the cervical spinal cord of 4-day embryos are mainly multipolar motor neuroblasts, whose axons are growing in the connective tissue (4). The transformation of bipolar into multipolar neuroblast is accompanied by a marked unraveling of chromatin, a small increase in nuclear volume and in the total amount of RNP particles. It is worth to note that a large spreading of chromatin takes place along the process including matrix cell-bipolar neuroblast-multipolar neuroblast transformations before any important change in RNP structures or increase in nuclear volume. The amount of DNA per nucleus of multipolar neuroblasts is the same as in anaphase plates, indicating that there is no cell engaged in mitotic cycle intermingled among them.

The large neurons in the head of the anterior horn of 9-day embryo can be viewed as immature motoneurons: most of their axons are functionally connected to muscle cells (11); electron microscopic studies demonstrate a large number of axo-dendritic synapses in the motor region of the spinal cord (19, 31); functional propiospinal connections are present at the cervical level in these embryos (19). Very important nuclear changes were found in the present study between 4 and 9 days of incubation. The large increase in nuclear volume that takes place in this period causes a dilution effect on RNP particles. However, there is a significant increase in the absolute amount of RNP structures per nucleus during this enlargement of nuclear volume. This increase is partially due to the augmentation in the abundance of perichromatin fibrils. Several studies show that perichromatin fibrils contain rapidly labeled RNA of non-nucleolar origin, probably hnRNA (1, 8, 15, 17, 21, 22, 26, 33). According to this view the enrichment in perichromatin fibrils found in motor-cell nuclei between 4 and 9 days of incubation reflects an increase in the transcriptional rate of non-nucleolar sites. Numerous observational and experimental works indicate that perichromatin granules are intranuclear storage and/or transport particles containing non-nucleolar RNA (25, 28, 33, 35, 36, 37). In agreement with this hypothesis the large increase in the amount of perichromatin granules that takes place between 4 and 9 days of incubation in the motor cells of the cervical spinal cord (37), can be interpreted as the result of a new equilibrium between the higher transcriptional rate of some genes involved in the acquisition of definitive neuronal traits

and the exit of this RNA to the cytoplasm. In fact, in experimental situations in which transcription and transportation or RNA to the cytoplasm are changed at different rates, accumulation of perichromatin granules are found when transcription proceeds faster than transportation, and depletion is seen in the inverse situation (36). Furthermore, in cells in which some genes are transcribed at very high rates, as puffs and Balbiani rings of nuclei containing polytene chromosomes, perichromatin granules are extremely abundant (33). These granules originate mainly in these loci (l.c.). Most differentiated cells have perichromatin granules (15); on the contrary, blastomeres (7) and undifferentiated matrix cells (37) are devoid or have very few of them. The increase in the rate of transcription of some genes is probably a very general phenomenon in differentiating cells (5). Thus, the increase in the number of perichromatin granules may be due to the intranuclear accumulation of RNA synthetized in some rapidly transcribed genes coding for most abundant proteins in differentiated cells or in their secretions.

Most important changes in nuclear ribonucleoprotein particles are related to the development of peripheral and central synaptic connections during neuronal differentiation. Numerous works demonstrate that differentiation affects the development of different regions of the brain and the spinal cord (30). Detailed studies on the nucleus vestibularis tangentialis show that immature neurons fail to survive in the absence of afferent connections (13, 33). The metabolic influences of synaptic connections on developing post-synaptic neurons were also demonstrated at the molecular level (3). The nuclear changes reported in the present study suggest that at least part of these transynaptic trophic and metabolic effects on the development of post-synaptic neurons are due to an action on gene expression.

Between 9 days of incubation and hatching there are important maturational changes in the motoneurons. The number and complexity of synaptic contacts increase sharply (19). Myelination begins on the 14th day of incubation, and most of the tracts become myelinated between the 18th and the 21st day of incubation (4, 6). During this period the bioelectric activity and the embryonic motility undergo important qualitative and quantitative changes (24). Growth of the embryonic body causes a marked lengthening of the axons of the motoneurons and a concomitant increase in the nuclear volume. The main nuclear differences between motoneurons of embryos of 9 and 21 days of incubation are: a steady increase in nuclear and nucleolar volume, an augmentation of the total amount of non-nucleolar RNP particles, and a progressive unraveling of chromatin. The numerical density of perichromatin granules increases continuously in this period (37). All these changes suggest that the above mentioned morphological and physiological transformations are accompanied by a sustained enhancement of transcriptional activity in the nucleolus and in the extranucleolar compartment. However, no sharp transition of nuclear parameters is found in this period; all changes are the continuation at slower rates of the trends initiated in previous stages of development.

The decrease in compact chromatin found between multipolar neuroblasts of the peripheral layer of 4-day embryos and motoneurons of 21-day embryos can only be explained by modifications of chromatin disposition, because in this period there is no diminution of the DNA content per nucleus.

It is important to note that in all types of cells studied in the present work the nucleolus is restrained to a few positions inside the nucleus. This result suggests that in spite of the large mobility of the nucleus, the nucleolus is a fairly still structure.

REFERENCES

1. Bachellerie J.P., Puvion E. and Zalta J.P., 1975. Ultrastructural organization and biochemical characterization of chromatin-RNA-protein complexes isolated from mammalian cell nuclei. *Eur. J. Biochem.*, **58**, 327-337.
2. Bernhard W., 1969. A new staining procedure for electron microscopical cytology. *J. Ultrastruct. Res.*, **27**, 250-265.
3. Black I.B. and Geen S.C., 1973. Trans-synaptic regulation of adrenergic neuron development: inhibition by ganglionic blockade. *Brain Res.*, **63**, 281-302.
4. Cajal S.R., 1909. Histologie du système nerveux de l'homme et des vertébrés. A. Maloine. Vol. I.
5. Davidson E.H., 1976. Gene activity in early development. Academic Press. 2nd edition.
6. El-Eishi H.I., 1967. Biochemical and histochemical studies on myelination in the chick embryo spinal cord. *J. Neurochem.*, **14**, 405-412.
7. Fakan S. and Odartchenko N., 1980. Ultrastructural organization of the cell nucleus in early mouse embryos. *Biol. Cell.*, **37**, 211-218.
8. Fakan S., Puvion E. and Spohr G., 1976. Localization and characterization of newly synthesized RNA in isolated rat hepatocytes. *Expil. Cell Res.*, **99**, 155-164.
9. Fujita S., 1964. Analysis of neuron differentiation in the central nervous system by tritiated thymidine autoradiography. *J. Comp. Neurol.* **122**, 311-328.
10. Fujita H. and Fujita S., 1963. Electron microscopic studies on neuroblast differentiation in the central nervous system of domestic fowl. *Z. Zellforsch.*, **60**, 463-478.
11. Hamburger V., 1975. Cell death in the development of the lateral motor column of the chick embryo. *J. Comp. Neurol.*, **160**, 533-546.
12. Hamburger V. and Hamilton H.L., 1951. A series of normal stages in the development of the chick embryo. *J. Morphol.*, **88**, 49-92.
13. Levi-Montalcini R., 1949. The development of acoustic-vestibular centers in the chick embryo in the absence of afferent root fibers and of descending fiber tracts. *J. Comp. Neurol.*, **91**, 209-241.
14. Lyser K., 1968. Early differentiation of motor neuroblasts in the chick embryo as studied by electron microscopy. II. Microtubules and microfilaments. *Develop. Biol.*, **17**, 117-142.
15. Monneron A. and Bernhard W., 1969. Fine structural organization of interphase nucleus in some mammalian cells. *J. Ultrastruct. Res.*, **27**, 266-278.

16. Moyne G., Nash R.E. and Puvion E., 1977. Perichromatin granules and other nuclear components in isolated rat hepatocytes treated with cortisol and cycloheximide. *Biol. Cell.*, **30**, 5-16.

17. Nash R.E., Puvion E. and Bernhard W., 1975. Perichromatin fibrils as components of rapidly labeled extranucleolar RNA. *J. Ultrastruct. Res.*, **53**, 395-405.

18. Oppenheim R.W., 1975. The role of supraspinal input in embryonic motility: a re-examination in the chick. *J. Comp. Neurol.*, **160**, 37-50.

19. Oppenheim R.W., Chu-Wang I. and Foelix R., 1975. Some aspects of synaptogenesis in the spinal cord of the chick embryo: a quantitative electron microscopic study. *J. Comp. Neurol.*, **161**, 383-418.

20. Pearse A.G.E., 1968. Histochemistry theoretical and applied. Vol. 1, 647-648. J. and A. Churchill.

21. Petrov P. and Bernhard W., 1971. Experimentally induced changes of extranucleolar ribonucleoprotein components of interphase nucleus. *J. Ultrastruct. Res.*, **35**, 386-402.

22. Petrov P. and Sekeris C.E., 1971. Early action of α-amanitin on extranucleolar ribonucleoproteins as revealed by electron microscopic observation. *Exptl. Cell Res.*, **69**, 393-401.

23. Peusner K.D. and Morest D.K., 1977. Neurogenesis in the nucleus vestibularis tangentialis of the chick embryo in the absence of primary afferent fibers. *Neuroscience*, **2**, 253-270.

24. Provine R.R., 1972. Ontogeny of bioelectric activity in the spinal cord of the chick embryo and its behavioral implications. *Brain Res.*, **41**, 365-378.

25. Puvion E. and Lange M., 1980. Functional significance of perichromatin granule accumulation induced by cadmium chloride in isolated rat liver cells. *Exptl. Cell Res.*, **128**, 47-58.

26. Puvion E. and Moyne G., 1978. Intranuclear migration of newly synthesized extranucleolar ribonucleoproteins. A high resolution quantitative autoradiographical and cytochemical study. *Exptl. Cell Res.*, **115**, 79-88.

27. Puvion E., Moyne G. and Bernhard W., 1976. Action of 3'Deoxyadenosine (Cordycepin) on the nuclear ribonucleoproteins of isolated liver cells. *J. Microscop. Biol. Cell.*, **25**, 17-32.

28. Puvion E., Viron A. and Bernhard W., 1977. Unusual accumulation of ribonucleoprotein constituents in the nucleus of cultured liver cells after hypothermal shock. *Biol. Cell.*, **29**, 81-88.

29. Sauer F.C., 1935. Mitosis in the neural tube. *J. Comp. Neurol.*, **62**, 377-405.

30. Smith D., 1977. The effect of deafferentation on the development of brain and spinal nuclei. *Prog. Neurobiol.*, **8**, 349-367.

31. Stelzner D.L., Martin A.H. and Scott G.L., 1973. Early stages of synaptogenesis in the cervical spinal cord of the chick embryo. *Z. Zellforsch.*, **138**, 475-488.

32. Underwood E.E., 1970. Quantitative stereology. Addison-Wesley. London.

33. Vazquez-Nin G.H. and Bernhard W., 1971. Comparative ultrastructural study of perichromatin- and Balbiani ring granules. *J. Ultrastruct. Res.*, **36**, 842-860.

34. Vazquez-Nin G.H., Chavez B. and Tomas-Martin C., 1973. A preferential staining method for chromatin in electron microscopy. *J. Microscopie*, **16**, 243-246.

35. Vazquez-Nin G.H., Echeverria O.M., Molina E. and Fragoso J., 1978. Effects of ovariectomy and estradiol injection on nuclear structures of endometrial epithelial cells. *Acta Anat.*, **102**, 308-318.

36. Vazquez-Nin G.H., Echeverria O.M. and Pedron J., 1979. Effects of estradiol on the ribonucleoprotein constituents of the nucleus of cultured endometrial epithelial cells. *Biol. Cell.*, **35**, 221-228.

37. Vazquez-Nin G.H., Ortega-Rangel J.A. and Echeverria O.M., 1980. Nuclear aspects of neuroblast differentiation in the chick embryo. *Biol. Cell.*, **39**, 143-146.

38. Vazquez-Nin G.H. and Sotelo J.R., 1966. Neurofibrillar differentiation during embryonary growth. *J. Comp. Neurol.*, **128**, 313-332.

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