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Experimental neurocytomas

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Summary. Four ethyl-nitrosourea (ENU)-induced oligodendroglioma-like tumors of the rat showing large rosettes on haematoxylin-eosin stain were studied by means of immunohistochemistry and electron microscopy, and their features compared with six human intraventricular neurocytomas. The similarities between the experimental and human tumors studied support the hypothesis that most of the so-called ENU-induced oligodendrogliomas in the rat are primitive neuroectodermal tumors with the tendency to differentiate toward a neuronal phenotype, and also suggest that the ENU-model of neurocarcinogenesis is useful for the induction of experimental neurocytomas.

Key words: Neurocytoma, Oligodendroglioma, ENU, Experimental brain tumors

Introduction

In clinical neuropathology, central neurocytoma is now considered as a well-defined tumor that may create problems from the point of view of histological diagnosis, since its resemblance to oligodendrogliomas makes it very difficult to identify the neuronal nature of the tumor cells by the usual histological techniques (Hassoun et al., 1982; Pearl et al., 1985; Townsend and Seaman, 1986; Nishio et al., 1988; Poon et al., 1988; Barbosa et al., 1990; Patil et al., 1990; Vaquero et al., 1992a).

This neoplasm was first described in 1982 by Hassoun et al. who defined it as benign tumor consisting of uniform neuron-like cells in a patch of fibrillary stroma. Since the characterization of these tumors, it has been established that a great number of neoplasms previously classified as intraventricular oligodendrogliomas could now be reclassified as intraventricular neurocytomas. At present, it is accepted that a central neurocytoma can be suspected when an intracranial tumor with histological features of oligodendroglioma shows tumor cells arranged around nucleus-free fibrillary zones, resembling the large rosettes described by Borit et al. in pineocytomas (Borit et al., 1980; Vaquero et al., 1992a). Although ultrastructural studies can demonstrate the neuronal character of the tumor, it has recently been pointed out that immunohistochemical demonstration of synaptophysin can be useful in establishing an accurate pathological diagnosis when large rosettes are present in a tumor showing the histological appearance of oligodendroglioma (Barbosa et al., 1990; Vaquero et al., 1992a).

On the other hand, our present experience with the so-called ethyl-nitrosourea (ENU)-induced oligodendrogliomas in the rat shows that these experimental tumors can express neuronal markers, such as synaptophysin, suggesting their neuronal character (Vaquero et al., 1992b).

Because the confusing oligodendrogliomatous aspect of a tumor of neuronal nature seems to be a common feature of both human central neurocytomas and murine ENU-induced oligodendrogliomas, we have studied the morphological similarities between these tumors, with the hypothesis that some of the so-called ENU-induced oligodendrogliomas could be classified as experimental neurocytomas.

Materials and methods

Among twenty intracerebral tumors with histological appearance of oligodendrogliomas induced in Wistar rats by means of transplacentary administration of ENU (50 mg/kg IP, at 17 day of gestation), we selected four tumors showing large rosettes with haematoxylin-eosin technique. These tumors appeared in the offspring after a latency of between 8 and 13 months. In addition, we have reviewed six human intraventricular neurocytomas for the purpose of studying the morphological

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similarities between these murine and human tumors.

In addition to the haematoxylin-eosin techniques on paraffin-embedded material, in all the tumors, immunohistochemical techniques for detection of synaptophysin, GFAP and vimentin were performed. Polyclonal rabbit anti-GFAP, and monoclonal mouse anti-vimentin and synaptophysin were used as primary antisera. All antibodies except synaptophysin (Euro-Diagnostic, Holland, dilution 1:50) were obtained pre-diluted from Bio-Genex Laboratories (San Ramon, CA, USA). Briefly, the staining procedure was as follows: 5-μm thick sections were deparaffinized in xylene, rehydrated in a graded ethanol series, and rinsed in phosphate-buffered saline (PBS), pH 7.6. They were placed in 3% H_2O_2 in water for 10 minutes to block endogenous peroxidase activity, and immersed in PBS for 30 minutes. The subsequent incubations were as follows (all at 37 °C in humid chamber): a) normal serum from the species in which the secondary antibody was made, 10 minutes; b) primary antibodies 30 minutes, three 5-minute washes in PBS; c) biotin-labelled goat anti-mouse antibody (in monoclonal antibodies) or biotin-labelled goat anti-rabbit antibody (in polyclonal antibodies) for 20 minutes, three 1-minute washes in PBS; and d) Avidin-biotin-peroxidase or streptavidin-alkaline phosphatase complex (Bio-Genex) 20 minutes, three 1-minute washes in PBS. Then, incubation in substrate took place using 3-amino-9-ethyl-carbazole or fast red TR. A nuclear counterstaining with haematoxylin was applied after substrate reaction. In all cases, positive stain control was done with cerebellum for GFAP and synaptophysin, and with skin for vimentin. Negative controls were carried out, substituting primary antibodies for 1-2 drops of normal serum in the course of the procedure.

Samples were taken of four human and two experimental tumors for ultrastructural study. They were fixed in 3% glutaraldehyde and embedded in Vestopal W. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined by transmission electron microscopy.

Results

In the four experimental tumors the haematoxylin-eosin technique disclosed large rosettes, with tumor cells arranged around nucleus-free fribrillary zones. This finding was the main histological feature of the human neurocytomas. In all the cases tumor cells showed a rounded nucleus and a small cytoplasm, frequently with a honeycomb appearance, suggesting a diagnosis of oligodendroglioma (Figs. 1, 3, 4).

Immunohistochemical studies disclosed a similar pattern of synaptophysin expression in both experimental and human tumors. This marker was positive in the tumor neuropil, especially in the acellular areas. Isolated cells, showing a strong cytoplasmic immunostain were also observed (Figs. 2, 5).

The GFAP marker was constantly negative on tumor cells. Nevertheless, isolated GFAP-positive cells were detected in both experimental and human tumors. These immunostained cells were interpreted as reactive astrocytes trapped in the tumor.

Table 1. Number of tumors showing positivity/number of studied tumors. Isolated cells showing positivity to GFAP were considered as trapped astrocytes. In all the cases, positivity to synaptophysin was found on tumor neuropil and on tumor cells. In all the experimental tumors of the series, most of the tumor cells showed strong cytoplasmic positivity to vimentin.

	GFAP	SYNAPTOPHYSIN	VIMENTIN
EXPERIMENTAL TUMORS	0/4	4/4	4/4
HUMAN NEUROCYTOMAS	0/6	6/6	0/6

Vimentin was constantly negative on tumor cells of human neurocytomas, but this marker was always present in cells of experimental tumors (Fig. 6).

Electron microscopy of the human tumors showed a uniform population of cells with rounded nucleus and scarce cytoplasm of neuronal aspect. A well-developed rough endoplasmic reticulum and a great number of free ribosomes were a constant finding (Figs. 7, 8). A neuropil, mature in appearance, surrounded the tumor cells and the blood vessels, and synaptic endings were occasionally observed on the surface of tumor cells. This ultrastructural pattern was also found in the four cases of experimental tumors (Figs. 9, 10). Frequently, neurotubules and dense-core vesicles were found within the processes of tumor cells, in both human and experimental tumors (Figs. 11, 12).

Discussion

Our present study shows that a number of ENU-induced tumors in the rat, composed of rounded uniform cells and generally interpreted as oligodendrogliomas, exhibit large rosettes and synaptophysin expression, suggesting a correspondence with the so-called central neurocytomas of human pathology. Furthermore, ultrastructural studies disclose great similarities between the experimental and human tumors that we have analyzed here.

In a previous work, we described a strong synaptophysin expression in most of the so-called ENU-induced oligodendrogliomas of the rat, suggesting their neuronal character (Vaquero et al., 1992b). According to this interpretation, morphological features of neuronal differentiation, such as the presence of large rosettes on haematoxylin-eosin (Rubinstein, 1982), could be expected in some ENU-induced oligodendroglioma-like tumors. Our present data demonstrate that, in addition to this finding, some of the ENU-induced brain tumors can show immunohistochemical and ultrastructural features in common with human central neurocytomas. Although a discordance seems to exist between human and

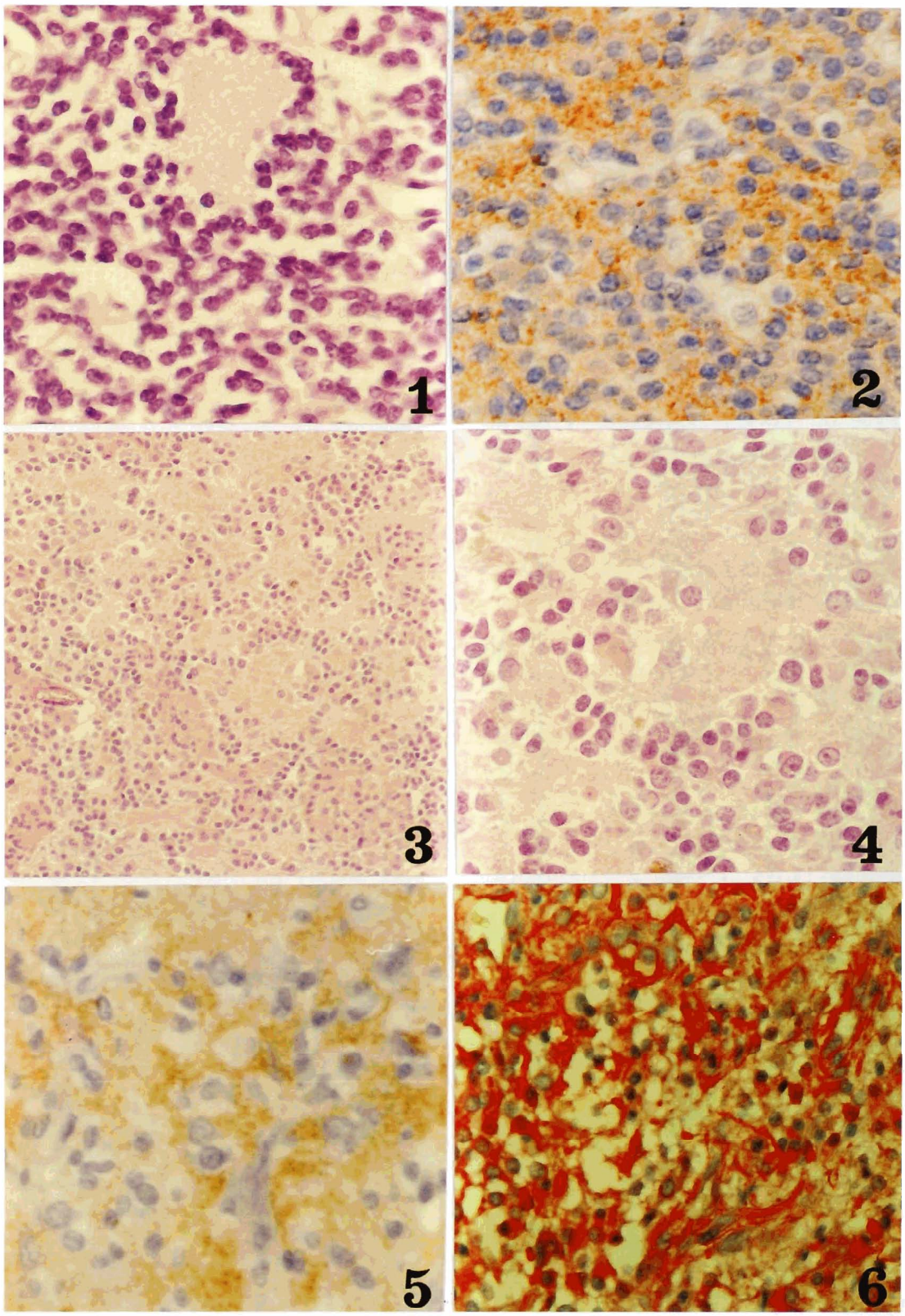


Fig. 1. Human neurocytoma showing small, rounded cells arranged around nucleus-free fibrillary zones. H-E. x 250

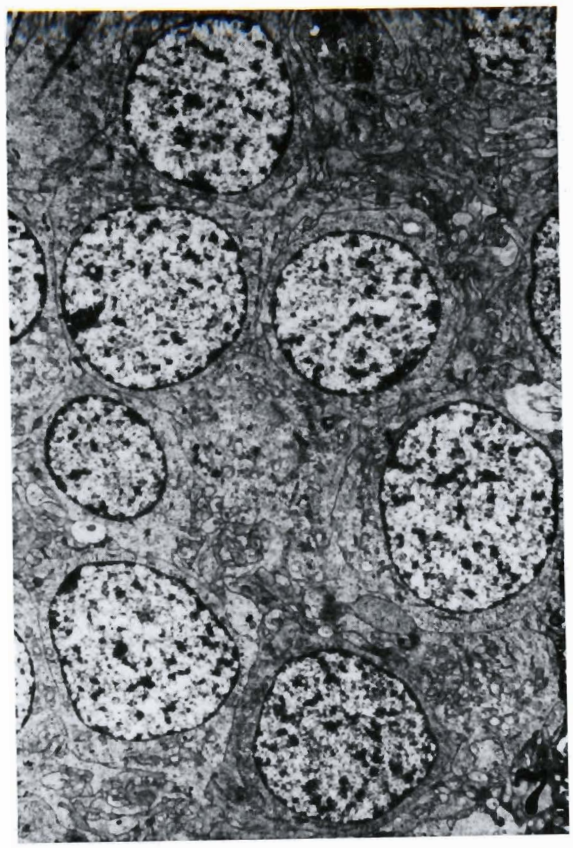
Fig. 2. Synaptophysin expression in the tumor neuropil of human neurocytomas. x 250

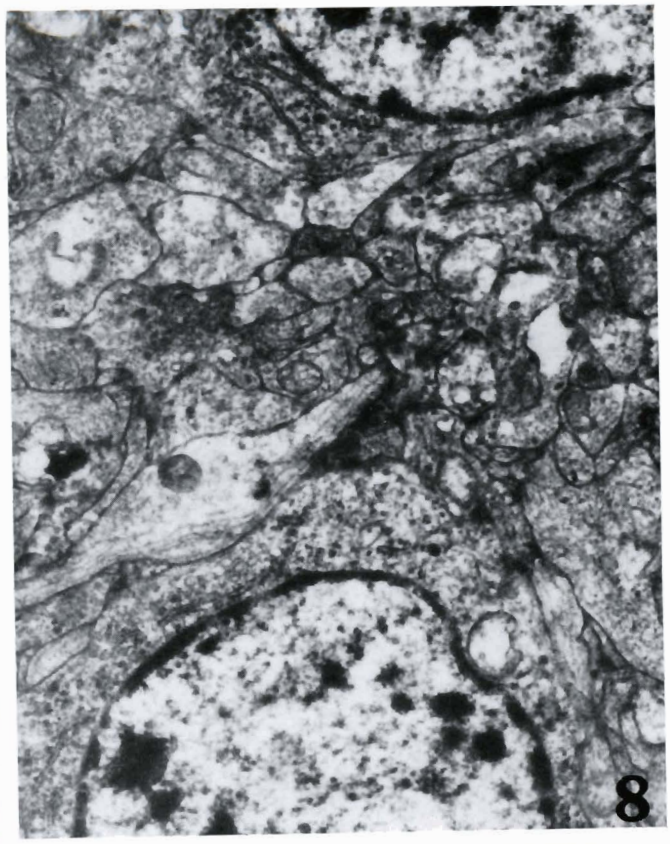
Fig. 3. Histological aspect of an ENU-induced tumor. Rounded cells showing a honeycomb appearance, and tumor cells arranged around nucleus-free zones, can be seen. H-E. x 125

Fig. 4. Detail of a large rosette in an ENU-induced tumor. H-E. x 250

Fig. 5. Synaptophysin expression in the neuropil of an experimental tumor. x 250

Fig. 6. Strong vimentin positivity in the tumor cells of an ENU-induced tumor. x 250

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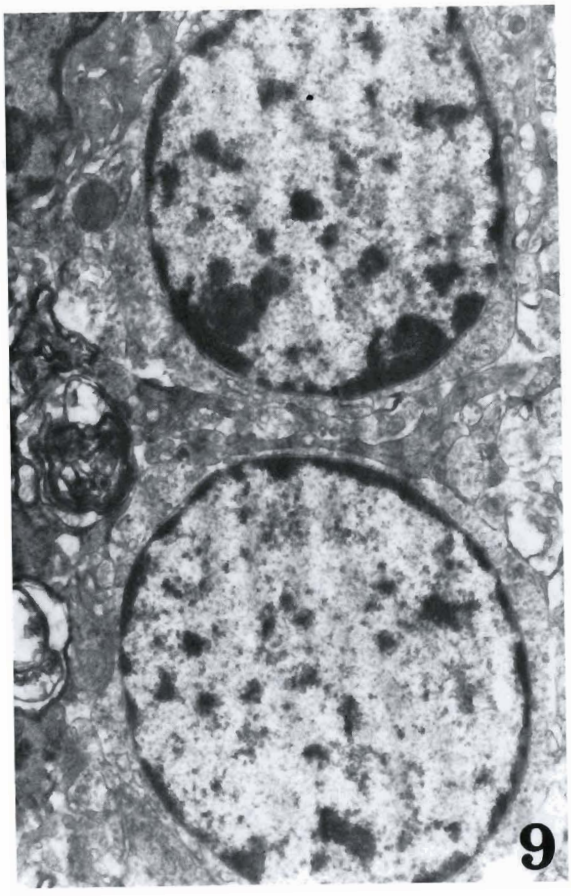
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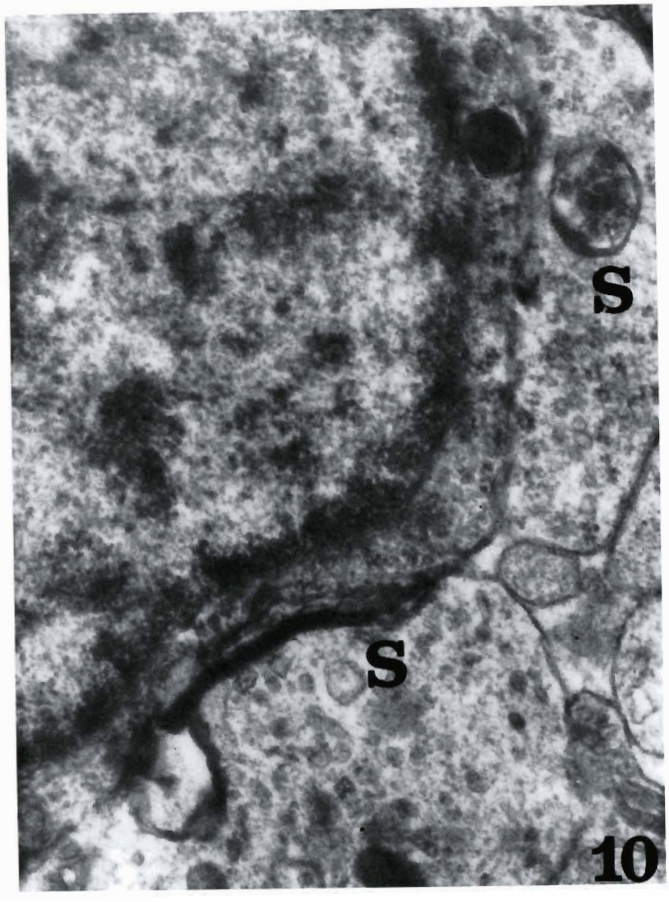
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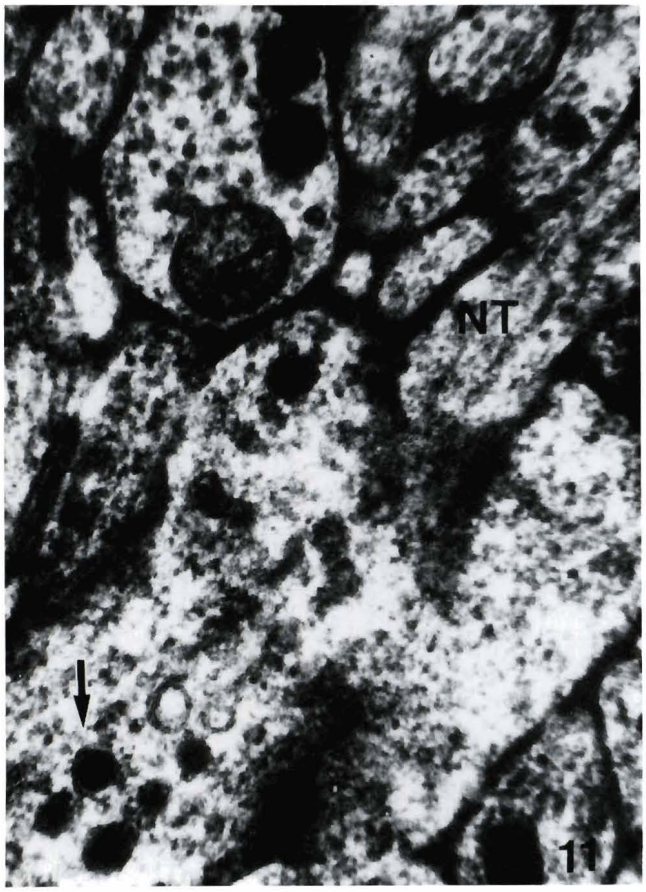
Fig. 7. Ultrastructural aspect of a human neurocytoma, showing uniform cells with rounded nuclei. x 3,000

Fig. 8. Ultrastructural details of a human neurocytoma. Tumor cells show a uniform rounded nucleus and a cytoplasm neuronal in appearance. A dense neuropil, with normal mature aspect, can be seen. x 6,000

Fig. 9. Ultrastructural details of an ENU-induced tumor. Rounded tumor cells with similar appearance to tumor cells of human neurocytomas can be seen. x 6,000

Fig. 10. Experimental tumor. Synaptic endings (S) over a tumor cell can be seen. x 12,500



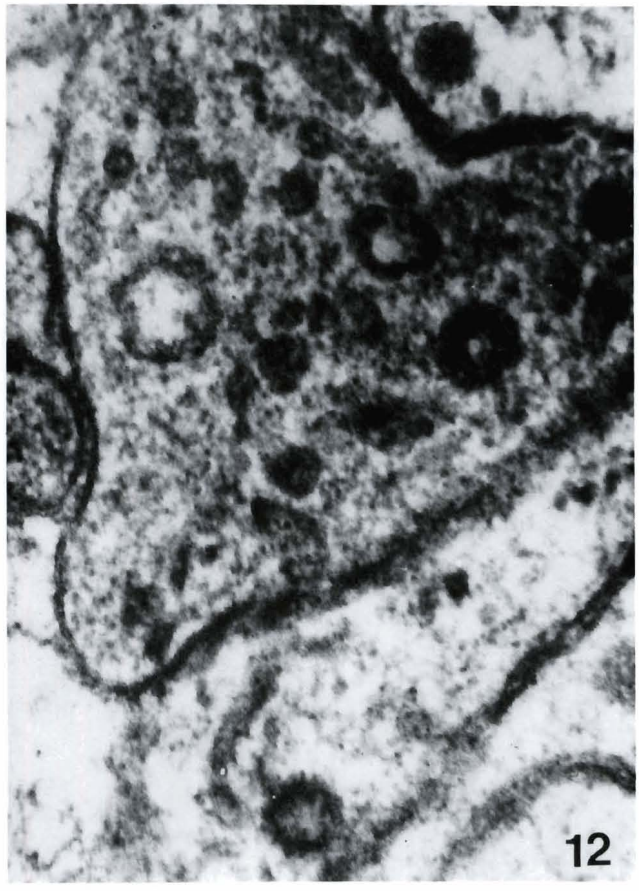


Fig. 11. Ultrastructural detail of tumor neuropil in human neurocytomas. Processes of tumor cells, containing neurotubules (NT) and dense-core vesicles (arrow), can be seen. x 30,000

Fig. 12. Ultrastructural detail of an ENU-induced tumor. In the tumor neuropil, processes containing electron-dense bodies and dense-core vesicles can be seen. x 50,000

experimental tumors regarding vimentin expression, it is possible that the presence of this marker in experimental neoplasms and its lack in human tumors is due to a lesser differentiation of tumor cells in the former, because it is well known that vimentin expression predates that of properly neuronal markers in neuronal cells (Molenaar and Trojanowski, 1991).

In any case, based on the data that we have obtained in the present study, we suggest that the ENU model of neurocarcinogenesis is usseful for the induction of experimental neurocytomas, and can contribute to a broader knowledge of human tumors with neuronal differentiation.

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