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CELL DEGENERATION AND NECROSIS IN EXPERIMENTAL GLIOMAS

P. L. LANTOS AND G. J. PILKINGTON

From the Department of Neurological Studies and the Bland-Sutton Institute of Pathology, Middlesex Hospital Medical School, London W1P 8AA

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Summary.—Cell degeneration and necrosis in ethylnitrosourea-induced gliomas of rats were examined using light and electron microscopy. Two types of cell loss were observed: massive necrosis and individual cell death. Massive necrosis was influenced by the size, malignancy and histological type of the gliomas: it occurred most frequently in large, malignant pleomorphic gliomas and ependymomas. Proliferation of endothelial cells, narrowing of vascular lumina and degenerative changes affecting vessel walls were thought to be the major factors causing necrosis. Individual cell death occurred throughout the neoplasms irrespective of their size. Progressive degenerative changes, involving both the nucleus and cytoplasm, preceded cell death. Macrophages (both microglial and monocytic in origin), reacting astrocytes and, to a lesser extent, neoplastic glial cells engulfed and digested the necrotic cells and their remnants.

SCHERER (1938, 1940), studying the development and growth patterns of gliomas, distinguished primary, secondary and tertiary structures in them. The primary or proper structures are intrinsic features of neoplastic glial cells and are, therefore, not influenced by surrounding tissues. Rosettes of ependymomas, for example, are primary structures: they occur irrespective of the site of the tumour.

Secondary structures, on the other hand, are brought about by pre-existing local tissue patterns and do not result from inherent growth characteristics of neoplastic cells. One type of secondary structure is the accumulation of tumour cells along the interfaces of brain parenchyma, around neurones and blood vessels. These subpial, subependymal, perineuronal and perivascular arrangements, occurring also at the periphery of gliomas, may represent the proliferating edge of the tumour. Another type of secondary structure is brought about by the restricting effect of nerve tracts on the growth of gliomas: therefore perifascicular, intrafascicular and interfibrillary growth patterns are seen. The morphology of an astrocytoma may thus be determined by the arrangement of the nerve fibres among which the neoplastic astrocytes proliferate.

Extensive necrosis and haemorrhage in malignant gliomas will give rise to tertiary structures: the consequent mesenchymal reaction will modify cellular arrangements and add new cells to the tumour, resulting in a mixed cell population.

Russell and Rubinstein (1971) have noted that, in addition to massive necrosis of the coagulation type, cell degeneration of varying severity is also present in malignant gliomas. In areas undergoing degeneration and necrosis scavenging microglial cells proliferate. These cells, engulfing sudanophilic material, become transformed into compound granular corpuscles and are thus responsible for the yellow discoloration along the border of necrotic areas.

Since regressive changes, including de-

generation and necrosis, can significantly alter the appearance of gliomas, Zülch (1965) considers a knowledge of their characteristics to be helpful in the diagnosis of neuroectodermal tumours. Moreover such information can be used in differential diagnosis: certain regressive changes occur in some neoplasms, but not in others. Necrosis is frequent in glioblastoma multiforme, the most malignant of all gliomas, but smaller areas of necrosis can occur in ependymomas and oligodendrocytomas. Zülch also formulates the rule that tissue destruction progresses slowly in the relatively benign neuroectodermal tumours, while it occurs suddenly, resulting in necrosis, in malignant neoplasms.

Degeneration of individual cells is a frequent phenomenon in gliomas: the mucoid degeneration in oligodendrocytomas and the cytoplasmic swelling in protoplasmic astrocytomas are the obvious examples. Degeneration accompanied by liquefaction leads to cyst formation, as is frequently observed in ependymomas and astrocytomas. An extreme variety of cyst formation occurs in some cerebellar astrocytomas in children: in these gliomas the actual tumour mass is a small nodule in the wall of a large cyst (Russell and Rubinstein, 1971).

Calcification, another regressive change, usually denotes slow growth: it occurs in low grade gliomas, particularly oligodendrocytomas. In the latter tumours calcium becomes deposited both in the stroma and in the blood vessels: these calcified vessels are likely to rupture, often causing fatal intracerebral haemorrhage. Blood vessels in gliomas can also undergo hyalinization: this change is not infrequent in ependymomas and oligodendrocytomas.

These examples show that regressive changes, particularly cell degeneration and necrosis, are important factors in determining the morphology and growth of gliomas.

The purpose of this paper is to describe cell degeneration and necrosis in experimental gliomas induced by N-ethyl-N-nitrosourea (ENU) and to discuss their importance in the development of these neoplasms.

MATERIALS AND METHODS

Gliomas were induced transplacentally by either a single i.v. injection of 30 mg of ENU/kg body wt or a single i.p. injection of 40 mg of ENU/kg body wt into pregnant BD-IX rats on the 15th day of gestation. The ENU was dissolved in a 3mM citrate buffer containing 0·9% (w/v) NaCl at pH 6·0. The offspring were killed by whole-body perfusion *via* the ascending aorta when they developed neurological signs of neoplasia. The rats were perfused with a mixture of glutaraldehyde and formaldehyde, one-half strength Karnovsky fixative, at pH 7·4 (Karnovsky, 1965) for 20–30 min at room temperature. The brains were left *in situ* and immersed in the same fixative for a further 4 h before being dissected out and examined for tumours. Coronal slices of brain were processed for paraffin wax embedding and sections of 5 μm cut and stained with haematoxylin and eosin. Sections were examined under the light microscope and necrotic regions counted and measured by the use of an eyepiece graticule.

Tissue blocks of 1 mm^3 for electron microscopy were washed in 0·2M cacodylate–sucrose solution for 12 h at pH 7·4 before post-fixation with 1% osmium tetroxide in phosphate buffer for 1 h at 4°C. Dehydration was *via* ascending grades of ethanol and subsequent embedding in either Spurr or Epon resin. Thin sections were stained with uranyl acetate and lead citrate and examined in either an A.E.I. 801 or an Hitachi HU12A electron microscope.

RESULTS

Light microscopy

Fifty tumours from 38 rats were examined: the occurrence of more than one neoplasm in any one rat was not infrequent. Histologically the tumours were gliomas of various types: ependymomas (3), oligodendrocytomas (3), anaplastic gliomas (1), and pleomorphic gliomas (40); the latter tumours were composed of mixed glial cell populations (Lantos, 1972). Neoplasms developed in the subcortical white matter of the cerebral hemispheres and in the hippocampus, but occurred most frequently adjacent to the ventricles (80%). The size of the

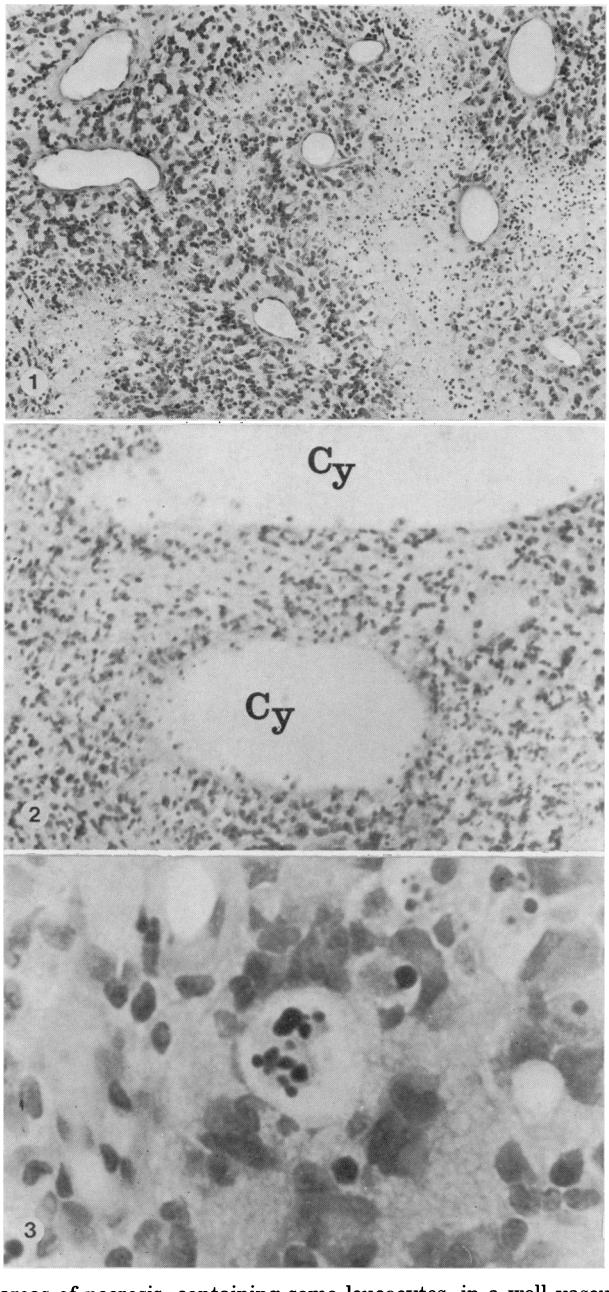
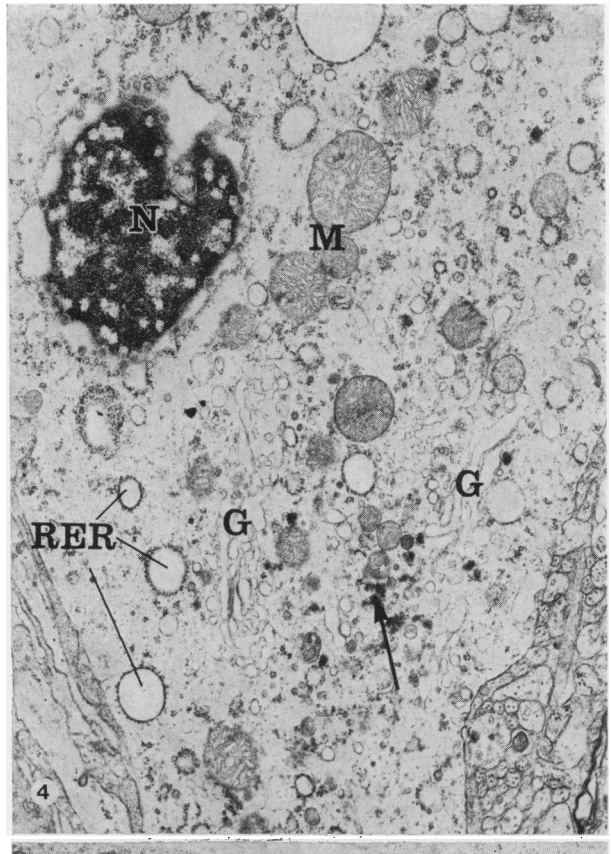


FIG. 1.—Extensive areas of necrosis, containing some leucocytes, in a well vascularized, predominantly ependymomatous region of a pleomorphic glioma. Cells have survived around blood vessels. H. and E. × 230.

FIG. 2.—Cysts (Cy) are present in a pleomorphic glioma. H. and E. × 270.

FIG. 3.—A necrotic cell is seen in the centre of the picture. H. and E. × 930.



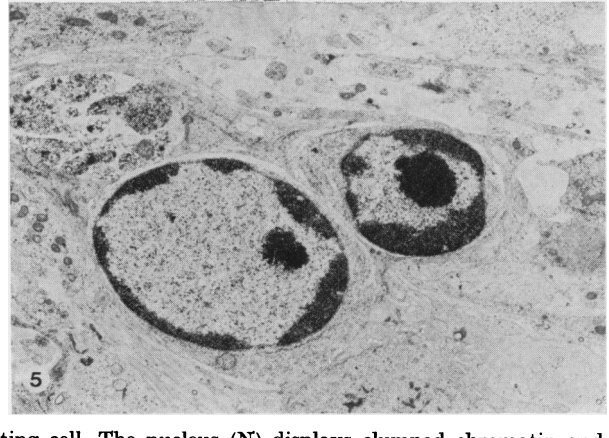


FIG. 4.—A degenerating cell. The nucleus (N) displays clumped chromatin and a dilated nuclear envelope. The cisternae of the rough-surfaced endoplasmic reticulum (RER) and of the Golgi complexes (G) are dilated and vesiculated. The mitochondria (M) become circular and a dense material accumulates in the light cytoplasm (arrow). ×12,600.

FIG. 5.—Two cells showing advanced degeneration: the chromatin forms dense clumps beneath the nuclear membrane. ×5070.

neoplasms varied between 0·5 mm and 9·0 mm in diameter.

Two types of cell loss were observed: massive necrosis involving many cells and individual cell death. Necrotic areas showed loss of structural details and cellularity: an exudate containing polymorphs and macrophages was frequently present in these regions (Fig. 1). Areas of necrosis developed in the centre of the neoplasm in most cases and their size varied between 0·144 mm^2 and 2·66 mm^2. Necrosis was seen in 13 of the tumours (26%) and an average of 2·6 necrotic areas per glioma were observed.

Cyst formation (Fig. 2) was evident in the larger tumours, particularly in ependymomas. Haemorrhages frequently occurred together with cysts and areas of necrosis. Cells were seen to survive only around blood vessels in areas of extensive necrosis (Fig. 1).

Individual necrotic cells (Fig. 3) were seen throughout the gliomas irrespective of their size. All tumours were well supplied with blood vessels. They did, however, show varying severity of abnormality (including endothelial proliferation): these changes have been previously described (Cox, Pilkington and Lantos, 1976). The extent of necrosis was related to both the size of the neoplasms and to their degree of malignancy: necrotic areas occurred most frequently in large gliomas of high-grade malignancy. In addition, the histological type was also important: areas of necrosis were present in pleomorphic gliomas and ependymomas, but not in oligodendrocytomas.

Electron microscopy

Large malignant tumours usually exhibit necrotic regions, which can be easily examined with the light microscope. In addition, individual cells throughout the tumours may undergo irreversible degenerative changes leading to cell death: the various stages of this process are observed with the electron microscope.

Aggregation of the chromatin into large clumps is one of the earliest manifestations of degeneration (Fig. 4). The ground substance of the cytoplasm may become lighter but show accumulations of a dense material. All cytoplasmic organelles are affected in the degenerative process. The cisternae of the rough-surfaced endoplasmic reticulum (RER) become dilated and even circular. Detachment of ribosomes and disintegration of the cisternae also occur. The cisternae of the Golgi complex become dilated and vesiculated. The mitochondrial cristae display irregularity and fragmentation. Microtubules are disrupted, but filaments appear to be more resistant.

At a more advanced stage the chromatin becomes condensed and accumulates beneath the nuclear membrane (Fig. 5). With the breakdown of the nuclear membrane the nucleus disintegrates into separate fragments (Fig. 6). The cytoplasm contains disrupted cisternae and vacuoles, disintegrated microtubules and unidentifiable debris. Macrophages, containing numerous lysosomes frequently surround these irreversibly damaged cells (Figs. 7, 8).

DISCUSSION

Bessis (1964) attempted to classify the phases of cell death in his classical study. Cell death, like the cell cycle, can be divided into several periods: the causative factors may, first, lead to reversible change followed by irreversible damage. Cytoplasmic vacuolation and oedema due to alterations in membrane permeability may result in cell rupture, or nuclear lesions—clumping of chromatin and karyorrhexis—may dominate the regressive changes.

The detection of irreversibly damaged cells is difficult (Cooper, 1973). Microcinephotography, staining by vital dyes and binding of isotopes are some of the techniques most frequently used. Advanced degenerative changes are easily recognized in the electron microscope, but it is difficult to establish at which point a

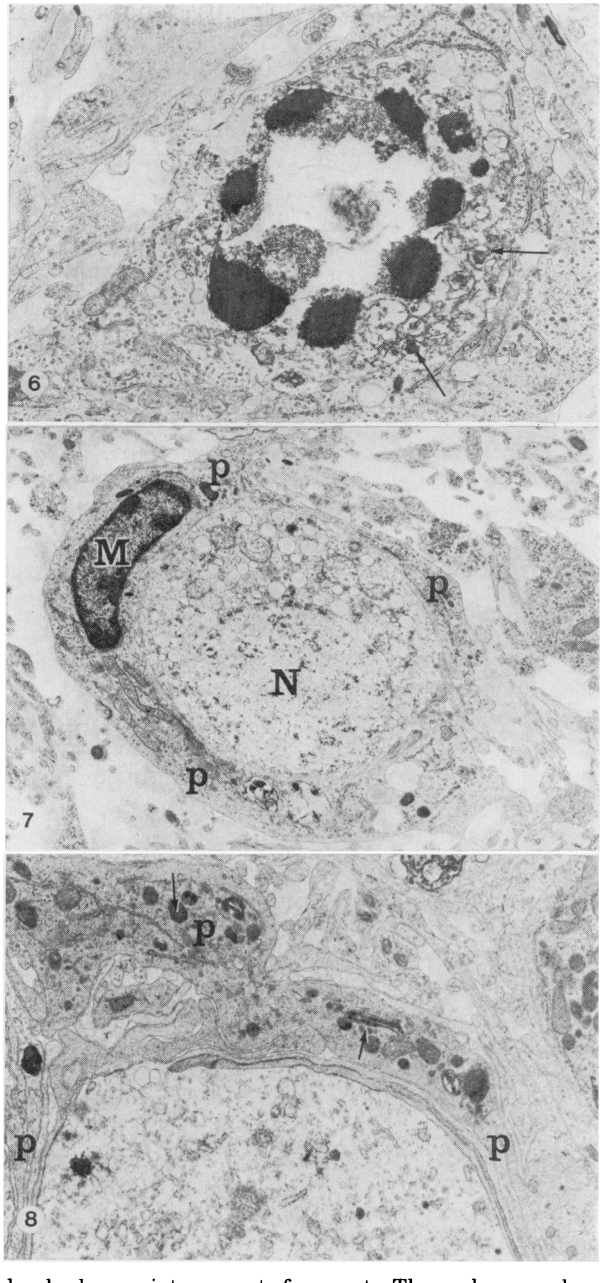


FIG. 6.—The nucleus has broken up into separate fragments. The nuclear membrane has disintegrated and the cytoplasm contains some unrecognizable debris (arrows). × 12,600.

FIG. 7.—A macrophage (M) with its long, thick processes (P) engulfs a necrotic cell whose nucleus (N) is barely distinguishable. × 7200.

FIG. 8.—Part of a necrotic cell is surrounded by layers of cell processes (P) of a macrophage. These contain many lysosomes (arrows). × 11,200.

degenerating cell may be irreversibly damaged.

Cooper, Bedford and Kenny (1975) classified the causes of cell death in malignant tissues: physical displacement of cells, intrinsic factors and extrinsic factors were distinguished. They could, however, act simultaneously and cell death may result from a series of events rather than a single, well-defined cause. Physical displacement, including migration and defoliation of cells from the tumour mass, may lead to cell loss. Intrinsic factors operating independently of the environment include cell ageing, differentiation, chromosome abnormalities and biochemical errors of neoplastic cells. Inadequate oxygenation and nutrition together with the host immune defences are the most important extrinsic factors.

These factors, however, may become irrelevant if the vascular supply of the neoplasm deteriorates: sudden occlusion of blood vessels, haemorrhage and defective vascularization may cause extensive necrosis. The blood supply of neoplasms, therefore, plays a prominent role in determining cell loss and, consequently, the growth of the neoplasm. Folkman (1975) has divided the growth of neoplasms into avascular and vascular phases. In the latter stage a diffusible angiogenetic substance is produced without which the tumour ceases to grow. Rubin and Casarett (1966) described different types of vascular patterns in tumours: peripheral vascularization with and without penetrating vessels and central vascularization. Both forms of peripheral vascularization may be associated with central necrosis, while individual cell death is the rule in centrally vascularized neoplasms. These authors could also distinguish between recent and old necroses: vascular pattern might be preserved in the former and destroyed in the latter.

The sequence of events culminating in cell death can be better studied in small neoplasms: once the tumour has reached a critical size the fate of individual cells is determined mainly by the blood supply. Electron microscopy is particularly useful in the study of necrosis of discrete cells in well vascularized areas of tumours (Cooper *et al.*, 1975). The main features of irreversibly damaged cells of Hodgkin's lymphoma included progressive cytoplasmic vacuolation and loss of cell contents, while lysosome activation and phagosome formation were late events. Other authors, however, reported mitochondrial swelling, disruption of endoplasmic reticulum, nuclear oedema and irregularities of the plasma membrane (Archibald and Frenster, 1973). Shrinkage necrosis (Kerr, 1971)—condensation of cytoplasm and nuclear chromatin while the cell organelles remain relatively preserved—also occurred in these lymphomas (Peckham and Cooper, 1973).

In ENU-induced gliomas two types of cell loss can be distinguished: massive necrosis and individual cell death. Impaired blood supply is responsible for the extensive necrosis: the blood vessels of malignant gliomas undergo changes—endothelial proliferation and vascular degeneration—which render them liable to occlusion and rupture. Outside pressure by neoplastic cells on the blood vessels and toxic metabolites released by the tumour may also impede blood flow. Haemorrhage and liquefaction will also result in necrosis. That necrosis is caused by the lack of an angiogenetic factor (Folkman, 1975) is unlikely, since these gliomas are well supplied with blood vessels and proliferating endothelial cells are also present (Cox *et al.*, 1976). It is the poor quality of these blood vessels rather than their numerical deficiency which is responsible for necrosis.

The mechanism of individual cell death in gliomas is more obscure: here inadequate nutrition, chromosome abnormalities, ageing, defoliation of neoplastic cells and immunological responses may separately or collectively cause cell death.

The fate of dead neoplastic glial cells varies, but in most cases they disintegrate spontaneously or become ingested by macrophages. Macrophages in gliomas—both microglial cells of the brain and monocytes originating from the blood—

engulf and digest necrotic cells and their breakdown products (Lantos, 1975). Reacting astrocytes in and around gliomas also have a phagocytic function: their abundant cytoplasm contains an assortment of inclusion bodies and debris (Lantos, 1974). Moreover, neoplastic glial cells were also seen to phagocytose necrotic material.

Acid phosphatase is important both in the autolysis of dying cells and phagocytic activity of macrophages. A fine structural study on the distribution of this enzyme in ENU-induced gliomas showed the highest acid phosphatase activity in macrophages: both primary and secondary lysosomes, the latter occasionally forming large conglomerates, were present. In addition, reacting astrocytes contained many secondary lysosomes of various size and activity (Lantos, 1974*a*).

The importance of cell death as a cause of cell loss from neoplasms has been well recognized. Necrosis has, however, another important effect on the growth of neoplasms: it facilitates the detachment of viable cells of the tumour and normal cells of surrounding tissues. This process, in turn, paves the way to invasion and metastasis (Weiss, 1977).

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