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14. We thank G. Hooper for the design of the data acquisition system, S. Davis for valuable criticism, E. Crump for editing the manuscript, and S. Williams for preparing the figures. This research was supported by Department of Energy grant 81ER10924 and NSF grant PCM 8012060.

19 July 1982

Sensitivity to Carcinogenesis in Nude Mice: Skin Tumors Caused by Transplacental Exposure to Ethylnitrosourea

Abstract. *Female athymic nude mice and their phenotypically normal littermates were exposed transplacentally to ethylnitrosourea. Skin tumors (papillomas and sebaceous adenomas) developed on the nude mice with an almost tenfold greater incidence than on their haired littermates. Skin tumors were also induced on nude mice but not haired controls by direct intraperitoneal treatment with ethylnitrosourea. These results indicate that nude mice have higher than normal susceptibility to carcinogenesis under some circumstances.*

The athymic nude (*nu/nu*) mouse provides a natural model for studying the role of thymus-dependent immune surveillance in tumorigenesis. Contrary to expectations, several carcinogenesis studies have not revealed that nude mice are more susceptible than phenotypically normal (*nu/+*) controls to a variety of spontaneous or chemically induced neoplasms, including epidermal and subcutaneous tumors (*1*, *2*). In the study reported here, BALB/c *nu/nu* mice and their *nu/+* littermates were exposed systemically (transplacentally or intraperitoneally) to the direct-acting alkylating carcinogen ethylnitrosourea (ENU). Transplacentally treated *nu/nu* mice developed significant, dose-dependent numbers of skin tumors; their *nu/+* littermates, by contrast, had a tenfold lower incidence of these tumors. Skin tumors also appeared on *nu/nu*, but not *nu/+*, mice treated intraperitoneally as adults with ENU. These results indicate that the nude mouse is more sensitive than normal to tumorigenesis in certain situations.

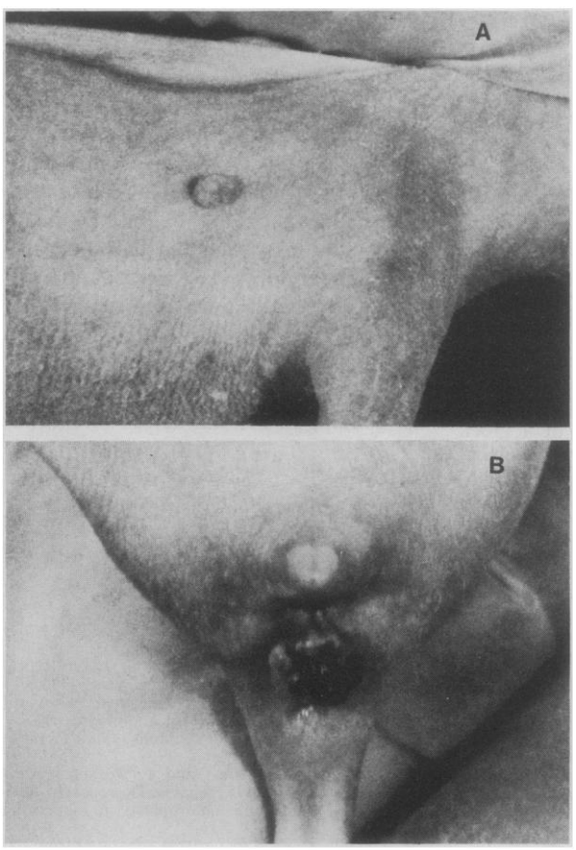

Fig. 1. (A) Sebaceous adenoma on nude mouse 12 months after transplacental exposure to ENU (50 mg/kg). (B) Papilloma 20 months after transplacental exposure to ENU (10 mg/kg).

The BALB/c nude mouse colony was derived from breeders purchased from Gibco Animal Resources Laboratory (Madison, Wisconsin). Skin transplantation experiments confirmed the congenicity of the *nu/nu* and *nu/+* mice. The colony was maintained free of pathogens (*3*) and there were no deaths due to infection. Ethylnitrosourea was synthesized (*4*) and stored at −80°C; solutions in trioctanoin were prepared within 1 hour of injection. On day 15 of gestation *nu/+* females impregnated by *nu/nu* males were injected with ENU (50 or 10 mg/kg) in the high lateral abdomen. In addition, 6-week-old *nu/nu* and *nu/+* females were given two intraperitoneal injections of ENU (50 mg/kg per injection) 1 week apart.

Only female progeny of the treated females were saved, since the responses of males and females were expected to be similar and since BALB/c males are difficult to house because of fighting. All mice were inspected daily and killed when moribund or at the end of the experiment. Most of the moribund animals had neoplasms of lung, lymphoid, or other tissues. At necropsy the skin of *nu/+* mice was searched for tumors by palpation, by wetting with alcohol, and by skinning the mice and examining the skin's lower surface. Since these searches revealed several small tumors comparable to the smallest tumors seen on the *nu/nu* mice, the method ensured discovery of most tumors on *nu/+* mice. The skin tumors were fixed in Bouin's solution, sectioned at 5-μm intervals, and stained with hematoxylin and eosin.

Of the 53 *nu/nu* mice exposed transplacentally to ENU at a dose of 50 mg/kg, 24 (45 percent) developed skin tumors, compared to only 3 of 58 *nu/+* littermates ($P < .01$) (Table 1). At the lower dose of 10 mg/kg fewer tumors appeared, but again the difference was significant ($P < .01$). Average age at death was similar for all mice and for skin tumor bearers in each treatment group, so differences in tumor incidences cannot be attributed to unequal survival times.

Of the 24 *nu/nu* females exposed intraperitoneally to a total of 100 mg of ENU per kilogram, four (17 percent) developed skin tumors (Table 1). No skin tumors were found on *nu/+* mice given

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Table 1. Skin tumors on *nu/nu* and *nu/+* mice exposed to ENU transplacentally (t.p.) or by direct intraperitoneal (i.p.) injection.

Treatment	Genotype	Number of mice	Age at death (months)*		Number of mice with skin tumors	Number of tumors per mouse with tumors*	Number of tumors		
			All mice	Tumor-bearers			Papillomas	Sebaceous adenomas	Mixed (papillary and sebaceous)
ENU (50 mg/kg, t.p.)	*nu/nu*	53	11.9 ± 3.5	13.2 ± 2.8	24†	1.4 ± 0.7 (range, 1 to 3)	10	18	6
	nu/+	58	10.6 ± 3.4	13.3 ± 3.0	3	1	0	2	1
ENU (10 mg/kg, t.p.)	*nu/nu*	48	17.7 ± 4.1	17.4 ± 4.3	11†	1.5 ± 0.9 (range, 1 to 4)	6	10	0
	nu/+	71	17.0 ± 5.0	15.0 ± 0	2	1.5 ± 0.7 (range, 1 to 2)	4	0	0
ENU (100 mg/kg, i.p.)	*nu/nu*	24	11.9 ± 3.0	13.5 ± 1.9	4	1	1	2	1
	nu/+	24	10.8 ± 3.5		0				
None	*nu/nu*	19	16.4 ± 2.9	22	1	1	0	1	0
	nu/+	11	15.5 ± 3.1		0				

*Mean ± standard deviation. †Significantly higher incidence than in *nu/+* littermates ($P < .01$, chi-square test with Yates' correction).

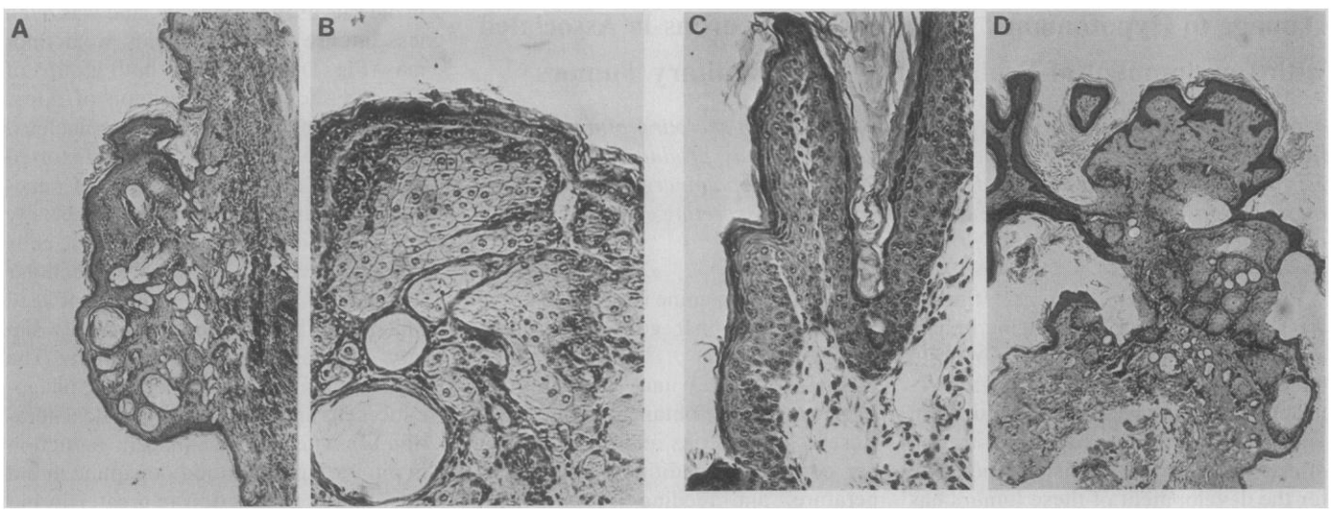

Fig. 2. Photomicrographs of sections of skin tumors resulting from transplacental exposure to ENU. (A) Sebaceous adenoma from *nu/nu* mouse 13 months after exposure to 10 mg/kg (×40). (B) Sebaceous adenoma from *nu/+* mouse 16 months after exposure to 50 mg/kg (×240). (C) Squamous cell papilloma from *nu/nu* mouse 11 months after exposure to 50 mg/kg (×240). (D) Squamous cell papilloma with underlying sebaceous proliferation from *nu/nu* mouse 13 months after exposure to 50 mg/kg (×40). Stain: hematoxylin and eosin.

ENU intraperitoneally. One tumor appeared on an untreated *nu/nu* female 22 months old.

Most of the skin tumors in *nu/+* mice were identified at necropsy; the tumors on the nude mice were more obvious. Thirty-two (63 percent) of the *nu/nu* skin tumors were noted before death. The mean latency between chemical exposure and appearance of these tumors was 7.4 ± 2.6 months (range, 4 to 13 months) for the mice receiving the high dose of ENU and 11.7 ± 5.3 months (range, 5 to 17 months) for the mice given the low dose. In gross appearance the tumors ranged from flattened white bumps and papillae to larger wartlike structures (Fig. 1). They occurred at random on all parts of the body. Most were small and slow-growing, attaining less than 2 mm in largest dimension by the time of death. Eleven tumors (two in the group treated intraperitoneally and nine in the group treated transplacentally) grew to 5 mm or more. No tumors regressed. The tumors were diagnosed as squamous cell papillomas, sebaceous adenomas, or of mixed type (Fig. 2). Tumors from *nu/nu* and *nu/+* mice were histologically similar (Fig. 2, A and B).

These results do not conflict with the reported lack of special susceptibility of nude mice to chemical carcinogenesis, including skin tumorigenesis after topical application of carcinogens or tumor promoters (*1*, *2*). To our knowledge, transplacental and intraperitoneal exposure of BALB/c nude mice to ENU has not been tried before (*5*). Nude mouse skin does not respond to phorbol tumor promoter (*6*), which may explain why other investigators, using skin tumorigenesis protocols involving initiation and promotion or repeated treatment with polycyclic aromatic hydrocarbons, observed similar tumor incidences on nude and normal mice or saw fewer tumors on the nude mice (*1*, *2*). The preponderance of sebaceous adenomas in our experiment suggests that our tumorigenesis model is biologically distinct from the two-stage skin carcinogenesis system, which produces a preponderance of squamous cell papillomas. The high susceptibility of our nude mice to skin carcinogenesis induced by systemic ENU may reflect unique properties of nude mouse skin, lack of immune surveillance, or other special characteristics of the *nu/nu* genotype.

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29 April 1982; revised 16 July 1982

Damage to Hypothalamic Dopaminergic Neurons Is Associated with Development of Prolactin-Secreting Pituitary Tumors

Abstract. *Old female rats with spontaneous prolactin-secreting pituitary tumors (prolactinomas) and young females with prolactinomas produced by prolonged estrogen treatment had damaged tuberoinfundibular dopaminergic neurons. Since these neurons inhibit the function of pituitary prolactin-secreting cells, their destruction may lead to development of prolactinomas.*

Prolactin-secreting pituitary tumors are frequently observed in aging female and male rats, with an incidence of up to 80 percent in old Sprague-Dawley females. Prolactinomas can be induced in young rats by prolonged administration of estrogen. The mechanism responsible for the development of these tumors has been elusive (*1*), but we now report evidence that such tumors are associated with a loss of hypothalamic dopaminergic neurons, which normally inhibit the function of prolactin-secreting cells (lactotrophs) in the anterior pituitary. Hypothalamic dopamine is produced mainly in tuberoinfundibular dopaminergic (TD) neurons of the arcuate nucleus in the medial basal hypothalamus. Most of these neurons terminate in the external layer of the median eminence (ME), from which dopamine is released into the portal vessels that go to the anterior pituitary (*2*).

Sprague-Dawley and Wistar Furth female rats were obtained from Harlan Research Industries and maintained under controlled conditions of light, temperature, and feeding (*3*). Of the 44 Sprague-Dawley females, 31 developed spontaneous prolactinomas at 26 to 28 months of age, as indicated by a significant increase in the mean weight of the anterior pituitary and in the concentration of serum prolactin (Table 1). Histological examination revealed that these pituitaries were hemorrhagic, with disruption of parenchymal cells and capillary integrity. The mean weight of the prolactinomas was more than three times greater than that of the pituitaries of tumor-free anestrous females 24 to 27 months of age (old controls) or diestrous females 3 to 4 months of age (young controls). Serum prolactin concentrations in old rats with prolactinomas were approximately six times higher than in old controls and 13 times higher than in young rats. In old control rats pituitary weights and serum prolactin concentrations were also significantly higher than in young rats. The mean concentration of dopamine in the ME of old rats with prolactinomas was significantly lower than in old controls, and the dopamine concentration in the ME in both groups of old rats was lower than that in young rats (Table 1).

Dopamine fluorescence (*4*) in the external layer of the ME was not as intense in old rats as in young rats, and was even less intense in old rats with prolactinomas (Fig. 1). In addition, both groups of old rats showed degeneration of dopaminergic neurons in the arcuate nucleus, as indicated by the presence of distorted fluorescent fibers and deposits of punctate autofluorescent material—usually considered indicative of phagocytic cells and lipofuscin (*4, 5*). Increased cellular lipofuscin is often seen in tissues of aged individuals but does not necessarily signify decreased cellular function. The presence of distorted fibers and phagocytic cells indicates neuronal degeneration (*4*). There is a significant reduction in pituitary portal blood dopamine in old rats, and the reduction is greater in old rats with prolactinomas (*6*). These observations suggest that functional dopaminergic neurons are lost during aging and that this loss increases as animals develop spontaneous prolactinomas.

Damage to TD neurons also was found in young rats with prolactinomas produced by long-term estrogen treatment (*7*) (Table 1 and Fig. 1). Compared to effects in young control rats, this treatment resulted in significantly greater anterior pituitary weight and serum prolac-

Table 1. Median eminence (ME) dopamine, serum prolactin, and anterior pituitary weight in Sprague-Dawley and Wistar Furth rats with and without prolactin-secreting tumors. Dopamine and prolactin concentrations were measured by radioenzymatic assay (*19*) and by radioimmunoassay (*3*), respectively. Numbers of rats are given in parentheses; values are means ± standard errors. Overall significance ($P < .001$) was determined by analysis of variance: for ME data, $F(4, 27) = 9.69$; for prolactin data, $F(4, 68) = 9.08$; and for AP data, $F(4, 62) = 28.48$. Subsequent t-tests were used to compare individual groups (all differences significant at $P < .05$, except ME data of old and young Sprague-Dawley anterior pituitary).

Group	Tumor type	Dopamine in ME (ng/mg protein)	Serum prolactin (μg/ml)	Anterior pituitary weight (mg)
Young Sprague-Dawley and Wistar Furth	None	127.1 ± 21.9 (8)	0.054 ± 0.006 (17)	9.3 ± 0.3 (22)
Old Sprague-Dawley	None	54.5 ± 3.6 (6)	0.129 ± 0.012 (12)	12.1 ± 0.6 (10)
Old Sprague-Dawley	Anterior pituitary	37.2 ± 1.7 (6)	0.702 ± 0.078 (19)	40.9 ± 10.2 (11)
Young Sprague-Dawley	Anterior pituitary (estrogen-induced)	32.4 ± 4.3 (6)	1.375 ± 0.230 (14)	59.2 ± 4.0 (8)
Young Wistar Furth	MtT · W_{15}	67.8 ± 7.2 (6)	7.780 ± 2.904 (11)	6.5 ± 0.4 (16)

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