immediate, although probably temporary, increase of food intake. It remains to be determined whether other anorexigenic tumors induce diet aversions, although it seems unlikely that this effect would be unique to the PW-739 tumor. Further studies should also evaluate the extent to which learned food aversions contribute to the overall anorexic effect of tumor growth.

Our results are reminiscent of the work of Rozin and co-workers (*16*) showing that the anorexia and weight loss of rats with vitamin deficiencies are associated with a learned aversion to the deficient diet. Rats deficient in thiamine, for example, demonstrate an aversion to their thiamine-deficient diet by spillage and a strong preference for any new diet offered them. Rozin concluded that the anorexia characteristic of many vitamin deficiencies reflects, at least in part, a learned aversion to the deficient diet since the anorexia symptoms disappear when a new diet is offered.

Food aversion learning enables rats and many other species to learn to select needed nutrients (*16*) and avoid toxins (*17*). This mechanism allows organisms to associate the delayed internal effects of toxins and imbalanced nutrients with the taste of consumed foods and to adjust their intake and preferences accordingly. However, this mechanism may be triggered under inappropriate circumstances, as in the case of the tumor-bearing animal which associates its tumor-induced discomfort and illness with its food. Food aversion learning seems to play a role in the anorexia produced by certain tumors. Since food aversion learning occurs in humans in a variety of circumstances (*4, 18*), these findings may be of clinical importance. An understanding of the factors controlling the acquisition of these aversions may enable assessment of the degree to which they contribute to cancer anorexia and may suggest methods of preventing them.

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12. Differences between the two control groups in their preference for associated diets are evident. Two factors contributed: (i) baseline palatability of the two diets is different, with the AIN diet being preferred to NIH-07 because it is high in sucrose. A counterbalanced diet design does not require diets of equal palatability (*11*); (ii) we have found, using a variety of complete diets, that exposure to a diet tends to produce a small reduction in consumption of that diet (relative to a novel diet) during a 24-hour choice test. Preferences for associated diets are lower than preferences for the same diets when they are novel. This applies to 24-hour choice tests, which are apparently long enough to overcome initial neophobic effects. These effects are small relative to the effects of tumor growth on diet preference, and, since tumor-bearing animals were compared with appropriate controls, these differences do not detract from our demonstration of specific aversions as a consequence of tumor growth. Differences in diet palatability in studies 2 and 3 also affected absolute consumption by tumor-bearing animals during the test, with those offered the familiar, aversive diet and an unpalatable choice showing somewhat less recovery than those offered a more palatable choice. In all studies, tumor-bearing animals' food intake was not reliably lower than that of controls.
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15. In a separate study, tumor-bearing animals had continuous access to Purina monkey chow during the latter stages of tumor growth. When offered a choice between a novel diet (NIH-07) and the familiar diet they had eaten before the tumor implant (Purina rat chow), they preferred the familiar diet (85 percent). Normal, naïve rats given this choice consumed more NIH-07 (26 percent of diet consumed was Purina rat chow).
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The Testicular Feminized Rat: A Naturally Occurring Model of Androgen Independent Brain Masculinization

Abstract. *Although genotypically male (XY), the testicular feminized rat develops as an anatomic female because of an inherited deficiency in intracellular androgen receptors that prevents androgen imprinting of sexual primordia. However, the ability of testicular feminized rats to exhibit male-like sexual behavior and little feminine sexual behavior suggests that the brain can be masculinized without androgens.*

It has been generally concluded that the inherent program of sexual differentiation in both sexes of mammals is female. If androgens are present during sexual development then both genetic males and females will be organized for masculine reproductive organs (*1*), hepatic enzymes (*2*), hypothalamic control of gonadotropin secretion (tonic) (*3*), and sexual behavior (*4*), whereas absence of either gonad during the critical developmental period allows for the expression of the inborn female programs (*1–4*). Recently, it has been suggested that androgens per se are not necessarily required for masculine organization of the brain, and that it is estrogen that organizes the brain as male. According to the "conversion hypothesis," androgen secreted by neonatal males is converted to estrogen, and it is this metabolite that is active intracellularly (*5*). The administration of estrogen to neonatal animals can defeminize the brain (*4, 6*), and estrogen antagonists can block the masculinizing effects of neonatal androgens (*7*).

A naturally occurring model for studies of hormonal controls of sexual differentiation is the testicular feminized (Tfm) animal. Testicular feminization is a hereditary defect found in humans (*8*), cattle (*9*), mice (*10*), and rats (*11*) in

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which genetic males develop as phenotypic females. The absence of androgen-dependent masculine differentiation in the Tfm animal is due to an inherited insensitivity of the target organs to androgens; this insensitivity is reflected at the cellular level by a deficiency of androgen-binding protein (*10*). The Tfm animals have an XY karyotype, no Barr body (*10, 11*), and H-Y antigen (*12*). However, with the exception of very small underdeveloped inguinal testes that secrete subnormal amounts of testosterone, they have no male reproductive structures (*8–11*). Instead, they develop a vagina, clitoris, mammary glands and nipples (*8–11*), and a feminine pattern of hepatic steriod metabolism (*13*).

To test the "conversion hypothesis" that estrogens and not androgens masculinize the mammalian brain, we used androgen-insensitive Tfm rats. These animals have no deficiency in estrogen receptors (*14*) and thus are normally responsive to estrogens (*10*). We reported previously that such neurally controlled functions as body growth and gonadotropin secretion rates are not feminized in the Tfm rat. Body weight gain in Tfm animals is intermediate between that of males and females (*15*) and, like in the normal male, pituitary gonadotropin secretion is apparently tonic in the Tfm rat, whereas it is cyclic in the female (*16*). Furthermore, there is some evidence that the hypothalamus is defeminized in the Tfm human (*17*) and Tfm mouse (*18*). If estrogen in the neonatal animal, in the presence of severely reduced levels of androgen receptors, is sufficient for the masculinization of the brain, then the Tfm animal should exhibit masculine sexual behavior with little or no female sexual behavior. Indeed, we report here that sexual behavior centers in the brains of some Tfm rats are masculinized in the absence of androgen imprinting.

We obtained rats of the King × Holtzman strain (Stanley-Gumbreck pseudohermaphrodites). We used 12 adult Tfm males and five each of their normal male and female littermates. The animals were housed individually in constant temperature rooms (23°C) on a reverse light-dark cycle (14 hours of light and 10 hours of darkness).

All animals were tested for feminine and masculine sexual behavior both before and after they were gonadectomized. Four weeks after the operation one group of animals (chosen randomly) was injected daily with testosterone propionate (10 mg/day for 7 days) and 4 to six weeks later was given a single injection of estradiol benzoate (0.5 mg).

Table 1. Female sexual behavior of male, female, and Tfm rats. Testosterone was administered (10 mg/day) for 7 days; estradiol was administered as a single injection (0.5 mg). The lordosis quotient [mean ± standard error of the mean (S.E.M.)] is for the animals responding to the stimulus.

Sex	Number of animals responding (%)	Lordosis quotient
	Intact rats	
Male	0	0
Female	Not tested	
Tfm	17	13.7 ± 3.1
	Castrated rats injected with testosterone	
Male	0	0
Female	40	27.3 ± 10.6
Tfm	25	7.9 ± 4.5*
	Castrated rats injected with estradiol	
Male	40	15.3 ± 3.6
Female	100	77.6 ± 8.3†
Tfm	75	29.9 ± 8.3*

*Significantly different from females of the same treatment group ($P < .01$). †Significantly different from males of the same treatment group ($P < .02$).

For the second group of animals the treatment was reversed, with the estradiol benzoate being given 4 to 6 weeks before the testosterone propionate. Sexual behavior was tested 6 hours after the last testosterone injection and 48 hours after the single estradiol injection (*19*). All trials were run for 30 minutes under red illumination in an open-top rectangular wooden box with a fitted transparent plastic front.

To enhance the feminine sexual response, the test animals were stimulated by rectal probing and manual palpation of the flanks and perineum immediately preceding introduction to the test chamber containing a vigorous stud male (*20*). Feminine sexual behavior was measured by the lordosis quotient (LQ = number of lordoses × 100/number of copulation attempts). Lordosis was defined as full arching of the back with head and rump raised; copulating attempts included mounts with pelvic thrusts, intromissions with penile insertion and kick back with extensive genital grooming, and ejaculations with a long final thrust and slow dismount.

In testing for masculine sexual behavior all animals were subjected to peripheral electric shocks in an attempt to increase the rate of copulatory behavior (*21*). A stimulus female (in estrus) was presented 30 seconds before the first shock and the occurrence of intromissions and ejaculations were recorded. (The absence of a penis in the female and Tfm rats makes physical intromission impossible. Nevertheless, the other behavioral correlates listed above still may be elicited.) Significance was calculated by the unpaired Student's *t*-test.

As expected (*4, 22*), male and female rats generally exhibited their homotypical sexual behavior regardless of the hormonal milieu (Tables 1 and 2). The intact, untreated Tfm rats displayed no significant sexual behavior. Our finding that treatment with testosterone or estradiol induced only low levels of lordosis (LQ < 30 percent) indicates that the brain of the Tfm rat is defeminized (*23*). In contrast, 75 percent of the Tfm animals that were treated with estradiol displayed intromissive behavior and 42 percent showed ejaculatory responses.

The ability of the Tfm rat to respond to

Table 2. Male sexual behavior of male, female, and Tfm rats. Testosterone was administered (10 mg/day) for 7 days; estradiol was administered as a single injection (0.5 mg).

Sex	Intromissions		Ejaculations	
	Number of animals responding (%)	Mean ± S.E.M.*	Number of animals responding (%)	Mean ± S.E.M.*
		Intact rats		
Male	100	26.7 ± 2.5	100	2.5 ± 0.4
Female	40	7.0 ± 0.5†	0	0
Tfm	58	4.1 ± 2.0†	0	0
		Castrated rats injected with testosterone		
Male	100	35.2 ± 3.6	100	1.8 ± 0.5
Female	100	7.6 ± 3.6†	0	0
Tfm	75	10.9 ± 2.4†	17	2.0 ± 1.5
		Castrated rats injected with estradiol		
Male	100	24.4 ± 2.0	100	1.9 ± 0.1
Female	40	7.0 ± 2.0†	0	0
Tfm	75	15.7 ± 6.1	42	2.7 ± 1.5

*Mean ± S.E.M. of intromissions or ejaculations for animals responding within a 30-minute test period. †Significantly different from males of the same treatment group ($P < .01$).

the large pharmacological doses of testosterone administered here suggests that the animal may not be completely insensitive to androgens (*10, 24*). The Tfm rat will not exhibit masculine behavior when treated with physiological levels of testosterone (*22, 23*). However, our findings are consistent with reports that testosterone-activated sexual behavior is due to aromatization of the androgen to estrogen in the brain (*5, 25, 26*). Since the ejaculatory behavior of the Tfm rat was more responsive to a single dose of estradiol than to seven large doses of testosterone, it is possible that the testosterone-induced male behavior was mediated by estrogen metabolites of testosterone aromatization. In this context, it should be noted that the Tfm mouse has normal levels of brain aromatase (*27*).

The occurrence of at least a partially defeminized and masculinized brain in an otherwise phenotypically female Tfm animal suggests that perinatal androgens are not required to masculinize the developing brain. It seems reasonable to assume that the testes of the Tfm animals are the source of estrogens during the perinatal period as they are during adulthood (*15*), or that the testes of these animals are the source of testosterone (*10*) that is subsequently aromatized to estrogens (*27*) in the brain. The estrogens so formed then bind to neural cytosolic estrogen receptors (*14*) and masculinize the differentiating brain by directing genome readout.

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Asbestos as a Cofactor in Carcinogenesis Among Nickel-Processing Workers

Abstract. *It has been widely assumed, especially in the absence of other explanations, that lung cancer and nasal sinus cancers observed among nickel smelter workers are the result of the carcinogenicity of nickel. Although there may be such influence, supplementary hypotheses are also possible. The nickeliferous ores from at least one major smelter in New Caledonia (excess numbers of cancers have been found in these smelter workers) are derived from serpentinized host rocks which contain large amounts of chrysotile asbestos. Analysis indicates that nickel ores from this area are heavily contaminated by these fibers. The deposits are mined for their nickel content, but workers may be exposed to the asbestos fibers contained in the deposits. Hygiene measures limited to the avoidance of nickel may be inadequate under such circumstances and should be reevaluated so as to prevent the inhalation of asbestos-containing dusts.*

A recent report concerning the incidence of carcinoma of the lung among nickel workers of New Caledonia (*1*) attracted the attention of investigators in the field of metal carcinogenesis to the effects of nickel compounds. These effects have been well documented and reported for both worker populations and laboratory animals (*2*).

Uncertainties exist concerning the exposure of workers to nickel and the appearance of malignant disease (*3*). The distribution of respiratory tract cancer in some instances has not followed a clear pattern of exposure to nickel compounds but was more closely correlated to "total dust" with specific carcinogens not identified (*4*). It has been difficult to explain varying observations in different mining areas, in different smelters, and in a number of countries. There have been other curious inconsistencies about nickel carcinogenesis. Perhaps most important, the excess numbers of lung and nasal sinus cancers have been almost exclusively found among employees of nickel smelters, and the hazard did not follow the metal from its refining to metalworking plants. General improvements in housekeeping and dust control in smelters, made before the risk was even suspected, without control of specific agents or processes, were found to sharply reduce or eliminate later incidence of cancer (*5*). Despite these measures, since there clearly has been increased cancer risk in nickel smelters, nickel has been considered the cause.

In August 1976 we were asked by the International Metalworkers Federation (*6*) for advice concerning what might be done about an increasing burden of cancer among workers belonging to its affiliated union in New Caledonia, employed at the large nickel-mining and -smelting operation at Nouméa. Some of our past studies have alerted us to the importance of the large geological literature on the complex mineralogical nature of metal ore deposits—for example, the contamination of Lake Superior with amphibole gangue minerals (*7*) and the identification of chrysotile asbestos in crushed stone used as road surfacings in Maryland (the materials were derived from serpentine rock formations) (*8*).

In March 1979 Dr. Julian Lee of Sydney, Australia, visited us and provided clinical evidence in support of the increased incidence of lung cancer in workers in the New Caledonia smelter (*1*); patients from Nouméa, flown to Sydney for treatment, had been operated on. Moreover, he heard reports from surgeons on Nouméa that pleural mesothelioma had occurred among some employees of the facility. This observation reminded us of the inclusion of a case of mesothelioma in a nickel worker in the review by McDonald and McDonald of

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