cited above, the participation of the fibroblast is essential for success. This symbiotic association of mast cell and fibroblast forms the basis of the 'mast-cell cycle', described elsewhere[2].

If, then, a mast-cell reaction in precancerous mouse skin is to be interpreted as an immunological phenomenon, it would seem necessary to postulate, first, the release of antigenic material from the hyperplastic epidermis and to envisage its contact with distant lymphocytes; second, the re-entry of these lymphocytes into an area which is still yielding specific antigen; and, third, the reunion to occur in the vicinity of young collagen-forming fibroblasts. Viewed in this way, a mast-cell reaction in mouse skin is not an obligatory feature of carcinogenesis: it neither favours nor hinders the development of cancer. Nevertheless, as an 'index of promotion' it may shed light on the mechanism whereby an initiated epidermis gradually acquires the property of invasive growth[3,4].

The Origin of Experimental Brain Tumours: A Sequential Study

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Summary. A sequential study of rat brains treated transplacentally with the neurotropic carcinogen ethylnitrosourea reveals small foci of cell proliferations from the age of 8 weeks. These lesions consist mainly of undifferentiated cells of the subependymal plate type. They occur in those areas in which gliomas develop and represent the earliest, histologically detectable, changes in the development of brain tumours.

Ten years ago Druckrey et al.[2] reported that a single i.v. injection of *N*-ethyl-*N*-nitrosourea (ENU) into pregnant rats induced tumours and malformations in the offspring. Since then ENU, a simple nitrosamide, has proved to be the ideal carcinogen in the study of experimental neural tumours. A single dose of this compound administered to foetal and neonatal rats induces a high incidence of neoplasms selectively and consistently in the nervous system. The strong carcinogenic action of ENU on the nervous tissue, however, is restricted to the perinatal period: no tumour develops when ENU is administered to pregnant rats before the 12th day of gestation and the susceptibility of the nervous system to ENU also decreases with increasing age[3,4].

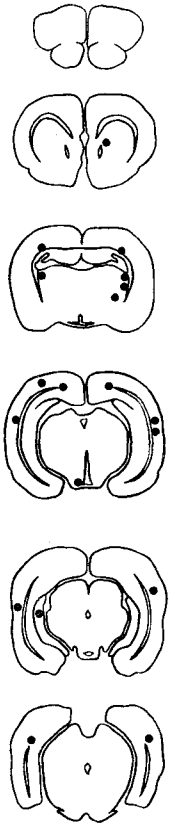

Fig. 1. Diagrammatic representation of the distribution of lesions described in the text.

Histologically the tumours are gliomas of the central nervous system and schwannomas of the cranial and peripheral nerves, although neuroblastomas have also been reported[5]. Multiple tumours are frequent: neoplasms of macroscopic size, microtumours and early neoplastic proliferations are all present in the same animal[6]. Cerebral tumours are not distributed haphazardly but occur in certain preferential sites: the periventricular area and the subcortical white matter of the cerebral hemispheres are most frequently involved.

Since most studies have been carried out on large, fully developed tumours which eventually killed the animals, after an average of 245 days, very little is known about the early stages of tumour growth. A sequential analysis of ENU-induced schwannomas of the trigeminal nerves was recently published[7], but similar studies are lacking on the earliest stages of the development of cerebral gliomas. The purpose of the present communication is to describe the development of such ENU-induced gliomas.

Material and methods. A single i.p. injection of 40 mg of ENU per kg of body weight was injected into pregnant BD-IX rats on the 15th day of gestation. The ENU was dissolved in a 3 m*M* citrate buffer containing 0.9% (w/v) sodium chloride, pH adjusted to 6.0 at 20 °C. Con-

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trol rats were injected with the buffer alone. The offspring were killed by whole body perfusion with 10% formal-saline at ages 2, 4, 6, 8, 10 and 12 weeks. The brains were removed and processed for light microscopy. Serial sections of 5 μm were cut, stained with haematoxylin and eosin and scanned with a light microscope. In each age group five treated brains and one control were examined.

Results. The earliest lesions, small collections of cells, are seen first in 8-week-old rats; the number of animals showing these foci are 1/5, 4/5 and 5/5 in the 8, 10 and 12-week-old groups respectively. The number of these early cell proliferations varies from 1 to 5 in any one animal. On average 1.6 lesions are found in the 10-week-old rats and 2.0 in the 12-week-old group.

The distribution of the lesions is shown in Figure 1. The areas most frequently involved are the outer aspects of the lateral ventricles (Figure 2), the angle of the ventricle between the corpus callosum and the caudate nucleus and the subcortical white matter adjacent to the hippocampus. In 2 cases increased cellularity is seen in the cortex of the parietal lobes notably around neurones (Figure 3).

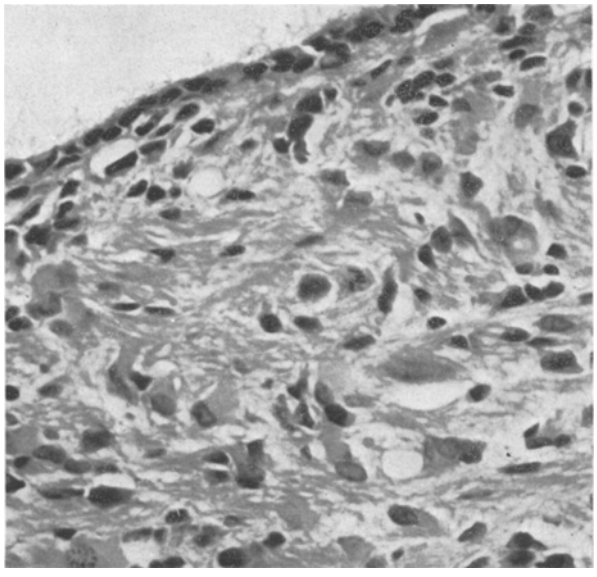

Fig. 2. Part of an early neoplastic cell proliferation adjacent to the wall of the lateral ventricle. Cells in mitosis are present. × 450.

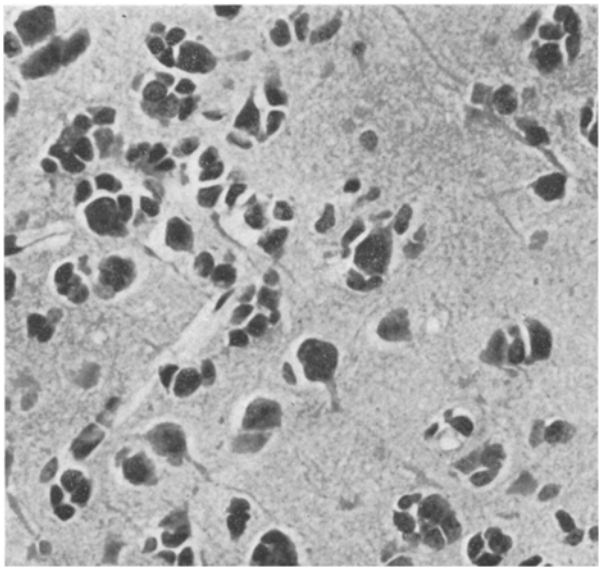

Fig. 3. Small groups of cells with hyperchromatic nuclei are seen in the cortex, notably around neurones. × 450.

The identification of the cells is difficult at light microscope level. They do, however, show features of undifferentiated cells: large, hyperchromatic nuclei, sparse cytoplasm and high nuclear-cytoplasmic ratio. Mitoses, although present, are not plentiful. Occasional cells display the features of oligodendrocytes or astrocytes. No significant differences are found between the lesions in 10-week-old and 12-week-old rats. Treated rats of the younger age groups and control animals do not show these changes.

Discussion. The early cell proliferations occur in those areas in which gliomas develop and they, like gliomas, are frequently multiple[6,8,9]. These findings therefore suggest that the small groups of cells have already been committed to neoplastic change: they represent the earliest, morphologically detectable stages of tumour development. It is likely that most, if not all, of these lesions will progress to fully developed gliomas.

Electron microscope investigations of early cell proliferations reveal (LANTOS, unpublished) that most of the cells are similar, if not identical, to the subependymal plate cells[10]. Moreover, undifferentiated subependymal plate cells are also present in large, pleomorphic gliomas of the periventricular regions together with various glial cell types[11]. These findings substantiate the hypothesis that the undifferentiated, mitotically active cells of the subependymal plate are the most susceptible targets for the carcinogenic action of ENU. The transformed cells of the subependymal plate may remain in situ and, maintaining their multipotentiality, differentiate into different glial cell types to produce mixed gliomas of the periventricular areas. Transformed subependymal cells may also migrate into the white matter where, undergoing the same process of incomplete differentiation and neoplastic proliferation, they develop into mixed gliomas, oligodendrocytomas or, occasionally, astrocytomas.

The findings in rat brains are relevant to the development of gliomas in man. Although the subependymal plate of man persists in adult life, its mitotic activity is greatest in developing brains. Since cell division is a prerequisite for the development of cancer, foetal and neonatal brains are more susceptible than the brains of adults. It is therefore reasonable to suggest that foetuses of pregnant women exposed to environmental carcinogens are at risk. It has been established that nitrosamines, formed by the interaction of nitrites and secondary amines, are present in the environment[9,12]. These compounds may exert their carcinogenic action transplacentally on the susceptible, mitotically active cells of the subependymal plate of the developing foetal brain and the resulting brain tumours will appear in adult life many years after the carcinogenic stimulus.

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