

Gliosis and Glioma Distinguished by Acridine Orange

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ABSTRACT: Acridine orange fluorochrome of nucleic acids was applied to sections of cerebral tissue from 20 patients showing acute or chronic reactive gliosis. The results were compared with the findings in 39 well differentiated and malignant astrocytomas. The orange cytoplasmic fluorescence of ribonucleic acid is lacking in reactive astrocytes of all ages including gemistocytes, but is uniformly present in astrocytoma cells. Acridine orange is a useful supplementary stain for distinguishing between astrocytosis and astrocytoma, particularly for small cerebral biopsies showing scattered or diffusely infiltrating pleomorphic glial cells.

RÉSUMÉ: Distinction entre la gliose et le gliome au moyen de la coloration par l'acridine orange Des coupes de tissu cérébral provenant de 20 patients présentant une gliose réactionnelle aiguë ou chronique ont été colorées à l'acridine orange, un fluorochrome des acides nucléiques. Les résultats ont été comparés à ceux obtenus dans les astrocytomes bien différenciés. Dans les astrocytes réactionnels de tous âges, comprenant les gémistocytes, la fluorescence cytoplasmique orange de l'acide ribonucléique est absente, alors qu'elle est uniformément présente dans les cellules d'astrocytomes. Les cellules microgliales présentent une légère fluorescence orange. L'acridine orange est une coloration supplémentaire utile pour distinguer entre l'astrocytose et l'astrocytome, particulièrement sur des petites biopsies cérébrales présentant des cellules gliales pléomorphes dispersées.

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We previously demonstrated the use of acridine orange (AO) fluorochrome as a histochemical marker of nucleic acids in a wide variety of nervous system tumours in children.¹ In the glial series of cerebral neoplasms, we found that cytoplasmic ribonucleic acid (RNA) showed weaker fluorescence in well differentiated astrocytoma cells than in anaplastic cells of high grade astrocytomas. No AO-RNA fluorescence was demonstrated in reactive gemistocytes. The present study further considers both acute and chronic astrocytosis in relation to AO-RNA fluorescence, to better establish whether this technique might be useful in distinguishing between reactive astrocytosis and low grade astrocytoma. This distinction is of foremost importance in the treatment of patients, but often is difficult in small biopsies of lesions and in diffusely infiltrating, well differentiated astrocytomas. AO may provide another reliable criterion for this important distinction.

MATERIALS AND METHODS

Histological examples of reactive gliosis of the brain were selected from autopsies or surgical biopsies of 20 patients ranging in age from premature infants to 85 years. In 3 patients the gliosis was secondary to viral encephalitis: one case of sub-acute sclerosing panencephalitis (SSPE) and 2 cases of herpes

encephalitis. Gliosis associated with hepatic encephalopathy in one adult was examined. Dysraphic spinal cords of 2 infants showing extensive gliosis also were available for examination. The remaining 14 cases represented gliosis surrounding zones of cerebral infarction or hemorrhage. Six of these were considered acute, 9 days to 5 weeks after infarction; the other 8 were chronic, several months in duration. The ages of the lesions seen histologically correlated well with the clinical courses of the patients.

Tissue had been fixed in 10 percent buffered formalin and embedded in paraffin according to standard neuropathological techniques. Freshly frozen (cryostat) sections also were available in 3 cases. Tissue sections were stained with acridine orange, examined in the fluorescence microscope, and photographed according to methods previously described.² The phosphate buffer solution was maintained at a constant pH of 6.0.

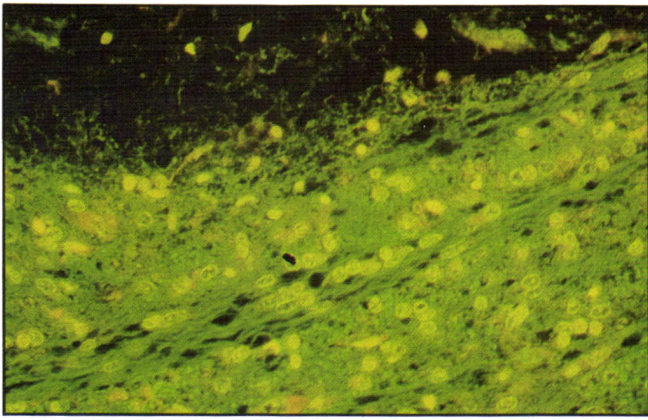
The 35 gliomas previously reported¹ using this technique were reviewed, together with four additional cases of astrocytoma in children. Comparisons were made with the cases of reactive gliosis on the basis of qualitative assessment of the intensity of the orange cytoplasmic fluorescence.

Immunoperoxidase reactions for glial fibrillary acidic protein (GFAP) were performed in several of the cases to confirm the identity of pleomorphic cells as glial. Hematoxylin-eosin stained sections were available for examination in all cases.

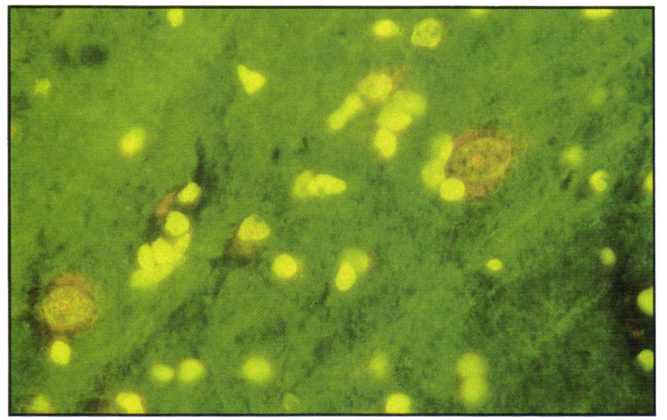
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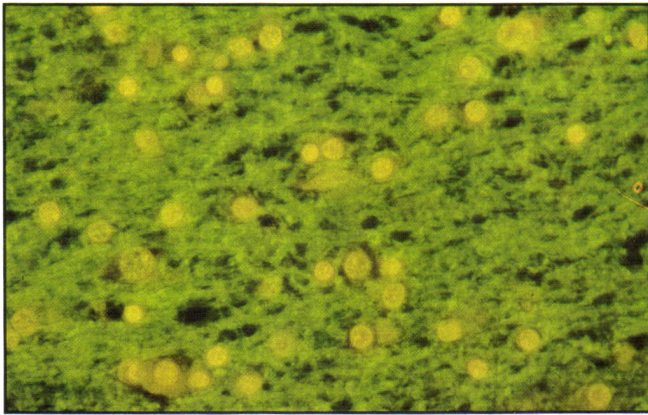
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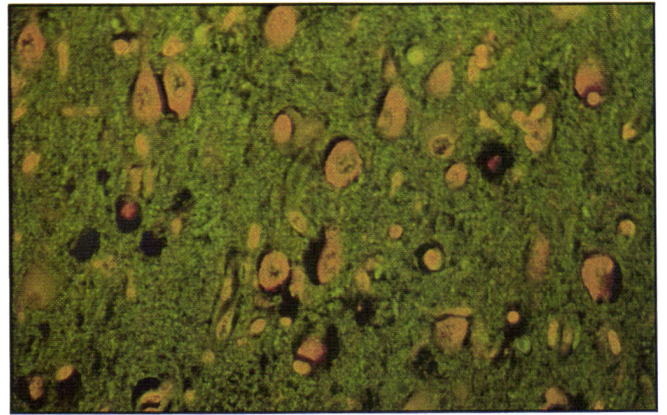
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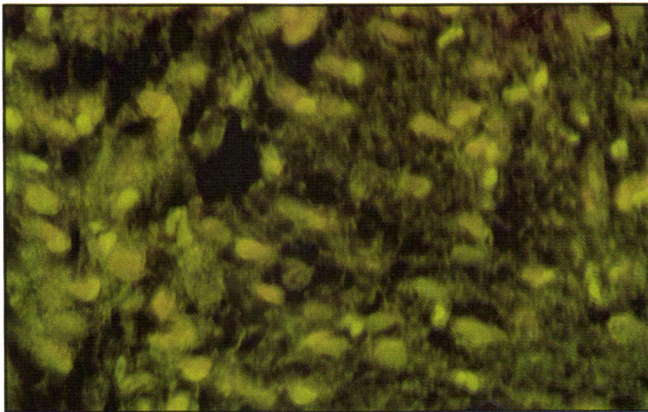
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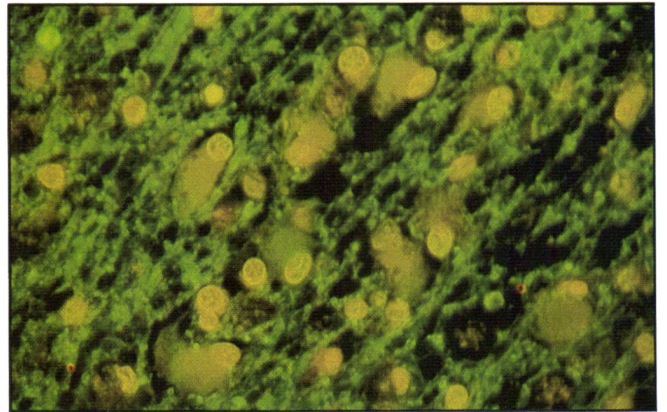
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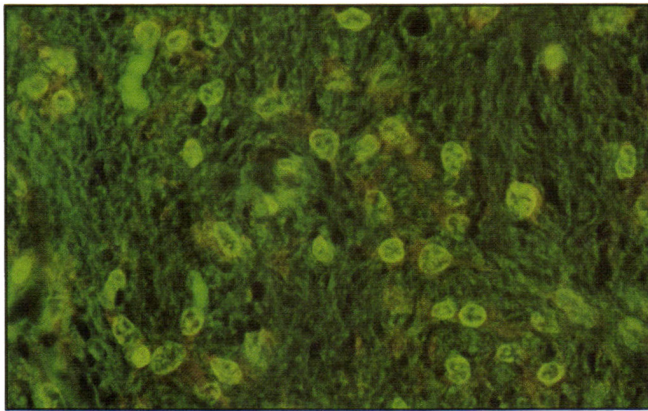
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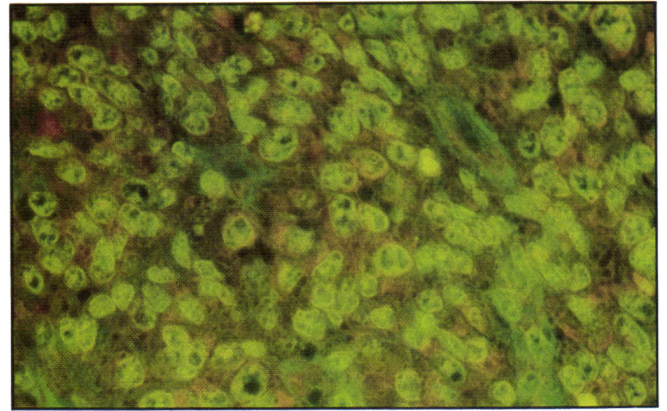
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Figure 1 — *Candida cerebritis* and infarction in pons of a 5-week-old infant born at 26 weeks gestation. A few neurons are identified by their orange cytoplasmic fluorescence, but the acute reactive astrocytes remain pale green. Acridine orange. X250.

Figure 2 — Frontal lobe biopsy of an 8-year-old boy with subacute sclerosing panencephalitis (SSPE) during a rapidly progressive course. Glial proliferation including many satellite astrocytes around neurons is seen. Neurons retain abundant RNA seen as orange cytoplasmic fluorescence, but most glial cells show none or very little. A few glial cells have weakly fluorescent orange cytoplasmic rims. Nuclear chromatin of all cells appears yellow. Acridine orange. Frozen section. X400. Also see Figure 9.

Figure 3 — Acute gliosis near zone of cerebral infarction 2 to 4 weeks old in parietal lobe of a 26-year-old man. No RNA is demonstrated in astrocytes by acridine orange. X400.

Figure 4 — Acute gliosis in hippocampus following multiple ischemic episodes in the last 17 days of life in a 69-year-old man. Neurons still exhibit strong AO-RNA fluorescence, but reactive astrocytes show none. Acridine orange. X250.

Figure 5 — Chronic gliosis in subcortical white matter of a 3-month-old boy with multiple cerebral infarcts due to cyanotic congenital heart disease and recurrent episodes of ischemichypoxic encephalopathy. Reactive astrocytes show no cytoplasmic AO-RNA fluorescence. Acridine orange. X250.

Figure 6 — Chronic gliosis around an infarct in the cerebral hemisphere of an 85-year-old woman. Clinical history suggests that this infarct occurred about 9 months previously. Many gemistocytes are seen, but AO-RNA fluorescence is not evident in these cells despite their abundant cytoplasm. Acridine orange. X400. Also see Figure 10.

Figure 7 — Well differentiated cerebellar astrocytoma in a 7-year-old boy. The scattered tumour cells are easily distinguished from reactive astrocytes by a definite orange cytoplasmic fluorescence. Acridine orange. X400.

Figure 8 — Malignant astrocytoma resected from the temporal lobe of a 16-year-old boy. The highly pleomorphic tumour cells exhibit brilliant orange cytoplasm owing to their high concentration of ribosomes. Acridine orange. X400.

RESULTS

The cytoplasmic orange fluorescence of RNA was absent or extremely faint in reactive astrocytosis, whether acute (Figures 1-4) or chronic (Figures 5, 6), in all cases of gliosis associated with cerebral infarction regardless of age of the patient. Even large gemistocytes with abundant cytoplasm appeared green. The reactive astrocytes in the case of hepatic encephalopathy showed no AO-RNA fluorescence. In the encephalitis cases, a few scattered glial cells showed faint orange tinges of their rim of cytoplasm (Figure 2), but most exhibited no orange colour, even as satellite cells in relation to neurons (Figure 2).

Nuclear deoxyribonucleic acid (DNA) emitted a luminous yellow fluorescence corresponding to the same chromatin pattern with hematoxylin-eosin stain. Neurons were easily distinguished by their strong cytoplasmic orange fluorescence and by their characteristic morphology (Figures 1, 2, 4). An orange nucleolus was visible in some cells.

Figures 9 and 10 illustrate the morphological appearance of reactive astrocytes from two of the cases studied with AO, after restaining with hematoxylin-eosin. The gliotic zones beneath the ependyma of the two dysraphic spinal cords examined (Figure 11) showed no orange cytoplasmic fluorescence in astrocytes, although neurons and ependymal cells clearly had orange-coloured cytoplasm.

By contrast with reactive astrocytes, astrocytoma cells exhibited definite orange cytoplasmic fluorescence (Figures 7, 8), tumours of low grade showing less intensity than those of high grade.¹ Three oligodendrogliomas had no demonstrable orange fluorescence in typical tumour cells, though the astrocytic tumour cells of these mixed neoplasms were characteristic of astrocytoma with AO.

COMMENT

Secretory cells have the highest content of RNA of all cells in the body. Among secretory cells, neurons have an abundance of ribosomes for the synthesis of neurotransmitters. Normal glial cells have very little RNA and, unlike neurons, it is almost all nucleolar.³ Normal oligodendrocytes have more ribosomes than do astrocytes,^{4,5} but oligodendrocytoma cells have less cytoplasmic RNA than do astrocytoma cells as previously shown by AO.¹ In vitro kinetic studies of labelled orotic acid and cytidine incorporated into RNA show much greater uptake of these precursors into neuronal than into glial fractions.^{3,6}

The reactive astrocytic proliferation in response to infarction or to other neural tissue injury is morphologically evident during the second week after the insult and continues for many months, as demonstrated by common histological staining techniques.⁵ Reactive astrocytes may become quite pleomorphic and closely resemble astrocytoma cells, especially in chronic reactions. Electron microscopy of hypertrophic reactive astrocytes (i.e. gemistocytes) shows that their abundant cytoplasm is filled with membranous organelles, filaments, glycogen, and fat, but contains few ribosomes.⁵ The lack of AO-RNA fluorescence of these cells is thus an expected finding. Oligodendrocytes and astrocytes, both protoplasmic and fibrillary varieties, of normal infant and adult brain similarly show no AO-RNA fluorescence.² Astrocytic proliferation is said to be the "scar formation" of the central nervous system. Proliferating fibroblasts forming scar tissue elsewhere in the body are filled with ribosomes and fluoresce strongly with AO. The difference may be attributed to the active secretion of collagen proteins by fibroblasts. The number of ribosomes needed for synthesis of a cell's own cytoplasm and organelles is very modest by contrast

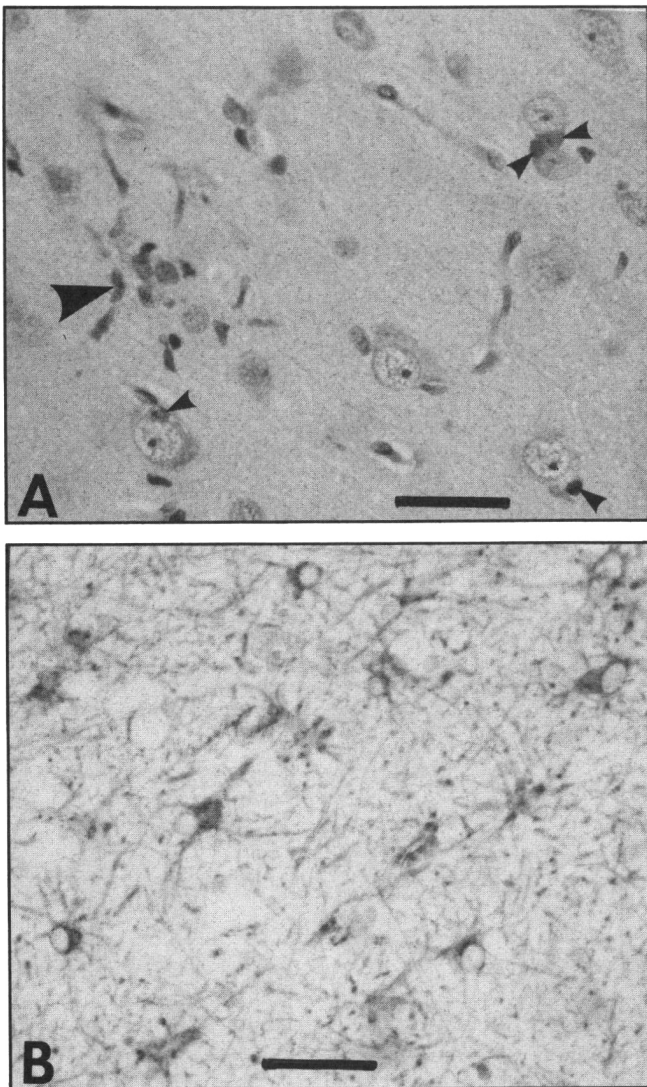


Figure 9 — Same case of SSPE as shown in Figure 2. (A) Cerebral cortex of frontal lobe shows satellite astrocytes around neurons (small arrowheads) and microglial nodules (large arrowhead) as part of intense gliotic reaction. Haematoxylin-eosin. (B) Subcortical white matter at margin of cortex also shows diffuse astrocytosis with immunoperoxidase stain for glial fibrillary acidic protein (GFAP). Calibration bar = 10 µm.

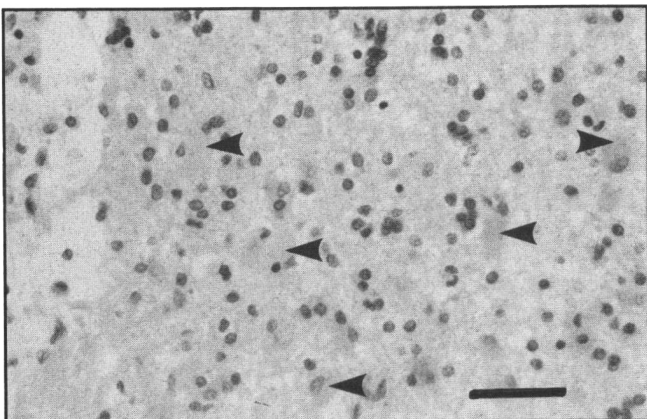


Figure 10 — Same case of chronic gliosis as shown in Figure 6. Zone of old infarction and cavitation is seen at left. Many reactive astrocytes are found, including large gemistocytes with abundant cytoplasm (arrowheads). Haematoxylin-eosin. Calibration bar = 10 µm.

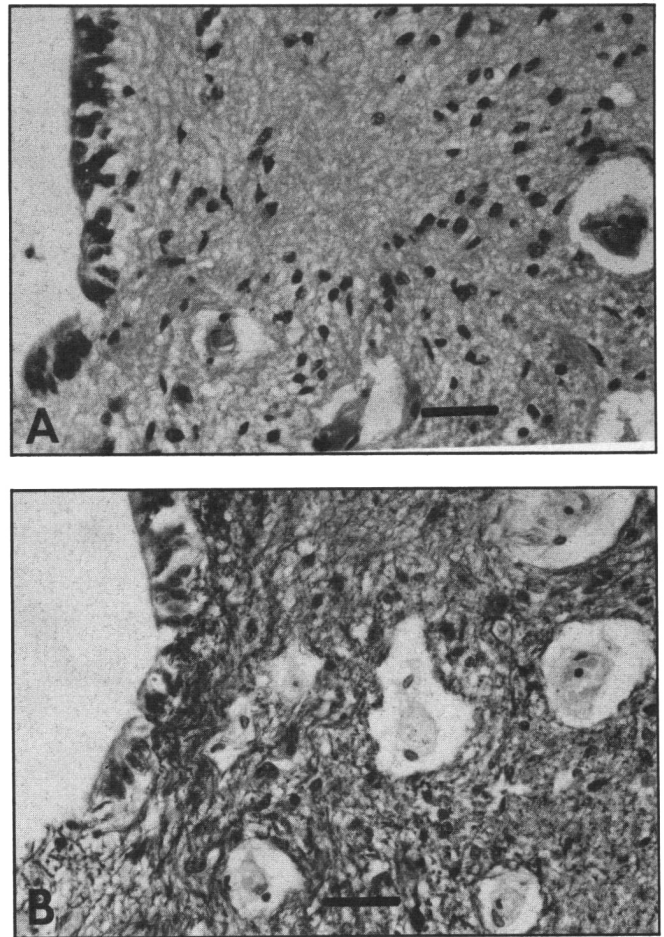


Figure 11 — Sacral segment of spinal cord of neonate dying with congenital myelomeningocele. The dysraphic spinal cord shows intense subependymal gliosis, but these cells exhibited no cytoplasmic AO-RNA fluorescence. (A) haematoxylin-eosin. (B) immunoperoxidase reaction for glial fibrillary acidic protein (GFAP) shows dense matrix of glial fibres. Calibration bar = 20 µm.

with the requirements for external secretory function, even during periods of rapid growth or mitosis.

A small amount of AO-RNA fluorescence was detected in cells other than neurons of the encephalitis cases. Many of these cells were microglial, by their characteristic morphology seen with hematoxylin-eosin staining (Figure 10). Microglial cells are of mesodermal rather than ectodermal origin. Large numbers of microglial cells may present a potential source of error in interpreting AO findings.

The AO technique is not useful in distinguishing oligodendrogloma cells from reactive astrocytes because of the lack of orange cytoplasmic fluorescence in both. However, the histological appearance of oligodendroglomas is so distinctive that special stains are usually not required. Some tumours are mixed oligodendrogloma/astrocytoma.

We conclude that AO is a useful supplementary stain for distinguishing reactive gliosis from low grade astrocytoma on the basis of a much stronger fluorescence in the neoplastic cells. The total staining technique was fewer steps than with hematoxylin-eosin and takes only 5-6 minutes. The simple and rapid AO method and its applicability to frozen as well as to paraffin sections of tissue make it versatile as a special stain during the course of neurological surgery.

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REFERENCES

1. Sarnat HB, Curry B, Rewcastle NB, Trevenen CL. Cytoplasmic RNA in nervous system tumours in children: A fluorochromic histochemical study using acridine orange. *Can J Neurol Sci* 1986; 13: 31-41.
2. Sarnat HB. L'acridine-orange: un fluorochrome des acides nucléiques pour l'étude des cellules musculaires et nerveuses. *Rev Neurol (Paris)* 1985; 141: 120-127.
3. Volpe P, Guiditta A. Biosynthesis of RNA in neuron-glia-enriched fractions. *Brain Res* 1967; 6: 228-240.
4. Peters A, Palay SL, Webster H de F. *The Fine Structure of the Nervous System: The Neurons and Supporting Cells*. Philadelphia: WB Saunders, 1976.
5. Duffy PE. *Astrocytes: Normal, Reactive, and Neoplastic*. New York: Raven Press, 1983.
6. Flangas AL, Bowman RE. Differential metabolism of RNA in neuronal-enriched and glial-enriched fractions of rat cerebrum. *J Neurochem* 1970; 17: 1237-1245.