Gliogenesis during embryonic development in the rat[1]

G. D. Das

Department of Biological Sciences, Purdue University, West Lafayette (Indiana 47907, USA), 6 June 1977

Summary. With the aid of thymidine-H^3 autoradiography gliogenesis in the rat brain was seen to start during embryonic stages, which might continue into the postnatal stages of development. Gliogenesis followed a caudo-rostral gradient closely following neurogenesis. Ependymogenesis was found to occur in parallel with gliogenesis.

Studies on gliogenesis in the mammalian central nervous system show that origin and differentiation of glia cells occur primarily during postnatal development[2–6]. However, some reports indicate that to some extent gliogenesis takes place during embryonic development of the animals[7–10]. The latter studies suffer from the shortcoming that they are addressed to a few single neural structures, and therefore fail to provide a comprehensive view of gliogenesis during embryonic period in the whole brain. In an attempt to overcome this shortcoming the present investigation was conducted.

Materials and methods. Sperm positive Wistar albino rats were singly injected with thymidine-H^3 on one of days 8 through 22 of gestation (dose: 5 μCi/g b.wt; spec. act. 6.7 Ci/mM). Their offspring were sacrificed at 6 weeks of age by perfusion with 10% neutral buffered formalin. The brains obtained from these animals were embedded in Paraplast and cut serially at 8 μm thickness in coronal as well as sagittal plane. The sections were processed for autoradiography and the slides were dipped in Kodak NTB-3 nuclear emulsion, and stored at 5 °C. After an exposure for 10 weeks the material was developed and post-stained with cresyl violet.

For microscopic analysis 2 levels in the sagittal plane, 1 midsagittal and 1 parasagittal were used. 6 sections at each of the 2 sagittal levels were examined, and the locations of heavily labelled glial cells were plotted. The intensity of the heavy labelling on the nuclei of the glial cells did not permit clearcut identification of different types of cells. Therefore, results include findings on all the glia cells collectively. The observations made in sagittal plane were corroborated with findings made on the coronally cut material.

The following considerations helped determine labelled cells as the labelled glial elements. a) They were small and did not show any cytoplasm. Whereas small neurons in any neural struture were larger than glia cells and had distinctly visible cytoplasm. b) They were found in both white and grey matter of the brain, and were identical in appearance (figure 1). c) In any given neural structure they were found after or at the termination of neurogenesis. On these bases the labelled glia cells were clearly distinguished from the small neurons.

Results. Gliogenesis, as indicated by the presence of intensely labelled elements, was seen as early as on day 17 of gestation in the caudal portions of the brain stem. On subsequent days of gestation it progressed through rostral portions of brain stem, midbrain, diencephalon, septal region, basal ganglia and olfactory structures, reaching a peak on days 20–21 of embryogenesis. In cerebral cortex, hippocampus and corpus callosum gliogenesis started on day 20 and reached its peak on the following day. In the

1 Supported by research grants NS-08817 and CA-14650 from N.I.H.

2 I. Smart and C. P. Leblond, J. comp. Neurol. *116*, 349 (1961).
3 M. Dalton, O. R. Hommes and C. P. Leblond, J. comp. Neurol. *134*, 397 (1968).
4 S. Gilmore, Anat. Rec. *171*, 283 (1971).
5 J. Paterson, A. Privat, E. Ling and C. P. Leblond, J. comp. Neurol. *149*, 83 (1973).
6 A. Privat, in: International Review of Cytology, vol. 40, p. 281. Ed. G. Bourne and J. Danielli. Academic Press, New York 1975.
7 S. Fujita, J. comp. Neurol. *120*, 37 (1963).
8 S. P. Hicks and C. D'Amato, Anat. Rec. *160*, 619 (1968).
9 J. W. Hinds, J. comp. Neurol. *134*, 287 (1968).
10 D. Biesold, G. Brückner and V. Mares, Brain Res. *104*, 295 (1976).

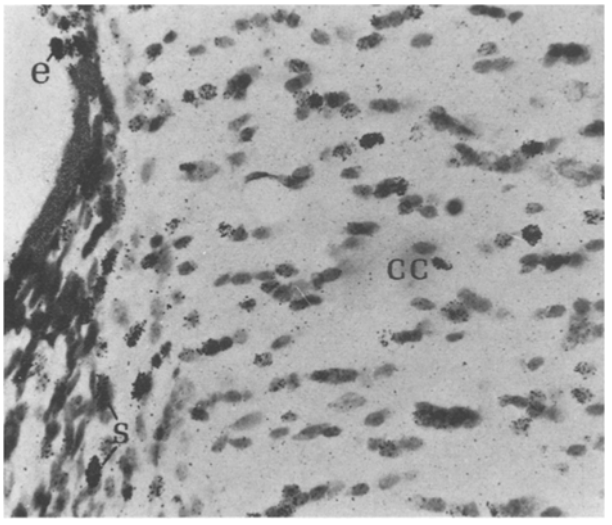

Fig. 1. Corpus callosum (cc) containing intensely as well as lightly labelled glia cells. Note intensely labelled subependymal cells (s) and ependymal cells (e) of the lateral ventricles. Animal injected with thymidine-H^3 on day 21 of gestation. ×300.

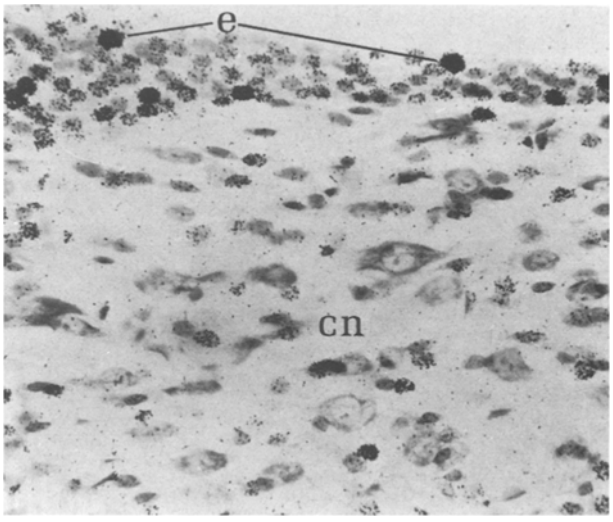

Fig. 2. Deep cerebellar nuclei (cn) showing intensely labelled glia cells, and roof of fourth ventricle containing intensely labelled ependymal cells (e). Animal injected with thymidine-H^3 on day 21 of gestation. ×300.

brains of animals receiving the radiochemical on day 22 of gestation, the number of labelled glia cells was reduced, indicating a decline in gliogenesis. From an overall point of view there were no differences in the white and the grey matter in gliogenesis. It seemed as if to sweep in broad strokes from caudal to rostral regions of the brain. An analysis of coronal sections did not reveal any mediolateral pattern of gliogenesis.

In the cerebellum intensely labelled glial elements were found in the deep cerebellar nuclei and the medullary layer surrounding them during the same period as described above, but not in the cerebellar cortex, which had only lightly labelled glial cells. This indicated that, in all likelihood, gliogenesis, in the sense of origin of glia cells out of last mitosis of their precursors and followed by their differentiation, was not present in the embryonic cerebellar cortex.

Another important fact observed was the presence of intensely labelled ependymal cells along the ventricular lumen in the brains of animals receiving thymidine-H^3 during embryogenesis. They, being highly specialized and different from their precursor neuroepithelial cells, must have arisen out of neuroepithelial cells during embryonic development. This represented ependymogenesis, and it was seen to run parallel to gliogenesis (figure 2).

Discussion and conclusions. The findings in this study have shown that gliogenesis during embryonic period is not confined to some selected structures, but is found to take place in the entire central nervous system except cerebellar cortex. Fujita[11] has demonstrated that gliogenesis in the cerebellar cortex is primarily a postnatal developmental event. The exact percentage of total glia cells formed during embryogenesis cannot be established from this study, but the results suggest this to be significant. In continuation with this, gliogenesis may be found during postnatal development and, at a low-rate, during adulthood[12-13].

Ependymogenesis was seen to follow a course parallel to gliogenesis, suggesting that these 2 histogenetic events may take place at the same time and, possibly, independently. This implies that as the last of the neuroepithelial cells undergo final mitoses to give rise to daughter cells determined to differentiate into the ependymal cells, the precursors of neuroglial elements, i.e. glioblasts, located in the embryonic white and grey matter undergo mitoses to give rise to the glial cells of embryonic origin. Some glioblasts or their undetermined daughter cells may continue to proliferate for long duration to contribute to gliogenesis during postnatal development.

11 S. Fujita, J. Cell Biol. *32*, 277 (1967).
12 O. R. Hommes and C. P. Leblond, J. comp. Neurol. *129*, 269 (1967).
13 H. Korr, B. Schultze and W. Maurer, J. comp. Neurol. *160*, 477 (1975).

Comparison of native and microwave irradiated DNA[1]

M. M. Varma and E. A. Traboulay

Bio-Environmetal Engineering and Sciences Research Laboratory, School of Engineering, Howard University, Washington DC 20059, USA, and School of Engineering and Applied Sciences, The George Washington University, Washington DC 20052, USA, 4 April 1977

Summary. Chemical changes induced in the testicular DNA of irradiated Swiss male mice are described. Parameters quantified were hyperchromocity and melting temperature. The data supports the possibility that microwave causes strand separation.

A portion of the population is being exposed to increasing number of devices which emit potentially hazardous microwaves. Most of these devices have gained their access to the consumer through medical, industrial and household applications.

Material and methods. The genetic damage caused by microwave exposure is not well documented. The purpose of this study was to determine structural changes in the testicular DNA induced by exposing to microwaves. Swiss mice, 56 days old (Charles River Breeding Laboratories, Wilmington, MA), were irradiated in a walk-in anechoic chamber at Walter Reed Army Medical Center, Washington, DC. The equipment and calibration procedures have been described elsewhere[2,3]. The irradiation frequency was 1.7 GHz, and the power density was 50 mW/cm^2 with an exposure time of 30 min. The results were compared with sham irradiated controls. The parameters quantified were a) hyperchromicity, and b) melting temperatures. A Gilford 2400 spectrophotometer was used for obtaining thermal profiles.

Each animal was laid supine on a platform in front of the waveguide in the near field. To avoid movement in the field during irradiation the mice were anesthetized with 1% phenobarbital. Care was taken so that only the testes were irradiated, the rest of the body being shielded with loaded urethane foam (Eccosorb-An). Sham irradiated controls were placed outside the waveguide during exposure of the irradiated group. Animals were observed during irradiation via a closed circuit television monitor. At the termination of the exposures, the males were sacrificed, the testes pooled and DNA isolated according to the procedure outlined by Britten et al.[4,5]. Because of the problems in introducing thermocouples in the field, the body temperature during irradiation was not monitored. However, rectal temperatures were taken before and after irradiation. Normal temperatures of Swiss mice varied between 35 and 37 °C. Rectal temperatures taken after exposure indicated an increase of 1–2 °C. Minimal temperature changes occurred in the sham irradiated controls.

1 This work was supported by grant No. N00014-A-0346-0002, from the Department of the Navy, Office of Naval Research, Arlington, VA.
2 M. M. Varma and E. A. Traboulay, Experientia *31*, 301 (1975).
3 M. M. Varma and E. A. Traboulay, Biological Effects of Non-Ionizing Radiation, Office of Naval Research, Arlington, VA, Contract No. N0014-73-A0346-0002, July 1975.
4 R. J. Britten, D. E. Graham and B. R. Neufeld, in: Analysis of Repeating DNA Sequences by Reassociation, Methods in Enzymology, vol. XXIX, p. 363. Ed. Grossman and Moldave. Academic Press 1974.
5 R. J. Britten and D. E. Khone, Science *161*, 529 (1968).