Data in table 1 for the spontaneous adenocarcinoma and table 2 for the sarcoma P 1798 indicate that, at least with respect to cytotoxicity the fixed cells provoke a similar immune response. High titers against the adenocarcinoma or the sarcoma cells were obtained from animals immunized with either formalin or glutaraldehyde fixed cells. In contrast immunization with frozen or freeze dried untreated cells led to much lower cytotoxic titers. The titers obtained after immunization with fixed cells suggest that their immunological potential is not compromised and hence that their immunologic determinants are largely intact. These fixed cells are clearly not viable cells, yet they maintain immunogenecity to a far greater extent than the cells rendered non-viable by freezing and subsequent thawing or by freeze drying. This suggestion is also supported by the fact that the fixed cells were non-oncogenic and protected against large doses of viable untreated homologous tumor cells.

The above evidence suggests that formalin or glutaraldehyde fixation of neuraminidase treated cells results in cell preservation without alteration of antigenecity. Although the use of liquid nitrogen freezers for slow cooling, with concurrent use of preservatives such as dimethyl sulfoxide[13] may result in immunizing cell preparations of equivalent efficacy to that of fresh cells, formalin or glutaraldehyde fixation of neuraminidase-treated malignant cells is a simple technique. In addition, with respect to animal or human tumors, a stock of cell can be harvested whenever the tumors are available, or at a specific point in time, treated immediately with neuraminidase, fixed with either formalin or glutaraldehyde, and used as a constant source of immunizing cells with avoidance of possible immunogenic alteration of cell lines maintained in culture. The use of neuraminidase-fixed tumor cells as a source of antigen in detection of autologous immunoglobulins or in the early detection of neoplastic proliferation is under investigation.

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Induction of malignant skin tumors in mice by topical application of ethylnitrosourea[1]

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Summary. A high percentage of squamous cell carcinomas were induced in the skin of Swiss-Webster mice by repeated topical application of ethylnitrosourea, indicating that this nitrosamide possesses a potent carcinogenic action when topically applied.

With the exception of methylnitrosourea (MNU)[4], most otherwise carcinogenic nitroso-compounds have not been proven to be effective skin carcinogens in laboratory animals[5–7], when topically applied, despite the fact that these substances do not require any metabolic activation to exert their activity[7]. We report the results of a sucessful attempt to induce malignant tumors in mouse skin by repeated application of 1-ethyl-1-nitrosourea (ENU) directly on skin.

52 randomly bred 8-week-old Swiss-Webster mice (27 males and 25 females) were housed by sex in groups of 10 in clear plastic cages on San-i-cell bedding. They were given Wayne pelleted diet (Allied mills, Chicago, Illinois) and tap water ad libitum and were maintained under standard hygienic conditions (constant temperature: 22 ± 2 °C and humidity; 12 h of artificial light per period of 24 h). The interscapular region was shaved (2.5 cm^2) and 33.4 µl of a 1% solution of ENU (0.334 µg) in pure distilled acetone (free of contamination by polycyclic aromatic hydrocarbons-PAH) was applied topically twice a week for 20 consecutive weeks with an automatic dispenser (Hamilton Co., Whittier, Calif.). The total dose of ENU received by each individual animal was 13.34 mg.

The solution of ENU in acetone was prepared freshly immediately prior to each treatment (pH 6.8–7.2 at room temperature). The animals were then observed daily for appearance and recording of tumors. They were allowed to die spontaneously or were killed when moribund. All were autopsied and all tissues and tumors were observed microscopically.

A group of 80 mice (40 males and 40 females of same age and origin) were used as untreated controls and maintained under the same conditions as the test animals. A total of 34 skin tumors (65.4% of all animals) were ob-

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Sex	Total No. of skin tumors	No. of squamous cell carcinomas	Δ % squamous cell carcinomas[a]	Survival time (weeks)[b] Treated	Controls	Δ % survival time[c]
Males	21 (77.8%)[d]	16 (59.2%)[e]	+ 23.4%	43.5 ± 12.7	81.0 ± 17.4	−46%
Females	13 (52.0%)[d]	12 (48.0%)[e]		53.7 ± 15.3	89.0 ± 21.8	−40%

[a] % increase of squamous cell carcinomas in males over females. [b] Mean ± SE. [c] Percent decrease in survival time of treated over controls. [d], [e] Percent of total number of animals in the group.

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

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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

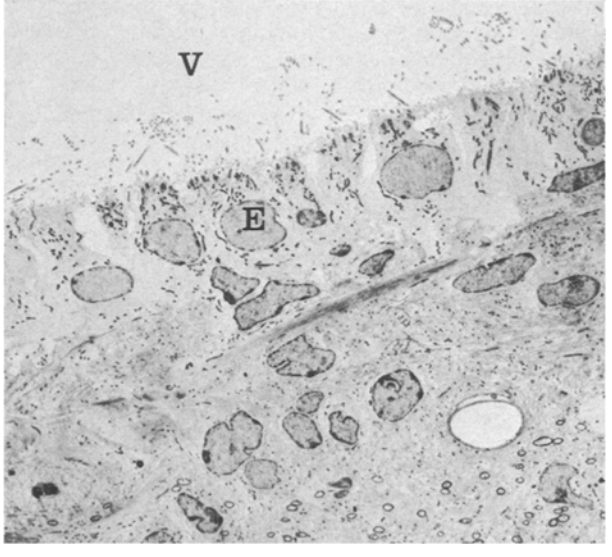

Fig. 1. An early lesion adjacent to the ependymal lining (E) of the lateral ventricle (V). × 2300.

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