Diverse Spectrum of Tumors in Male Sprague–Dawley Rats Following Single High Doses of N-Ethyl-N-Nitrosourea (ENU)

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In this study, 30-day-old male Sprague–Dawley rats, were inoculated intraperitoneally with a single dose of 45, 90, and 180 mg/kg of N-ethyl-N-Nitrosourea (ENU). A wide spectrum of neoplasms occurred. The most common tumors were those of the mammary gland and of the nervous system. Although the incidence of mammary tumors was highest in the two high-dose groups (90 and 180 mg/kg ENU), the incidence of neurogenic tumors was highest in the 45 mg/kg dose group. Mammary tumor development led to early death and precluded development of tumors of the nervous system, which require a longer latency period. A variety of neoplasms of other organs have been associated particularly with high doses of ENU, including ameloblastic tumors, carcinomas of the thyroid, prostate, kidney, pancreas, intestine, and lung, hemilymphatic tumors, and sarcomas. It is concluded that large doses of ENU are capable of expanding the tumor spectrum in young male rats beyond the target organs generally affected with lower doses, as described in earlier reports. (Am J Pathol 1984, 116:319–326)

N-ETHYL-N-NITROSOUREA (ENU) has been extensively used as a transplacental neurocarcinogen capable of inducing neurogenic tumors in nearly 100% of rats exposed prenatally under optimal conditions. Susceptibility increases with advancing gestation culminating at parturition time and declining postnatally. After 30 days of age it becomes difficult, if not impossible, to induce neurogenic tumors in rats with a single dose of ENU.[1–4]

In a recent study we used 30-day-old female Sprague–Dawley (CD) rats in an attempt to correlate molecular damage with tumor incidence in selected organs following single inoculations of high doses of ENU.[5] A surprisingly high incidence of mammary tumors (MTs) (up to 100% in the 90 and 180 mg/kg dose groups) resulted in early death and precluded the development of tumors of the nervous system (a prime target organ), which require a longer latency period than MTs.[6]

These findings prompted us to repeat the experiment using 30-day-old *male* rats. In this report we describe the spectrum of neoplasms induced with high single doses of ENU in susceptible young male CD rats.

Materials and Methods

Carcinogen

ENU crystals (synthesized by Dr. M. Chang, Veterinary Pathobiology Department, Ohio State University) were freshly dissolved in phosphate/citrate-buffered saline at pH 4.2 (1 part buffer to 14 parts saline) before every administration. The crystalline carcinogen was stored in a dessicator at −20 C

Animals

Four groups of 30 rats (three experimental groups and one control group) were used (Table 1). All rats were 30-day-old male specific-pathogen-free (SPF) cesarean-delivered (CD) Sprague–Dawley (CD) rats (Charles River Laboratory). They were housed in

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plastic cages (two rats per cage) in temperature-controlled rooms (24 C) provided with 10 hours electric light per day. They were fed autoclaved Purina Lab Chow 5010 C and water *ad libitum*. Rats in the three experimental groups were inoculated intraperitoneally with freshly prepared ENU solutions in single high doses of 45, 90, and 180 mg/kg, repectively. The control group was inoculated with an equal volume of phosphate/citrate-buffered saline solution at pH 4.2. Rats were observed daily and weighed weekly after treatment. Most rats died or were sacrificed because of tumors of the nervous system, hemilymphatic system, or mammary glands or because of cachexia. A complete necropsy was performed shortly after spontaneous death or euthanasia of moribund animals. The histologic sampling procedures were equal among all groups. Tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4–6 μ, and stained with hematoxylin and eosin (H&E). Special stains, such as Masson's trichrome, periodic acid–Schiff (PAS), and Movat's pentachrome, were used when indicated. Tumors of all organs were studied. Mammary neoplasms were classified according to the suggested classification of mammary tumors in rats by the World Health Organization.[7,8] Morphologic criteria for malignancy were pleomorphism, layering of secretory epithelium, infiltration of surrounding tissue, high mitotic activity, anaplasia, and metastases.

Tissues for electron microscopy were fixed in 4% glutaraldehyde, postfixed in osmium tetroxide, and, after processing, embedded in Epon, cut with an ultramicrotome (1-μ sections), and stained with 1% toluidine blue. Blocks were selected for electron-microscopic examination, and ultrathin sections were contrasted with uranyl acetate and lead citrate. Survival times were analyzed by one-way analysis of variance. Significant differences between means were determined by the least significant difference (LSD) test ($P = 0.05$).

Results

Incidence and Survival Time

The incidence of tumors in all ENU treatment groups (80–86%) was substantially higher than in the control group (7%) (Table 1). There was, however, no difference in either the tumor incidence or the number of tumors per rat among the three ENU dose groups. A significant difference existed in survival time between the control group and treated groups and also among treated groups. The mean survival time decreased with increasing doses of ENU (Table 1). Another difference among the three dose groups was the incidence of tumors of the mammary gland and nervous system (Table 2). The rats affected with MTs per dose group were 7 (23%) in the 45 mg/kg group, 14 (46%) in the 90 mg/kg group, and 12 (40%) in the 180 mg/kg group. While 19% of the total number of MTs occurred in the 45 mg/kg group, 40% occurred in the 90 mg/kg group, and 41% occurred in the 180 mg/kg group. The two highest ENU doses resulted not only in a higher incidence of mammary neoplasias but also in an increased incidence of carcinomatous and sarcomatous types of MTs. There was a direct correlation between dose of ENU and the percentage of malignant MTs (10%, 21%, and 27% malignancy per dose group). The overall mean MT induction time was 140 days, with a mean of 105 days in the highest ENU dose group (180 mg/kg). The mean survival time of the rats bearing malignant MTs was 280 (±5) days. The opposite was true for neurogenic tumors; 46% of the total number of neurogenic tumors developed in the low-dose group (45 mg/kg), 26% developed in the 90 mg/kg group, and 28% developed in the 180 mg/kg group. The number of rats per group affected by neurogenic tumors was 20 (41%) in the 45 mg/kg group, 14 (28%) in the 90 mg/kg group, and 15 (31%) in the 180 mg/kg group. The mean survival time of rats bearing neurogenic tumors per dose group was 447 days for 45 mg/kg group, 386 days for 90 mg/kg group, and 350 days for 180 mg/kg group.

Table 1—Number of Tumors and Survival Time in Male Sprague-Dawley Rats Treated With ENU Intraperitoneally at 30 Days of Age

Dose of ENU	No. of rats with tumors (30 rats per group; percentage in parentheses)	Mean number of tumors per rat	Mean survival time (days) (mean ± SEM)
0	2 (7)	0.1	491 ± 4
45 mg/kg	24 (80)	1.7	425 ± 16
90 mg/kg	26 (86)	1.2	367 ± 19
180 mg/kg	24 (80)	1.6	294 ± 14

Histologic Classification

The histologic classification and the number of various types of neoplasms are listed in Table 2.

Neurogenic Tumors

Tumors of the nervous system are similar to those described in detail in previous publications.[1–4] In addition to the neurogenic tumors mentioned (Table 2), 10% and 15% of the rats from the high-dose groups developed alterative changes such as focal malacia and gliosis.

Table 2—Incidence and Classification of Tumors Induced With ENU in Male Sprague-Dawley Rats Inoculated at 30 Days of Age

Neoplasias	Number of tumors per dose/group (30 rats per group)			
	0	45 mg/kg	90 mg/kg	180 mg/kg
Central nervous system	–	18	12	11
Oligodendrogliomas	–	12	8	9
Astrocytomas	–	2	–	2
Mixed gliomas	–	2	4	–
Ependimomas	–	2	–	–
Peripheral nervous system	–	7	2	4
Anaplastic neurinomas	–	7	2	4
Mammary gland tumors	–	10	21	21
Fibromas	–	5	7	7
Fibroadenomas	–	–	3	2
Fibrosarcomas	–	1	–	2
Adenocarcinomas	–	4	9	11
Carcinosarcomas	–	–	2	–
Thyroid gland	2	8	2	2
Follicular carcinomas	–	2	1	–
Follicular adenomas	–	3	1	–
C-Cell adenomas	2	3	–	2
Pituitary Gland	–	1	1	–
Chromophobe adenoma	–	1	1	–
Bone	–	–	–	1
Osteosarcoma	–	–	–	1
Odontogenic tumor	–	–	–	2
Ameloblastic odontoma	–	–	–	2
Pancreas	–	2	–	2
Islet cell carcinomas	–	1	–	2
Exocrine Adenocarcinoma	–	1	–	–
Prostate gland	–	–	1	2
Adenocarcinomas	–	–	1	2
Skin	–	2	–	1
Hemangiopericytoma	–	2	–	1
Liver	–	1	1	–
Hepatocellular carcinomas	–	1	1	–
Hemilymphatic system	–	2	3	4
Lymphosarcomas	–	2	1	2
Myeloid leukemias	–	–	2	2
Spleen	–	–	–	1
Hemangiosarcoma	–	–	–	1
Large intestine	–	1	–	–
Carcinomas	–	1	–	–
Kidney	–	1	–	2
Renal carcinomas	–	1	–	2
Adrenal gland	–	1	–	–
Pheochromocytomas	–	1	–	–
Lung	–	–	2	1
Adenomas	–	–	1	1
Squamous cell carcinomas	–	–	1	–
Peritoneum	–	–	–	1
Mesothelioma	–	–	–	1
Totals	2	54	46	53

Mammary Tumors

Benign MTs (42% of all MT) were either fibromas or fibroadenomas consisting of fibrotic encapsulated masses with various degrees of intercalated glandular structures (fibroadenomas). Malignant MTs were either fibrosarcomas, adenocarcinomas, or carcinosarcomas. Fibrosarcomas consisted of anaplastic fusiform fibroblastic cells with whorl formation and a high rate of mitosis (5–6 mitotic figures per high-power field). One fibrosarcoma had metastasized to the axillary lymph node, mediastinum, and lung. Adenocarcinomas (Figure 1) consisted of irregular ductular and acinar structures intercalated with a dysplastic stroma often heavily infiltrated with mast cells. The multilayered cells exhibited an increased nuclear–cytoplasmic ratio, pleomorphism, and a high rate of mitosis. Marked necrosis and cystic ductular

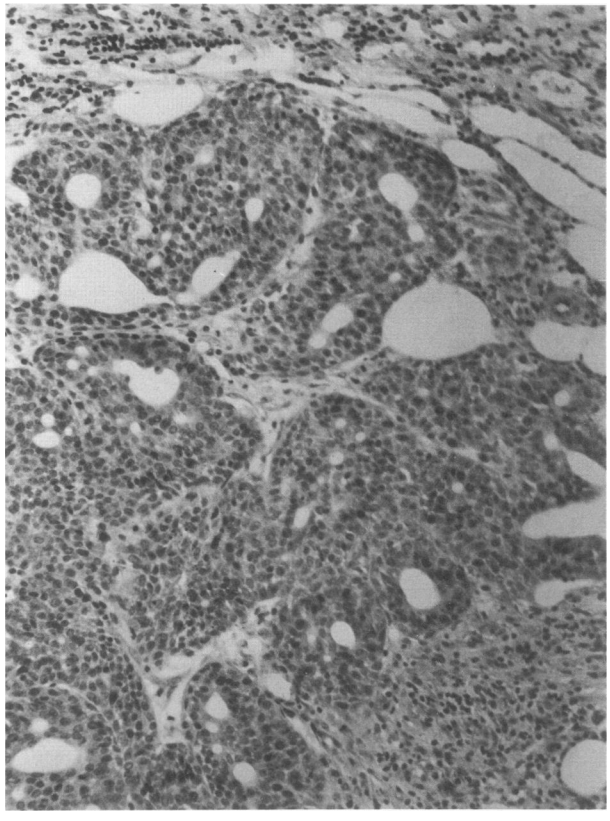

Figure 1—Mammary tumor adenocarcinoma showing ducts with multilayered neoplastic epithelial cells (H&E, ×120)

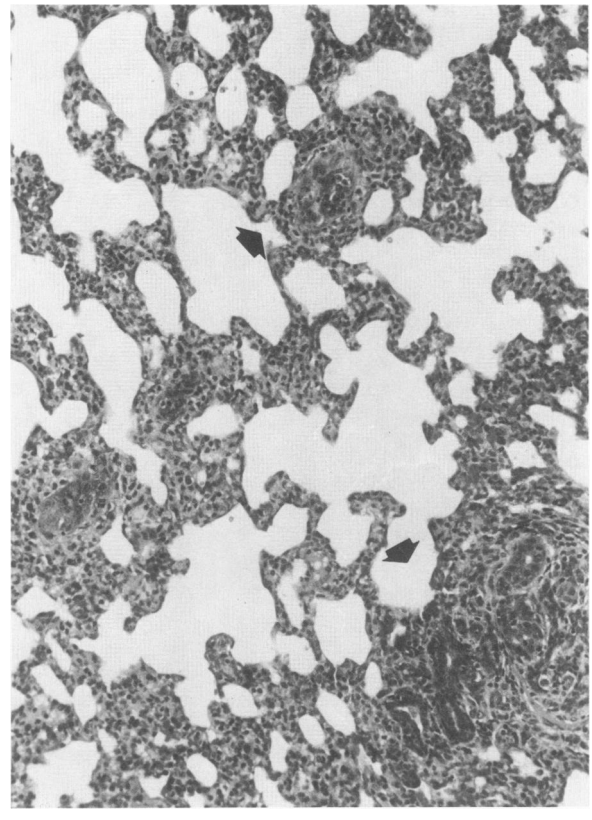

Figure 2—Mammary tumor adenocarcinoma metastases to the lung exhibiting vascular neoplastic emboli (*arrows*). (H&E, ×120)

structures filled with eosinophilic material were often associated with this type of tumor. Two of the rats with adenocarcinomas (one each in the 45 and 180 mg/kg ENU dose groups) had lung metastases characterized by multiple epithelial emboli and tumoral masses invading the lung parenchyma (Figure 2). The carcinosarcomas were characterized by malignancy of both epithelial and mesenchymal components. In addition, 8% of the rats from the treated groups free of mammary neoplasms developed mammary gland hyperplasia.

Electron micrographs of carcinoma cells exhibited the characteristic pattern of mammary epithelial cells. The cells consisted of a central nucleus with a large nucleolus. The free margin had numerous microvilli and desmosomal junctions. Free ribosomes and tubules of rough-surfaced endoplasmic reticulum (RER) were widely distributed throughout all parts of the cell. Some of the tubules formed cisternae. Numerous electron-dense secretory granules and a moderate number of lipid bodies occurred intracytoplasmically and within intercellular spaces.

Ameloblastic odontomas were detected in two rats (180 mg/kg ENU dose) with a survival time of 280 days. The tumors contained many developing teeth of various sizes and shapes resembling normal or atypical tooth germs, with enamel and dentin (Figure 3). The tumor contained dentine, enamel, and cementum in varying quantities and, in addition, epithelial and mesenchymal odontogenic tissues with scattered islands of bone and osteoid.

Hepatocellular carcinomas were encountered in 2 rats (45 and 90 mg/kg ENU dose groups) with a survival time of 415 and 320 days, respectively. The tumors had a trabecular pattern with moderate necrosis, telengiectasia, and fibrosis (Figure 4). In addition, multiple nodules and vascular emboli consisting of well-differentiated hepatoblasts were detected in the lung. In other, liver-tumor-free rats, hepatic changes consisting of focal cellular alterations, nodular hyperplasia, spongiosis hepatis, focal necrosis, biliary hyperplasia, and cholangiofibrosis were observed in 20%, 23%, and 30% of the treated group rats, compared with foci of cellular alteration in only 5% of the control group rats.

Thyroid tumors occurred in all dose groups and controls in equal number except for the 45 mg/kg group, where four times the number on the other

groups occurred. The mean survival time of rats with thyroid tumors was 450 (±15) days. Only 3 of the 14 tumors (all in experimental groups) had characteristics of malignancy. They were diagnosed as follicular carcinomas (2 in the 45 mg/kg and 1 in the 90 mg/kg ENU dose groups) and were characterized by a papillary pattern with invasion of the capsule and adjacent tissue. No metastases to other organs were detected.

Tumors of the pancreas were detected in 4 ENU-inoculated rats. The mean survival time of rats bearing these tumors was 320 (±10) days. Three were islet cell tumors recognized macroscopically as solitary nodules 0.5–1 cm in diameter. The tumors (one in the 45 mg/kg group and two in the 180 mg/kg group) were islet cell carcinomas characterized by the lack of a capsule, a well-defined neuroendocrine pattern, cellular anaplasia, and local invasion. Invasion of the surrounding exocrine pancreas, with aggregates of exocrine acini incorporated into the neoplasia, was clearly apparent (Figure 5). Hyperplasia of the islet cell of the pancreas was recognized in 10% of the additional rats from high-dose groups. One tumor was an adenocarcinoma of the exocrine pancreas, with a high degree of anaplasia and many mitoses. The exocrine pancreatic adenocarcinoma had metastasized to the mesenteric lymph nodes and liver.

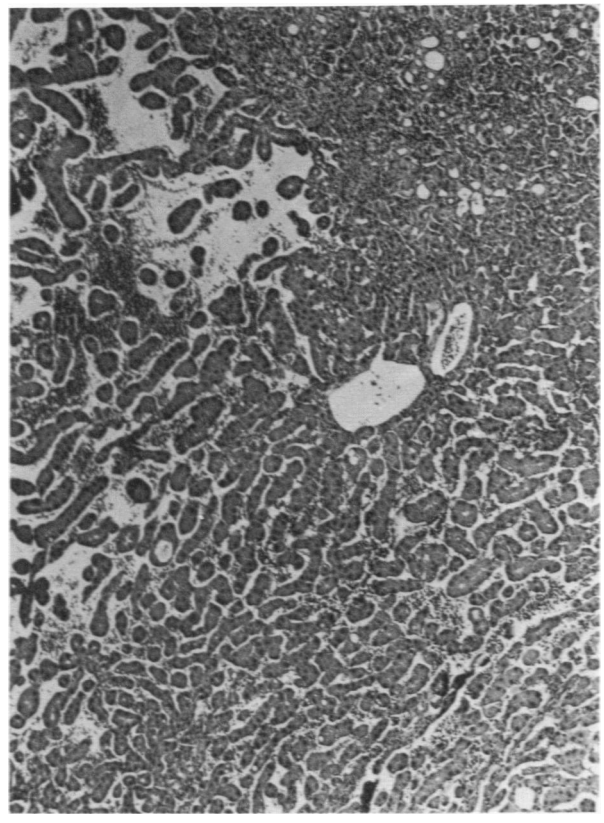

Figure 4 — Hepatocellular carcinoma, trabecular pattern. (H&E, ×48)

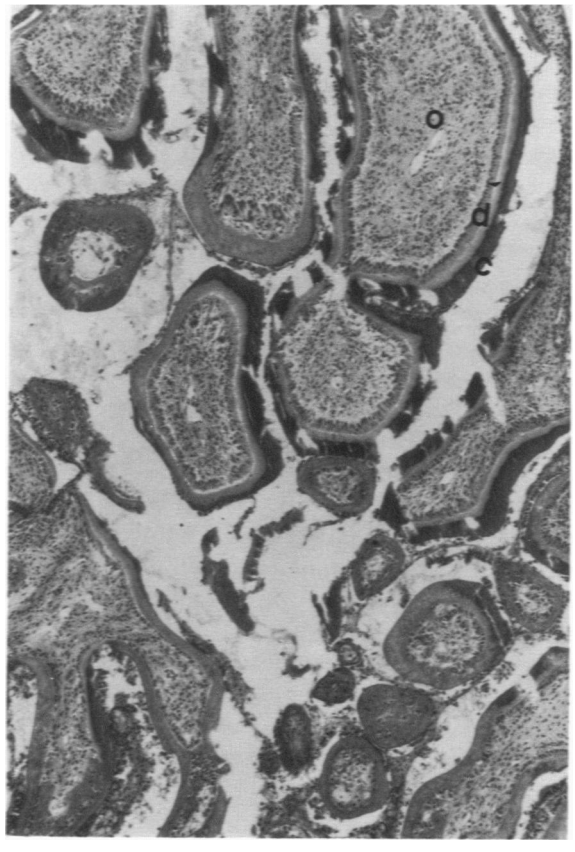

Figure 3 — Ameloblastic odontoma showing irregular structures consisting of mesenchymal odontogenic tissue (O), dentin (D) and cementum (C). (H&E, ×120)

In 1 rat a *carcinoma of the large intestine* was encountered, with metastases to the adjacent mesenteric lymph node. The animal was terminated at 510 days of age. An *osteosarcoma* arising from sacral vertebrae was diagnosed in 1 rat at the age of 330 days. This tumor metastasized to the lung, right atrium, and adrenal gland.

Adenocarcinomas of the prostate gland were found in 3 rats. The mean survival time of rats bearing this type of tumors was 260 (±2) days. These tumors were anaplastic and had metastasized to lymph nodes, liver, and lung and invaded adjacent tissue. Mucosal papillary hyperplasia of the urinary bladder and prostate gland hyperplasia with squamous metaplasia were observed in 10% of the additional rats from the 2 high-dose groups. None of those changes were observed in the control group.

A *hemangiosarcoma of the spleen* with a survival of 320 days had metastasized to the liver and kidney.

Hemangiopericytomas in the subcutis of the submaxillary region occurred in 3 ENU-exposed rats, with a mean survival time of 450 (±2) days.

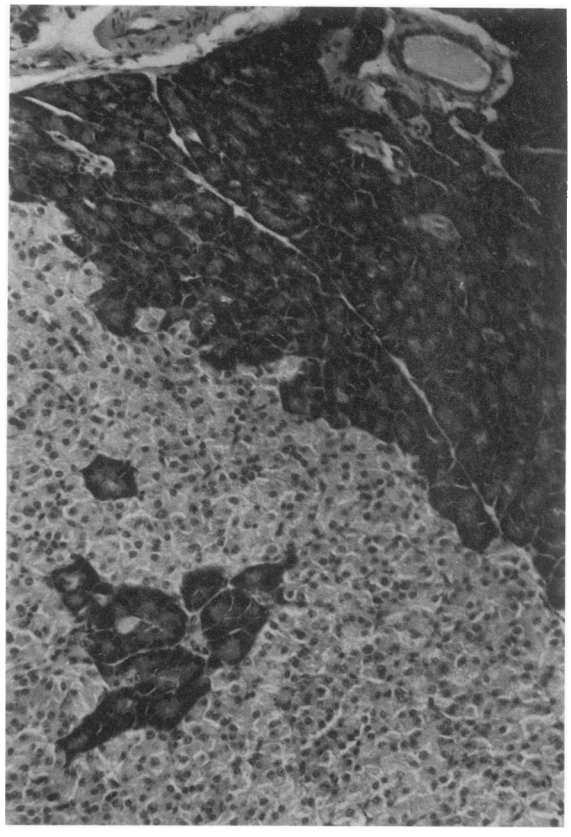

Figure 5—Pancreas. Islet cell carcinoma. Aggregates of exocrine acini incorporated into the neoplasia are clearly apparent. (H&E, ×120)

A *mesothelioma* was encountered in 1 rat with a survival time of 406 days. The tumor had spread throughout the serosal surface of the abdominal cavity.

Renal carcinomas developed in 3 exposed rats, with a survival time of 260 (±2) days. One of the tumors (45 mg/kg group) was a solid carcinoma developing in the cortex and invading the adjacent renal parenchyma. The other two tumors (180 mg/kg group) were solid clear-cell carcinomas occupying large areas of the corticomedulla at one pole of the kidney. They consisted of large polyhedral cells with small hyperchromatic nuclei and clear cytoplasm. Foci of small aggregates of polyhedral cells with eosinophilic granular cytoplasm were interspersed throughout. Local invasion of the surrounding renal tissue was apparent.

Primary *tumors of the lung* were detected in three instances: two were adenomas and one was a squamous cell carcinoma characterized by the presence of numerous keratin pearls, invasion, necrosis and inflammation. The survival time for the rat bearing a squamous cell carcinoma was 253 days.

Neoplasms of the hemilymphatic system were seen in 9 rats: five lymphosarcomas and four myeloid leukemias. All nine hemilymphatic neoplasms occurred in ENU-exposed rats and affected liver, spleen, kidneys, and lymph nodes. All the hemilymphatic neoplasms occurred in rats under 1 year of age (the mean survival time was 245 ± 5 days).

Discussion

The results indicate that single postnatal ENU exposure of 30-day-old male rats has the potential of inducing a wide spectrum of neoplasms in multiple, histogenetically unrelated tissues.

The most striking and unusual finding was the high incidence of MTs in male rats, not previously described as a consequence of transplacental or postnatal ENU exposure. In a previous experiment using female rats, 30% of the ENU-induced MTs were ovarian-hormone-independent.[5] Although the hormone responsiveness of the ENU-induced MTs in the male rats of the present study is not known, it is reasonable to assume that they are largely ovarian-hormone-independent, because they were transformed in the absence of substantial quantities of these hormones. *In vitro* experiments provided evidence that N-methyl-N-nitrosourea (MNU)-induced rat neoplastic mammary epithelial cells were able to grow in culture medium free of ovarian hormone.[9]

The inverse correlation between the doses of ENU and neurogenic tumor incidence can be explained by the shorter survival time in the high-dose groups precluding development of neurogenic tumors, which have a longer latency period than MTs. In the high-dose-treated groups we saw a high incidence of alterative changes of central nervous system, such as glial nodules associated with focal malacia, which can be considered early neoplastic proliferation. Fourteen rats from the 90 and 180 mg/kg dose groups in our experiments were terminated because of malignant MTs at 9 months of age (280 days), which is lower than the mean survival time of rats bearing neurogenic tumors in the high-dose groups (386 and 350 days, respectively). Early neoplastic proliferations of the central nervous system were recognized in 8 of these rats. Given a longer survival time, it is probable that true tumors could have developed from those changes.

Of special interest are the ameloblastic odontomas,

a tumor type not previously reported in CD rats associated with ENU exposure. This tumor occurs in man and has been reported with relatively high frequency in some areas of Africa. Malignant behavior has been recognized, and lung metastases have been described occasionally.[10] In dogs, this tumor has been encountered with low frequency, behaving as a slow-growing benign neoplasm.[11] Ameloblastic odontomas apparently arise from the primitive odontogenic epithelium, resembling the dental lamina.

Tumors of the liver have not been reported in experiments using ENU as a carcinogen. The liver has been generally considered to be a non-target organ in ENU carcinogenesis. However, we observed hepatocellular carcinomas in 2 ENU-exposed rats. The spontaneous incidence of liver tumors in male CD rats was reported to be 1% at 24 months of age,[14] a survival time which is considerably higher than that of rats from the ENU-treated groups.

Some of the tumors listed in Table 2 are reported to occur spontaneously in the rat.[12-14] Tumors of the exocrine pancreas, bone, and odontogenic apparatus were not encountered spontaneously in historical controls of CD male rats at 24 months of age.[13,14] Only 2 adenomas of the thyroid gland occurred in the control group. The reason no other tumors were noticed is the age of the animals (491 days) when the experiment was terminated. Spontaneous tumors in rats usually occur later in life, peaking at 2 years and beyond.[12-14]

The mean survival time for the ENU-exposed rats was approximately 1 year. Most of the malignant tumors occurred in the high-dose groups, in which none of the rats survived the first year of life. It is a fair assumption that these tumors are treatment-related. The number of animals used in the experiment and the low incidence of most tumors preclude statistical analysis of these neoplasms other than mammary and neurogenic tumors, where the high incidence is convincing.

It is expected that a systemically acting carcinogenic agent might increase the incidence of naturally occurring tumors and shift their appearance to a younger age. Also, exposure to a carcinogen may render spontaneously occurring benign tumors more malignant and anaplastic. Neoplasias affected in that manner were considered treatment-related. In support of the assumption that malignancies are treatment-related is the presence of proliferative changes (preneoplastic alterations) in organs and tissues of treated animals without tumors. Such proliferative changes occurred in the brain, liver, pancreas, mammary gland, and urogenital system. None of these changes occurred in the control group.

We conclude that malignant neoplasias of the nervous system, mammary gland, odontogenic apparatus, bone, prostate, liver, hemilymphatic system, kidney, pancreas (islet cell carcinomas and exocrine carcinomas), and thyroid gland in the ENU-treated groups were treatment-related.

Although neuroectodermal cells and epithelial cells of the mammary gland are the primary target cells for the oncogenic effect of ENU, high doses of ENU administered to young rats induce a wide spectrum of neoplasms. This indicates that ENU is capable of transforming a variety of cell types of different organs. In addition to the high doses, the age of the rats may play an important role in explaining these results. During the first weeks of life, the cells of many organs have a high turnover rate, including those of the male mammary gland.

The ENU-induced spectrum of neoplasms in multiple histogenetically unrelated tissues in our experiment could be related to the rate of DNA synthesis and cellular replication, which greatly augment the process of neoplastic transformation.[15-17]

References

1. Druckrey H, Ivankovic S, Preussman R: Teratogenic and carcinogenic effects in the offspring after single injection of ethylnitrosourea to pregnant rats. Nature 1966, 210:1378-1379
2. Koestner A, Swenberg JA, Weschler W: Transplacental production with ethylnitrosourea of neoplasms of the nervous system in Sprague-Dawley rats. Am J Pathol 1970, 63:37-56
3. Koestner A, Swenberg JA, Weschler W: Experimental tumors of the nervous system induced by resorptive N-nitrosourea compounds. Recent Advances in Brain Tumor Research, Progress in Experimental Tumor Research. Homburger Fed 1972, 7:9-30
4. Jones PL, Searle CE, Smith Thomas W: Tumors of the nervous system induced in rats by the neonatal administration of N-ethyl-N-nitrosourea. J Pathol 109, 1973
5. Su CM, Brash DE, Change MJW, Hart RW, D'Ambrosio SM: Induction of single-strand breaks plus alkalilabile bonds by N-nitrosourea in rat tissues in vivo: Ethylnitrosourea versus benzoylnitrosourea. Mutat Res 1983, 108:1-12
6. Stoica G, Koestner A, Capen C: Characterization of N-ethyl-N-nitrosourea induced mammary tumors in the rat. Am J Pathol 1983, 110:161-167
7. Searr RW, Torloni H: Histological typing of breast tumors. World Health Organization, International Classification of Tumors, No. 2, 1968
8. Young S, Hallowes RD: Tumors of the mammary gland, Pathology of tumors in Laboratory Animals. Part I. Tumors of the Rat. Edited by VS Turusov. France, IARC, 1973, pp 37-73
9. Cohen AL: Isolation and characteristics of a serially cultivated, neoplastic, epithelial cell line from the N-nitro-

somethylurea induced mammary adenocarcinoma. In Vitro, 1982, 18:565–575
10. Slavin G, Cameron DH: Ameloblastomas in Africans from Tanzania and Uganda: A report of 56 cases. Cancer 1969, 23:31–37
11. Langham RF, Mostosky UV, Schirmer RG: Ameloblastic odontoma in the dog. Am J Vet Res 1969, 31:1873–1876
12. Gilbert C, Gillman Y: Spontaneous neoplasms in the albino rat. S Afr J Med Sci 1958, 23:257–272
13. Squire RA, Goodman DG, Valerio MG, Fredrickson TN, Strandbert JD, Levitt MH, Lingeman CH, Harshbarger JC, Dawe CJ: Tumors, Pathology of Laboratory Animals. Vol 2. Edited by K Benirschke, FM Garner, TC Jones. New York, Springer-Verlag, 1978, p 1051
14. Gart JJ, Chu KC, Tarone ER. Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. J Natl Cancer Inst 1979, 62:957–974
15. Russo J, Wilgus G, Tait L, Russo IH: Influence of age and parity on the susceptibility of rat mammary gland epithelial cells in primary cultures to 7, 12-dimethylbenz(a)anthracene. In Vitro 1981, 17:877–884
16. Nagasawa H: Causes of age-dependency of mammary tumor induction by carcinogens in rats. Biomedicine 1981, 34:9–11
17. Greiner WJ, Bryan AH, Malán-Shilbley LB, Janss HD: aryl hydrocarbon hydroxylase and epoxide hydratase activities: Age Effects in mammary Epithelial cells of Sprague–Dawley rats. J Natl Cancer Inst 1980, 64:1127–1133

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