The Effect of Gonadal Ablation on Transplacentally Induced Neurogenic Tumors in Hamsters

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SUMMARY
The effects of gonadectomy on tumors induced transplacentally by the ethynitrosourea precursors, ethylurea and sodium nitrite, were investigated in hamsters. The pregnant hamsters were exposed to four daily doses of ethylurea (100 mg/kg) and sodium nitrite (50 mg/kg) administered from Day 12 to 15 of pregnancy. Weaned offspring were gonadectomized when they reached the age of 5 weeks.

Orchiectomized male progeny showed a multiplicity and greater frequency of peripheral nervous system tumors and of any other tumor types than did intact males or their ovariec-tomized and intact female siblings. The possible inhibitory effects of endogenous androgens on the development and growth of neurogenic tumors in the peripheral nervous system and the influence of an induced endocrinial imbalance on prenatally induced neoplasms are discussed.

INTRODUCTION
The hamster has been shown to possess a high degree of sensitivity to hormone imbalances, which influence the development of numerous tumor types, including those of tissues not normally recognized as being under direct hormonal control (1–3, 7–10, 20). Fortner (2) reported, for example, that gonadal ablation in hamsters resulted in a significant decrease in spontaneously occurring neoplasms of many types in both sexes. In the latter study, this reduction was most significant in terms of the gastrointestinal tract, liver, and pancreas; however, benign tumors of the adrenal cortex developed in greater proportions, particularly in males.

In a previous communication, we reported a greater incidence and multiplicity of neurogenic tumors of the PNS in the female progeny of hamsters exposed to ENU precursors during the late stages of pregnancy (18). A possible estrogenic influence on the development and growth of neurogenic tumors was thereby suggested (19). The present study dealt with the effects of gonadal ablation on prenatally induced neurogenic tumors in hamster offspring.

MATERIALS AND METHODS
Animals. Ten female Syrian golden hamsters, randomly bred in the Eppley Institute colony (University of Nebraska Medical Center, Omaha, Neb.), were mated and housed individually until 5 weeks after delivery. The offspring were then separated by sex, gonadectomized, and housed 5/group on sterilized San-i-Cel bedding in clear plastic cages. They were kept in a temperature-controlled room at 69–74°F, fed Wayne pelleted diet, and given water ad libitum.

Test Substances. Ethylurea (Aldrich Chemical Co., Inc., Milwaukee, Wis.) was dissolved in distilled water, together with sodium nitrite (Malinckrodt Chemical Works, St. Louis, Mo.) in concentrations of 2% and 1% (w/v), respectively, shortly before i.g. intubation.

Treatment. The pregnant animals were given 4 i.g. intubations of ethylurea, 100 mg/kg, and NaNO2, 50 mg/kg, simultaneously at the rate of 0.01 ml/g body weight. Treatment began on Day 12 of gestation and continued for 4 consecutive days, so that each animal received a total of 400 mg ethylurea per kg and 200 mg NaNO2 per kg. The details of mating and the administration techniques were described previously (17–19).

Gonadectomy. All surgery was performed under anesthesia with Diabutal (sodium pentobarbital; Diamond Laboratories, Des Moines, Iowa) delivered i.p. at 75 mg/kg. Orchiectomy consisted of removal of both the epididymis and testes through a lateral incision in the scrotal sac and subsequent ligature at the level of the spermatic cords. Ovariectomy was achieved via a single incision in the hypogastric region (linea alba), followed by bilateral excision of the ovaries at the level of the ends of the distal horns and closure with sutures.

Experimental Groups. From 10 litters, 117 newborns were delivered, and 85 siblings weaned and separated into 2 groups consisting of 40 females and 45 males. The effective number of animals listed in Table 1 represents those that survived either beyond 7 weeks of age or the 2nd postoperative week. Hamsters that died of postoperative stress during the 2 weeks after surgery were excluded. Males were particularly affected in this respect.

There were 47 female and 46 male untreated control hamsters (Groups 3 and 6). Data pertaining to the intact and control groups were reported previously (17). However, for comparative purposes they are repeated in Table 1. All animals, including treated mothers, were inspected regularly and weighed weekly throughout their lives. Grossly observed external tumors were recorded. Animals were allowed to die spontaneously or were sacrificed when moribund. All were completely autopsied and their organs, including the CNS and PNS, were fixed in 10% buffered formalin or in Cajal’s Brom-fornol solution, embedded in paraffin, and processed and stained with hematoxylin and
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eosin, Wilder’s reticulum, Gomori, Masson’s trichrome, cresyl violet, van Gieson’s, phosphotungstic acid-hematoxylin, or Bodian’s. Histological studies were performed routinely on all tumors, as well as on the lungs, liver, kidneys, spleen, stomach, bone marrow, urogenital organs, adrenal glands, and selected segments (coronal, horizontal, or sagittal sections) of the brain and spinal cord. All organs and tissues with gross pathological alterations were microscopically evaluated.

RESULTS

General Observations

The survival rates of the treated intact and gonadectomized groups are given in Chart 1. The life-spans ranged from 16 to 108 weeks in intact hamsters and from 15 to 61 weeks in those that were gonadectomized. As indicated, the orchietomized male progeny had significantly shorter average life expectancies (males, p < 0.05; Student’s t test) than did treated, intact animals. The orchietomized, treated males developed greater numbers and multiplicities of tumors (of any type) at significantly shorter average latencies than did intact, treated males (Group 4 versus Group 5). The percentages and multiplicity of all tumor types were also greater in orchietomized male siblings than in intact or orchietomized females (Group 4 versus Groups 1 and 2, respectively). The orchietomized females showed an increased percentage of all tumor types but a considerably smaller number of tumors per tumor-bearing animal. The average latent period for female progeny in both groups was shorter than in their male siblings.

Neurogenic Tumors

PNS Tumors. The orchietomized male progeny (Table 1) developed significantly greater incidences of PNS tumors than did intact male offspring (85% versus 56%; p < 0.05, x² test) and a greater number of neurogenic tumors per tumor-bearing animal (3.8 versus 1.4; p < 0.005, Student’s t test) at shorter latency periods (72 versus 51 weeks; p < 0.05, Student’s t test) (Table 1). The orchietomized progeny also developed a slightly increased rate of PNS tumors over their intact male and female siblings (at a rate of 73 versus 70 for the siblings), with longer latencies (41 versus 45 weeks), but with a considerably reduced multiplicity (2.6% in orchietomized versus 3.8% in intact females) of these tumors per tumor-bearing animal. The PNS tumors developed in greater proportions in the spinal nerves and their branches than in cranial nerves, with ratios of 3:1 for orchietomized and intact males (48 versus 17 and 20 versus 7 for PNS and cranial tumors, respectively), and 2:1 for orchietomized and intact females (46 versus 24 and 62 versus 31 for PNS and cranial tumors, respectively).

The distribution pattern of the PNS tumors has been described (17—19). Grossly, the tumors were prominent, well circumscribed, white, resilient nodular growths, of a solid consistency. They varied in diameter from 1 to 2 mm to 5 cm. The neoplasms were delineated by an incomplete fibrillary capsule, which was absent at the sites of invasion into surrounding structures. No metastases were observed.

Because of the multiplicity of tumors in individual animals, some neoplasms were grossly observed as mere tumefactions of the neural branch. However, upon histological examination, they proved to be genuine neoplasms. Frequently, the tumorous masses grew expansively into nodular conglomerates, making the exact location of origin impossible to determine.

Microscopically, the tumors, which originated at different sites, consisted of Schwann cells of various morphological profiles and architectural arrangements. However, according to previously described criteria (18, 22), they were basically fibrillary (Fig. 1, left) and reticular (Fig. 1, right) types. The Schwann cell elements, of which the fibrillary types were composed, were spindle or fusiform in shape and sparsely distributed in the fibrillary matrix. Areas of cellular elements with delicate processes, which formed either lamellary round structures or were arranged in a longitudinal fashion alternating with zones of whorled or rhythmic structural arrangements, were commonly seen in nerve sheath tumors. The extracellular compartment contained fibroblasts, fibroblasts, collagen and reticulin fibrils, various amounts of amorphous ground substance, and sometimes nerve fascicles, all of which are typical of plexiform neurofibromas in humans (Fig. 2). The regressive changes probably were initiated by microcystic degeneration in the individual cell, and progressed from large, unstained spaces to the creation of cystic cavitations. In general, the morphology of these neurinomas appeared similar to that of neoplasms described in previous reports (17—19).

CNS Tumors. Two gliomas were recorded in the orchietomized progeny. One was a mixed glioma and the other was an oligodendroglioma (see Table 1, footnotes).

The mixed glioma developed in the cervical segment of the spinal cord in a 50-week-old hamster. Grossly, the tumor was a white, partly hemorrhagic, dense area, which extended almost through the entire diameter of the spinal cord. It was 2 to 3 mm long and microscopically was a
**Table 1**

Treatment, incidence, and distribution pattern of neurogenic and other tumors in treated intact and gonadectomized hamsters with ethylurea (100 mg/kg) and NaNO\textsubscript{2} (50 mg/kg) delivered simultaneously by i.g. intubation to pregnant hamsters, was begun on Day 12 of gestation and continued for 4 consecutive days. Total amount of precursors equals 400 mg ethylurea per kg and 200 mg NaNO\textsubscript{2} per kg respectively.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Treatment</th>
<th>Effective no. of animals</th>
<th>Tumor-bearing animals</th>
<th>Neurogenic tumors in PNS\textsuperscript{c}</th>
<th>Spinal and peripheral nerves</th>
<th>Other neoplasms\textsuperscript{f}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>Ethylurea and NaNO\textsubscript{2} prenatally; ovarioectomy</td>
<td>37</td>
<td>75.7 3.0 44.0</td>
<td>73.0 2.6 24</td>
<td>45  (15–61)</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>Ethylurea and NaNO\textsubscript{2} prenatally; left intact</td>
<td>39</td>
<td>71.8 3.6 40.9</td>
<td>70.0 3.8 31</td>
<td>62  (21–60)</td>
<td>B</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>No treatment</td>
<td>47</td>
<td>15.0 1.0 63.0</td>
<td>85.0 3.8 17</td>
<td>48  (38–61)</td>
<td>C</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>Ethylurea and NaNO\textsubscript{2} prenatally; orchietectomy</td>
<td>20</td>
<td>85.0 4.2 51.1</td>
<td></td>
<td></td>
<td>D</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>Ethylurea and NaNO\textsubscript{2} prenatally; left intact</td>
<td>32</td>
<td>68.8 1.9 75.1</td>
<td>56.0 1.4 7</td>
<td>20  (16–108)</td>
<td>E</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>No treatment</td>
<td>46</td>
<td>17.0 1.0 75.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Tumor-bearing animals with neoplasia of any type.

\textsuperscript{b} Neurogenic tumor-bearing animals in peripheral nervous system.

\textsuperscript{c} Average number of tumors per tumor-bearing animal (T/TBA).

\textsuperscript{d} Average age, expressed in weeks, at which tumors were found and recorded.

\textsuperscript{e} Average number of neurogenic tumors per tumor-bearing animal (NT/TBA).

\textsuperscript{f} A, 3 melanomas of skin (46, 49, 61); 3 adenomas of thyroid (24, 45, 52); 2 nephroblastomas of kidneys (36, 39); 1 oligodendroglioma of brain (42); 1 mixed glioma of spinal cord (50); 1 parathyroid adenoma (52); 1 papilloma of forestomach (29); 1 squamous cell carcinoma of skin (57); B, 2 nephroblastomas of kidney (53, 54); 1 melanoma of eye (43); 1 ganglioneuroma of sympathetic trunk (31); 1 papilloma of trachea (36); 1 leiomyoma of uterus (50); C, 1 carcinoma of adrenal cortex (99); 1 carcinoma of thyroid (78); 1 hemangioma (cavernous) of ovary (88); 1 squamous cell carcinoma of uterus (99); 1 adenocarcinoma of uterus (99); 1 hemangioma (cavernous) of spleen (96); D, 3 adenomas of adrenal cortex (56, 56, 58); 1 carcinoma of adrenal cortex (49); 2 melanomas of skin (46, 56); E, 2 carcinomas of adrenal cortex (63, 97); 2 adenomas of adrenal cortex (78, 98); 2 adenomas of thyroid (69, 108); 1 melanoma of skin (malignant) (107); 1 melanoma of skin (74); 1 hemangioendothelioma of spleen (99); 1 hemangioma of spleen (100); 1 cholangiocarcinoma of liver (97); 1 cholangiocarcinoma of liver (107); 1 malignant lymphoma (histiocytic) (99); 1 adenoma of gallbladder (78); 1 squamous cell papilloma of forestomach (97); F, 2 malignant lymphomas (histiocytic type) (87, 100); 2 pheochromocytomas of adrenal glands (116, 116); 2 fibrosarcomas of subcutis (76, 84); 1 adenoma of adrenal cortex (105); 1 adenoma of gallbladder (84). (Numbers in parentheses in this footnote indicate age in weeks at which animal died.)

mixture of neoplastic oligodendrocytes and astrocytes with predominating areas of either type of element. The neoplastic tissue was characterized by vascular proliferation (Fig. 3), necrotic areas, and round calcium deposits (calciospherites).

The oligodendroglialoma occurred in the brain stem of a 42-week-old animal. Grossly, the neoplasm extended from the brain stem in the subcortical area and invaded the lateral ventricles. Microscopically, the oligodendrocytes had round, dense, stained nuclei surrounded by a clear halo of nonstained cytoplasm. This produced a boxed-cell effect (Fig. 4). The neoplastic oligodendrocytes were moderately uniform and closely packed or arranged in nests. Calcification was a conspicuous feature in the form of scattered spherules in degenerating patches particularly in the vicinity of blood vessels.

Other Tumors and Observations

In addition to neurogenic neoplasms, a number of other tumor types were observed in the gonadectomized hamsters and are summarized in Table 1. The incidences of extraneural tumors in untreated controls and treated, intact hamsters have been reported (17). Of interest were the accentuated rates of melanotic skin tumors and thyroid adenomas (3 in each case), as well as of benign adrenal tumors in ovariectomized females. The development of 2 nephroblastomas was also observed in intact females. In both intact and ovariectomized males, 4 adrenocortical tumors and 2 skin melanomas were found. Although similar in number, their incidence was slightly greater in castrated animals, with 20% of the latter presenting adrenal tumors versus 12% of the intact hamsters. Similarly, the melanotic
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Discussion

In recent communications we reported high incidences of PNS tumors transplacentally induced in hamster offspring, with a preponderance of these neoplasms in females (18, 19). The greater frequency and multiplicity of PNS tumors in female progeny at significantly shorter latencies than in males indicated that an age and sex dependence existed for these tumors. This further suggested the possible influence of estrogenic hormones on the growth and development of PNS tumors induced prenatally in hamsters.

Results in the present study revealed that gonadectomy influenced the overall tumor incidence and had the most profound effects on the incidence of PNS tumors in male progeny. Therefore, the significantly increased rates, multiplicity, and shorter latencies of PNS tumors in orchietomized males over their intact siblings indicate that androgens may have had an inhibitory effect on the growth and development of these tumors. The fact that these parameters were practically unchanged in ovarietomized females, when compared with their intact female siblings, indirectly supports this hypothesis and suggests that female sex hormones are not necessarily involved in the induction of PNS tumors.

The morphological features of PNS tumors in gonadectomized animals were essentially similar to those seen in intact animals, indicating that sex hormones may not affect the differentiation of these neoplasms. Cytologically, the PNS tumors were composed of Schwann cells, the cellular elements that originate from neuroectodermal anlage, e.g., the neural crest.

It seems that the carcinogenic action of ENU during preganatal life in hamsters affects preferentially the embryogenetic cellular derivatives of the neural crest since PNS rather than CNS tumors have been found almost exclusively in hamster progeny (14, 15, 18, 19). In this study melanomas coexisted with neurinomas in all instances, and melanocytes also are derivatives of the neural crest.

Gliomas were previously reported in hamsters that had been treated transplacentally with p.o. doses of ENU (5) but were consistently absent in hamsters exposed to the ENU precursors ethylnitrocarbocyanine during prenatal (19, 19) and adult life (17). Many tumor types were also not reported in more recent pretransplant experiments with ENU in hamster progeny (14, 15). The oligodendroglioma found in a female is, to the best of our knowledge, unique among the series of gliomas described in this species. Whether the hormonal environment of the host affected the development of gliomas is not known. Further assessment of findings in this study shows that melanomas tended to develop in ovarietomized females. Gonadectomy inhibited development of spontaneously occurring melanomas in males (2). The extent to which sex hormones influence melanoma development in certain rodents is apparent, since transplanted melanomas grow faster in male mouse recipients (21), while the opposite has proven true in studies with female hamsters (13, 16). According to the findings of Hornig (4), estrogens suppress melanogenesis through interference with the melanocyte-stimulating hormone from the pars intermedia of the hypophysis.

The appearance of benign thyroid tumors in ovarietomized females and intact males and their absence in prematurely exposed, nonovariectomized siblings indicate the nature of the hormonal environment of the host in which these neoplasms develop.

In contrast to previous reports (2, 11, 12) of higher incidences of tumors of the adrenal cortex recorded in male hamsters after gonadectomy, we did not observe higher rates of these neoplasms in either sex, but rather an increased incidence of adrenal nodular hyperplasia in males.

Fortner (2), in explaining the effects of gonadectomy on spontaneous tumor incidences in the hamster, found that this rodent has a decreased incidence of multiple tumor types (with the exception of benign cortical tumors in males). This observation suggests that both androgens and estrogens may stimulate the development of spontaneous neoplasms, which originate in various organs and tissues.

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References

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Fig. 1. Neurinoma in which the incomplete fibrillary capsule delineates neoplasia. Left, fibrillary and right, reticular types of neurinomas. Note similarity of structure with Antoni A and B in human counterpart. The neoplasm developed from brachial plexus. H & E, × 150.

Fig. 2. Neurinoma of a neurofibromatous variant, originating from ischiadic nerve (lumbosacral plexus). Note multiplicity of neural twigs and striking similarity to plexiform neurofibroma in man; also the longitudinal arrangements of Schwann cell processes at right. H & E, × 150.

Fig. 3. Mixed glioma composed of astrocytes and oligodendrocytes in which neither type of element predominated. Astrocytes were characterized by large nuclei and had moderate or abundant pink granular cytoplasm; oligodendrocytes had small, sometimes hyperchromatic nuclei, with marginal condensation of the chromatin and perinuclear clear halo. Origin: spinal cord in vicinity of medulla oblongata. H & E, × 180.

Fig. 4. Oligodendroglioma which extended from brain stem and invaded lateral cerebral ventricle and subcortical areas. Note round nuclei with marginal condensation of chromatin and intensely hyperchromatic nuclei surrounded by clear perinuclear halo. H & E, × 450.
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