served in the treated group. They consisted of 28 (53.8%) invasive squamous cell carcinomas-16 in males and 12 in females-4 were papillomas (3 in males, 1 in females) and 1 keratoacanthoma in a male. No skin tumors developed in the control group. The mean survival time of the animals are given in the table along with the numbers of tumors in each group. Among other tumors induced in this experiment were a substantial number of lymphomas and lung adenomas which were most likely responsible for the relatively short survival of the treated animals. A detailed pathological report will appear later.

A careful survey of the literature failed to provide any information regarding malignant skin tumors induced in mice by direct repeated application of ENU. The data reported here clearly indicate that not only are PAH capable of inducing malignant tumors in mouse skin by topical application, but also nitrosamides as well have this ability and may be as potent as PAH. In this study, ENU appears to have as an effect as did MNU as reported by Graffi and Hoffmann[4], in terms of numbers of tumors: 30 (69.7%). It is also possible to conclude from our results that ENU does not require a promoting agent such as croton oil to exert a tumorigenic influence as PAH do. From these results it can also be observed that the number of tumors is higher in the males, indicating a different susceptibility to the carcinogen based on sex; the survival time of the males is also shorter (Δ % = – 19%).

Further experiments are obviously needed to study the mechanism of this direct action which does not fit into the classical 2-stage carcinogenesis theory (initiation-promotion), and eventually disclose any difference and similarity between this action and that of PAH.

The role of the subependymal plate in the origin of gliomas induced by ethylnitrosourea in the rat brain

P. L. Lantos[1]

Department of Neurological Studies and Bland-Sutton Institute of Pathology, Middlesex Hospital Medical School, London, W1P 8AA (England), 13 October 1976

Summary. The fine structure of early cell proliferations induced transplacentally by ethylnitrosourea in the rat brain reveals that the cells show features of the undifferentiated cells of the subependymal plate: high nuclear-cytoplasmic ratio, scarcity of cell organelles and dominance of free over membrane-bound ribosomes. These findings suggest that most, if not all, gliomas induced by ethylnitrosourea originate from these primitive cells.

A single dose of N-ethyl-N-nitrosourea (ENU) injected into pregnant rats during the second half of gestation induces a high incidence of tumours selectively and consistently in the nervous system of the offspring[2]. The neoplasms are gliomas of the brain and spinal cord and schwannomas of the cranial and peripheral nerves. The cerebral gliomas develop most frequently in areas adjacent to the lateral ventricles and consist of a pleomorphic mixture of various glial cell types[3–5]. Although the morphology, including the ultrastructure, of these tumours has been extensively studied[6], very little is known about those changes which precede the appearance of gross neoplasms. However, Roscoe and Claisse[7], in a sequential in vivo-in vitro study, found cells in culture which showed the characteristics of malignant cells after a much shorter interval than the 245 days of latency. Histological examination of serial sections of rat brains at various intervals following the transplacental administration of ENU revealed small groups of abnormal cells in 8-week-old rats. These lesions, more frequently seen in 10- and 12-week-old animals, were thought to represent the earliest, histologically detectable changes in the development of brain tumours[8]. The purpose of this paper is to describe the fine structure of those lesions which have been found in 16-week-old rats in order to identify their constituent cells and to try to establish the role of the subependymal plate in the origin of gliomas.

Materials and methods. A single dose of 40 mg of ENU per kg of body weight was injected i.p. into pregnant BD-IX rats on the 15th day of gestation. The ENU was dissolved in a 3 mM citrate buffer containing 0.9% sodium

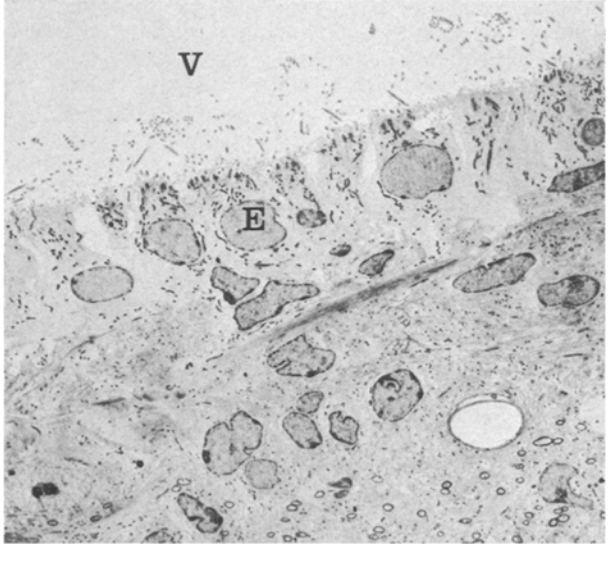

Fig. 1. An early lesion adjacent to the ependymal lining (E) of the lateral ventricle (V). × 2300.

1 Acknowledgments. I thank D. J. Cox and G. J. Pilkington for their skilful technical assistance.

2 H. Druckrey, S. Ivankovic and R. Preussmann, Nature, Lond. *210*, 1378 (1966).
3 S. Ivankovic and H. Druckrey, Z. Krebsforsch. *71*, 320 (1968).
4 A. Koestner, J. A. Swenberg and W. Wechsler, Am. J. Path. *63*, 37 (1971).
5 P. L. Lantos, J. neurol. Sci. *17*, 443 (1972).
6 P. Kleihues, P. L. Lantos and P. N. Magee, Int. Rev. exp. Path. *15*, 153 (1976).
7 J. P. Roscoe and P. J. Claisse, Nature, Lond. *262*, 314 (1976).
8 P. L. Lantos and D. J. Cox, Experientia *32*, 1467 (1976).

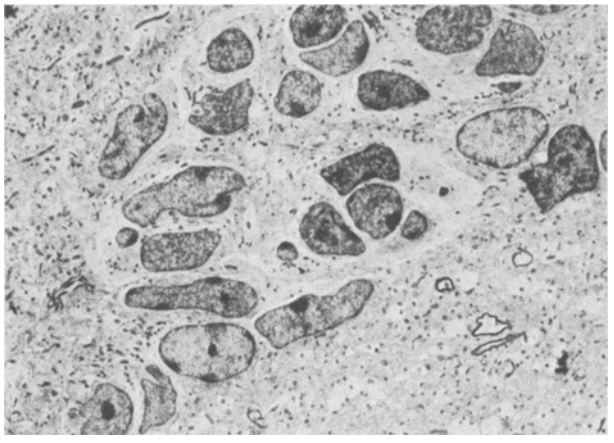

Fig. 2. A group of undifferentiated cells between the corpus callosum and the caudate nucleus. × 3900.

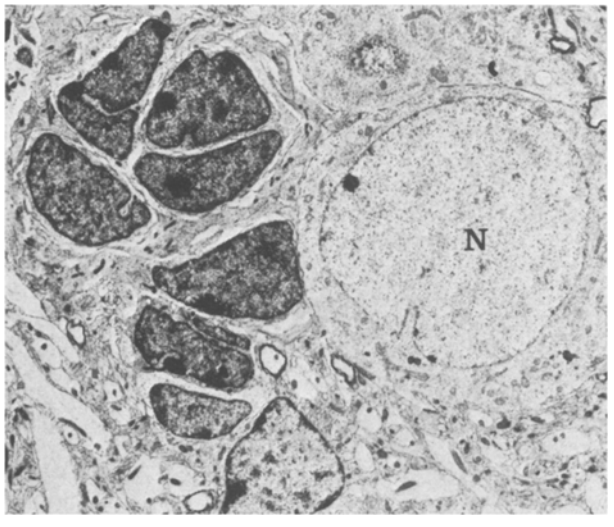

Fig. 3. Undifferentiated cells next to a neurone (N). × 5400.

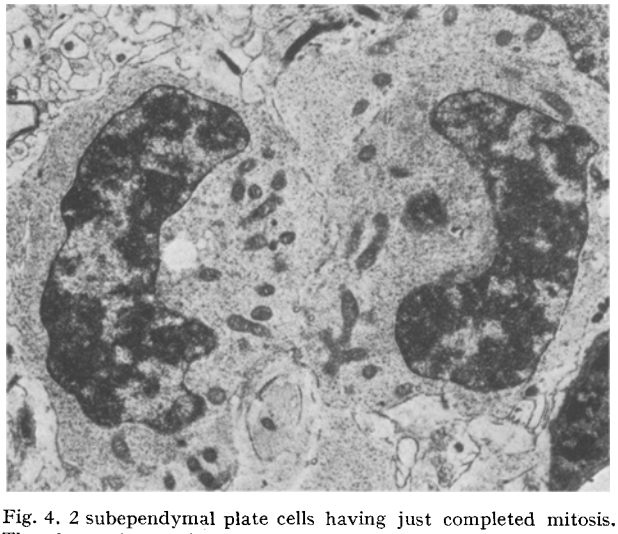

Fig. 4. 2 subependymal plate cells having just completed mitosis. They have high nuclear-cytoplasmic ratio and few cell organelles. × 14800.

chloride, pH adjusted to 6.0 at 20 °C. Control rats were injected with the buffer alone. The offspring were killed at the age of 16 weeks by whole body perfusion with one-half strength Karnovsky fixative[9]. The brains of 4 treated rats were removed and the subependymal plates of both hemispheres processed for electron microscopy. The blocks taken included the lateral aspect of the lateral ventricle and the angle between the corpus callosum and caudate nucleus: these are the areas in which the early cell proliferations were most frequently found in histological sections. Thin sections were double stained with uranyl acetate and lead citrate and examined with an Hitachi HU12A electron microscope.

Results. Of the 8 subependymal plate areas examined 5 show groups of cells which are absent in controls. These early lesions vary in size and occur in 3 sites: immediately adjacent to the ependymal lining of the ventricle (figure 1), in the angle of the ventricle between the corpus callosum and caudate nucleus (figure 2) and among neurones of the caudate nucleus (figure 3). The constituent cells, irrespective of the localization, are indistinguishable from subependymal plate cells, displaying their common characteristics: high nuclear-cytoplasmic ratio, scarcity of cell organelles and dominance of free over membrane-bound ribosomes (figure 4).

Discussion. These results show that the early lesions are collections of subependymal plate cells, thus providing evidence that most, if not all, gliomas induced by ENU originate from these primitive cells. This finding would explain both the distribution and the cytoarchitecture of these gliomas. The subependymal plate cells transformed by ENU will either remain in situ or migrate into the white matter subjacent to the cortex. These are the areas in which both the early lesions and gross gliomas most frequently occur. The early lesions consist chiefly of undifferentiated cells, but as the neoplastic process progresses various glial cells are seen at different stages of maturation reflecting the multipotentiality of the subependymal plate cell. Oligodendrocytes, astrocytes and ependymal cells are intermingled with undifferentiated and anaplastic glial cells. At a later stage of tumour development one cell type may become predominant enabling the tumour to be classified according to this cell type. This phenomenon can be attributed to the more rapid growth of one, particularly malignant, cell type[5]. Thus the morphology of the gliomas arising from the subependymal plate is determined by the diverging processes of differentiation and anaplasia resulting in a pleomorphic cell population. These findings lend some support to the hypothesis that carcinogenesis in certain cases may be an abortive attempt at differentiation on the part of a neoplastic stem cell[10].

The involvement of the subependymal plate in the origin of rat gliomas is significant for man: the subependymal plate with its mitotically active, undifferentiated cells also exists in primates[11] and in man[12].

9 M. J. Karnovsky, J. Cell Biol. *27*, 137A (1965).
10 P. Dustin, Jr, Cell Tiss. Kinet. *5*, 519 (1972).
11 P. D. Lewis, Nature, Lond. *217*, 974 (1968).
12 J. H. Globus and H. Kuhlenbeck, J. Neuropath. exp. Neurol. *3*, 1 (1944).