# PROLIFERATIVE ACTIVITY OF CULTURED RAT GLIAL CELLS AFTER IRRADIATION OF PROGENITORS OR MULTIPLYING AND DIFFERENTIATING GLIAL CELLS.

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## INTRODUCTION

Both glial and mesodermal cells participate in the formation of scar tissue after injury in the adult central nervous system. According to most authors, the glial response to brain injury is present in fetus and new born animals but incomplete. The mature scarring would be effective only on day 8 after birth in rat (1). The GFAP+ (Glial acidic Fibrillary Protein) cells in the rat fetuses cortex were first detectable on day 19 (2). Are there, early in gestation, target cells sensitive to the deleterious effects of irradiation?

## MATERIAL and METHODS

Irradiation (1 Gy) were carried out on 15 or 21 days pregnant rats or on nerve cells just after the beginning of culture. On day 1 after birth, the cortex cells were isolated and cultured in medium with 10% fetal calf serum (FCS) until 30% of confluency. The cells were synchronized in the G1 phase of the cell cycle by a 48 hours culture in medium with 0.1% FCS. This arrest was verified by BrdU incubation. The medium was then replaced by one with 10% FCS for the cells to enter the S phase and 20 hours later $^{3}H$ thymidine or BrdU were added for 6 hours. The radioactivity was measured in washed cells and BrdU+, GFAP+ cells by immunohistochemistry were counted on 5 microscopic fields. The MTT survival test was carried out on all cultures.

## RESULTS

The culture with 10% FCS promoted the survival of astrocytes (GFAP+) which represented 99% of the cells. After irradiation, the GFAP+ cells survived (MTT test) as well as the controls (figure 1). The cell cycle arrest with 0.1% FCS was effective as only 7% of cells were BrdU+. After return to 10% FCS, 35% of control glial cells were BrdU+. The proliferative activity of cells from fetuses irradiated on day 15 was increased by 100% and on day 21 to about 50% of the control

values (figure 2). The proliferative activity of the cells irradiated *in vitro* was similar to the control values.

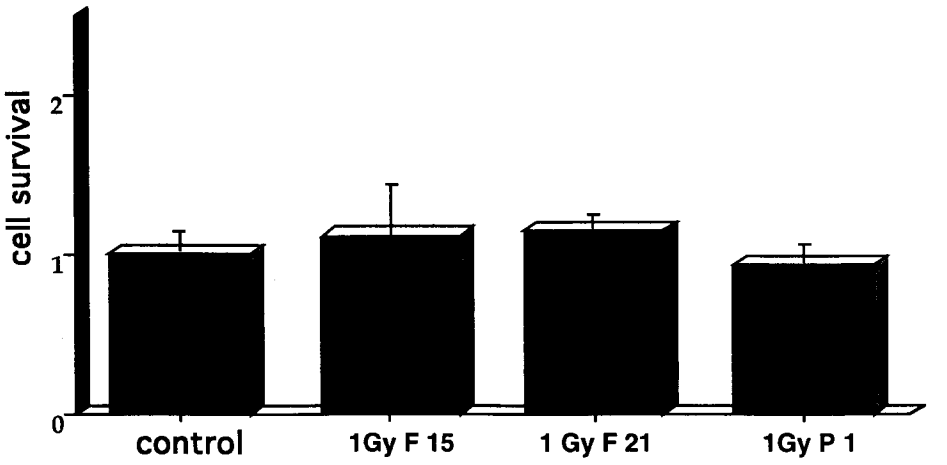


Figure1: Cell survival measured by MTT test relative to control survival. P 1: isolated nerve cells irradiated on day one postnatal; F 15 or F 21: nerve cells isolated from 1 day old animals exposed when they were 15 or 21 day old fetuses

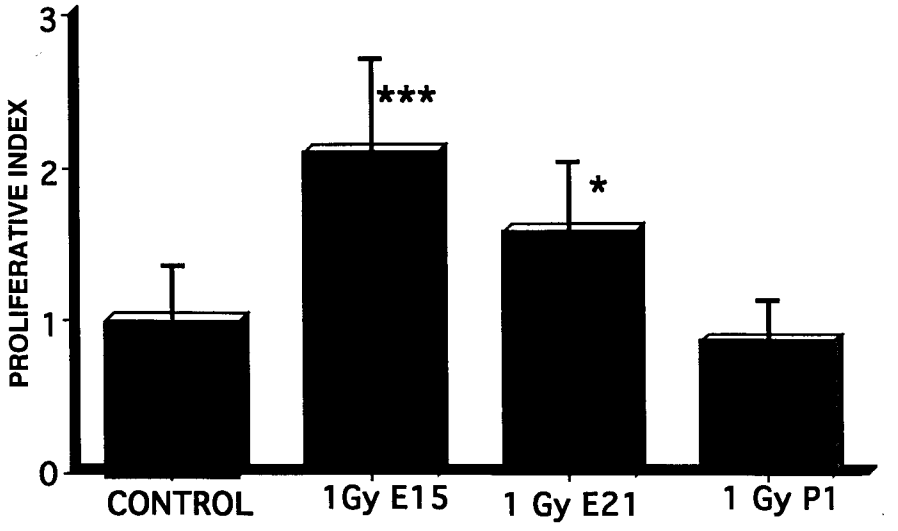


Figure 2: GFAP+ glial cells proliferation relative to the control one.
*** $p < 0.001$,
* $p < 0.05$

## DISCUSSION

The neurons death induced by irradiation was most effective on gestational day 15 when these cells were dividing actively (3) but glial cells present as progenitors were radioresistant (unpublished results) . On the other hand, few neurons were dividing on day 21 and few were killed by irradiation. In spite of glial cells division from progenitors, these cells were rather radioresistant in comparison with the proliferative neurons (unpublished results). The increase of proliferative activity induced by the irradiation on day 15 of gestation was very interesting because at that time the glial cells were mainly as progenitors. The increase in proliferative activity was lower on day 21 when glial cells started dividing. The progenitors of glial cells could be the target of ionizing radiation to induce proliferation. We can hypothetize that the exposure on day 15, the most radiosensitive period for the cortex neurons, kill a great number of these cells which may release directly or indirectly (via mesenchymal cells) factors like FGF (4) that stimulate progenitor glial cells to proliferate. On day 21pc, the decrease of both neurons radiosensitivity and the number of progenitor glial cells (sensitive to released growth factors) could explain the lower increase of the proliferative activity of the glial cells. On day one after birth, no change was observed with the cells irradiated *in vitro*. This could be either the consequence that glial cells and neurons had lost their close relationships or glial cells could not respond to irradiation at this age.

## CONCLUSION

This increase of proliferative activity after prenatal irradiation is very important because:

- the gliomas incidence could be enhanced
- a postnatal brain injury could recruit more reactive glial cells to proliferate.

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