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SEXUAL DIMORPHISM AND THE EFFECTS OF THE X-LINKED *Tfm* LOCUS ON HEXOBARBITONE METABOLISM AND ACTION IN MICE

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1 Normal males of the testicular feminized strain of mice (*Tfm*) had longer hexobarbitone-induced sleeping times than females, and hepatic hexobarbitone hydroxylase activity differed in that the K_m was higher and the V_{max} lower in the male.

2 Castration and androgen replacement studies indicated that testicular androgens were responsible for the sexual differences in drug metabolism found in this mouse strain.

3 Hepatic hexobarbitone metabolism and action were feminized in the intact, androgen-insensitive, genetically male *Tfm* mouse. Furthermore, hexobarbitone hydroxylase activities were less responsive to large doses of testosterone in *Tfm* mice than in normal males.

4 The *Tfm* mouse with a deficiency in androgen receptors responded to the enzyme-inductive effects of phenobarbitone and softwood bedding, indicating that these inducers do not act through the androgen receptors.

Introduction

A considerable body of evidence has accumulated indicating that there are sex differences in the metabolism and action of many xenobiotics in intact rats. Furthermore, studies have shown that the sexual dimorphism in microsomal enzyme activities are in part under the control of testicular hormones (Conney, 1967; Gillette, 1971; Kato, 1974). These sex differences are not present in sexually immature rats and have an age-dependent pattern of development, are abolished by orchidectomy, are reversed by antagonistic hormones (i.e., oestrogens), or are re-established by androgen replacement therapy following castration. Sexual differences in drug metabolism also have been described in the mouse (Vesell, 1968; Noordhoek, 1972; Brown, Bardin & Greene, 1978a). However, depending upon the strain, the sexual dimorphism in hepatic drug metabolism could be reversed. BALB/cJ males exhibit greater hepatic ethylmorphine *N*-demethylase activity than do females, while Crl:CD-1 females have the greater enzyme activity (Brown *et al.*, 1978a). Furthermore, there are a number of mouse strains that do not exhibit sex differences in hepatic hexobarbitone hydroxylase (Vesell, 1968; Noordhoek, 1972) and ethylmorphine *N*-demethylase (Brown *et al.*, 1978a) which is in contrast to their sexual dimorphism in cytochrome P450 levels.

Studies of sexual dimorphism in drug metabolism have shown that androgen is the agent responsible for producing the sex differences seen in the rat (Conney, 1967; Kato, 1974) and, probably, in the mouse (Noordhoek, 1972; Brown *et al.*, 1978a). Interestingly, depending upon the species and strain, testosterone will either induce or repress those sexually responsive components of the hepatic microsomal mono-oxygenase system (Noordhoek, 1972; Kato, 1974). How the same agent, i.e., testosterone, can produce the opposite effects on drug metabolism in rats and mice is unknown. A better understanding of the mechanism of action of androgen on the mono-oxygenase system might explain how testosterone could function as both a repressor and inducer. In this regard, the genetically male *Tfm* (testicular feminization) rodent develops as a phenotypic female because of an androgen insensitivity caused by an inherited insufficiency of androgen receptors in its target cells (Bardin, Bullock, Sherins, Mowscowicz & Blackburn, 1973). As it is believed that the actions of testosterone on hepatic microsomal mono-oxygenases are mediated by an androgen receptor similar to that in reproductive tissues (Bullock, Bardin, Gram, Schroeder & Gillette, 1971; Brown, Greene & Bardin, 1976), the *Tfm* animal becomes a useful model in which to study the mechanism of androgen action on drug metabolism. Unlike its normal littermates, the *Tfm* rat is not responsive to the inductive effects of testosterone on hepatic ethylmorphine *N*-demethylase (Bullock *et al.*, 1971), while in the *Tfm* mouse the androgen does not repress the enzyme as it does in the mouse littermates (Brown, Greene, Bullock & Bardin, 1978b). Yet, both the mouse and rat *Tfm* respond to the inductive effects of

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phenobarbitone (Brown *et al.*, 1978b; Sonawane, Yaffe & Shapiro, 1979). Thus, it would appear that the inductive and repressive actions of androgen on mono-oxygenases are mediated by an intracellular androgen receptor and that the receptor is not required for phenobarbitone-induction. In this paper we have studied the effects of the X-linked *Tfm* locus and the resulting deficiency in androgen receptors on hexobarbitone metabolism and action in the mouse.

Methods

Animals

Adult *Tfm* mice and their normal male and female littermates of similar age from our breeding stock were used in the experiments. Our mice originated from 12 breeding pairs generously supplied by Dr Leslie Bullock of the Milton S. Hershey Medical Center, Hershey, Pennsylvania, who in turn obtained her breeding pairs from the colony of Dr Susumo Ohno of the City of Hope Medical Center, Duarte, California. Mice housed on hardwood bedding in plastic cages were given water and commercial mouse diet *ad libitum* and were kept in air conditioned quarters, 20–23°C, with a photoperiod of 12 h light: 12 h dark.

Treatment

In the 'castration' experiment, 7 to 8 week old mice were bilaterally gonadectomized. The mice were anaesthetized by an injection of Chloropent (chloral hydrate, 162 mg/kg and pentobarbitone, 34 mg/kg) and a dorsal incision was made on both sides of the backbone. The isthmus of each fallopian tube was ligated with absorbable suture and severed through the ampulla, enabling the ovaries to be removed. Control animals of similar age were 'sham' castrated by surgically lifting the gonads from the body cavity and replacing them without interrupting blood flow. Animals were allowed to rest for 3 to 4 weeks before beginning further treatment. Testosterone was injected (s.c.) at 100 mg/kg in ethanol/corn oil, 1:4(v/v) in the late afternoon for 14 consecutive days. Hexobarbitone-induced sleeping studies were performed on the morning of day 10 and the animals were killed on the morning of day 15. In some mice, sodium phenobarbitone was injected (i.p.) at 50 mg/kg in 0.9% w/v NaCl solution (saline) in the late afternoon for 9 consecutive days. Hexobarbitone-induced sleeping studies were performed on the morning of day 5 and the mice were killed on the morning of day 10. Control animals received either the oil or saline diluent for the time periods specified above. (As there were no differences between the oil and saline-treated controls, data from these two groups were pooled.)

In the 'bedding' experiment all animals were born and maintained on hardwood bedding ('Beta-Chip', Northern Products Corp., Warrensburg, New York 12885, U.S.A.) until 3 months of age when half of the animals were put on white pine (softwood) bedding (Buckshire Feeds Ltd., Perkasie, Pennsylvania 18944, U.S.A.) for an additional 2 months. The remaining animals continued on the hardwood bedding. Hexobarbitone-induced sleeping studies were performed 10 to 12 days before the animals were killed.

Sleeping times

Sleeping times were measured from an intraperitoneal injection of hexobarbitone (125 mg/kg) to the restoration of the righting response, defined as the capacity of the animal when placed on its back on a flat surface to turn over on its paws 3 times in 15 s (Vesell, 1968).

Microsome preparation

Thirty to 60 s after decapitation and exsanguination, livers were perfused *in situ* through the posterior vena cava with 20 ml of ice-cold saline. Next, the livers were excised and placed in ice-cold saline. All subsequent steps were carried out at 0 to 4°C. Each liver was homogenized with 7 ml of ice-cold 0.1 M potassium phosphate buffer, pH 7.4, in a Teflon-glass tissue homogenizer with the aid of a motor driven pestle. The homogenate was centrifuged for 20 min at 10,000 *g* and the resulting supernatant was centrifuged for 60 min at 100,000 *g*. The resulting microsomal pellet was suspended in 2.8 ml of 0.1 M potassium phosphate buffer. The microsomal suspension was stored at −70°C and assayed for hexobarbitone hydroxylase the next morning.

Substrate purification

Within one day before assaying the microsomes, the radioactive hexobarbitone was purified on silica gel t.l.c. according to the procedure of Dr Barry Dvorchik (personal communications). [^{14}C]-hexobarbitone was applied to a pre-washed (methanol/acetone, 1:1, v/v) t.l.c. plate and developed in chloroform/acetone, 9:1 (v/v). The purified [^{14}C]-hexobarbitone was extracted twice with 2 ml of methanol and concentrated under nitrogen gas to 50,000 d/min per 20 μl methanol.

Hexobarbitone hydroxylase

Hepatic microsomal hexobarbitone hydroxylase was assayed by a modification of the procedure of Kupfer & Rosenfeld (1973) and Marietta, Vesell, Hartman,

Weisz & Dvorchik (1979). The radioactive hexobarbitone (50,000 d/min) was dried at the bottom of each incubation vessel. A 1.0 ml incubation mixture contained microsomes (1 to 2 mg of protein), potassium phosphate buffer (pH 7.4, 0.1M), $MgCl_2$ (10 mM), glucose-6-phosphate (12 mM), NADP (0.4 mM), glucose-6-phosphate dehydrogenase (1 I.E.U.) and hexobarbitone (0.67 mM). The microsomes were added last to initiate the reaction. Linear kinetic data for hexobarbitone hydroxylase were obtained with 5 different hexobarbitone concentrations (0.03 to 0.7 mM) and a microsomal suspension (0.5 to 4 mg microsomal protein/incubation) prepared from the livers of individual mice. The microsomes were incubated in a shaking water bath (37°C) in air for 10 min. The reaction was terminated by the addition of 0.2 ml of 2.5 M perchloric acid. Blanks containing hexobarbitone, the NADPH generating system and perchloric acid also were incubated. Next, 0.8 ml of chilled 1 M citrate-15% sodium chloride buffer, pH 5.5, and 3.5 ml of 1-chlorobutane were added to the incubation vessels with quick swirling. Vessel contents were immediately transferred to test tubes with Teflon screw caps. After vigorous shaking for 15 s, the test tubes were centrifuged for 5 min at 2000 *g*. The hexobarbitone containing organic (upper) phase was aspirated and discarded, and the aqueous phase containing the hexobarbitone metabolites was extracted twice more with 1-chlorobutane. Aliquots of the aqueous phase were mixed with Econo-Verse and monitored for ^{14}C disintegrations in a liquid scintillation counter. Sufficient counts were accumulated to achieve a standard deviation of $<0.5\%$. Michaelis constants and maximal velocities were determined from linear regression models of the data using the method of Lineweaver & Burk (1934). The correlation coefficients for all Lineweaver & Burk plots were positive, exceeded 0.95, and were found to be statistically significant.

Protein

Microsomal protein content was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) and bovine serum albumin was used as the standard.

Statistics

Groups were compared for statistically significant differences by two-way analysis of variance and *t* test.

Chemicals

Hexobarbitone, glucose-6-phosphate, NADP, glucose-6-phosphate dehydrogenase and testosterone were purchased from Sigma Chemical Company (St. Louis, Missouri, U.S.A.). Econo-Verse and 1-chlorobutane (HPLC grade) were from Fisher Scientific Company (Fairlawn, New Jersey, U.S.A.). Radioactive hexobarbitone (cyclohexenyl-3,5-dimethylbarbituric acid, 5-[2-^{14}C]), 8.58 mCi/mmol, was obtained from New England Nuclear (Boston, Massachusetts, U.S.A.). Sodium phenobarbitone and Chloropent were purchased from J.T. Baker (Phillipsburg, New Jersey, U.S.A.) and Fort Dodge Laboratories (Fort Dodge, Iowa, U.S.A.), respectively. All other reagents were of analytical grade.

Results

Effects of castration, testosterone and phenobarbitone on sleeping times

The data clearly demonstrate a sexual dimorphism in hexobarbitone-induced sleeping times in the intact mice (Table 1). In response to the same dose of hexobarbitone, males slept 32% longer than females. The genetically male *Tfm* mice exhibited the female sleep patterns; sleeping a significantly shorter time than males.

Bilateral gonadectomy abolished the sexual dimorphism in hexobarbitone-induced sleeping times. Sleeping times in orchidectomized males were reduced to that of females, which were unchanged following ovariectomy. Removal of the inguinal testes in the *Tfm* mice had no effect on sleeping times, which remained similar to those of females.

Table 1 Hexobarbitone-induced sleeping times (min) of male, female and *Tfm* mice

	Sham castrate	*Castrate*	*Castrate + testosterone*	*Castrate + phenobarbitone*
Male	62.0 ± 7.2†	43.6 ± 5.8	76.0 ± 3.1†	20.6 ± 4.8†
Female	42.2 ± 4.1*	40.2 ± 6.9	72.2 ± 6.6†	17.0 ± 1.9†
Tfm	40.4 ± 8.8*	42.6 ± 5.8	60.8 ± 7.6*†	17.7 ± 5.1†

Results are expressed as means ± s.d. of 10 mice per group. Mice were treated daily with either diluent, testosterone (100 mg/kg) or sodium phenobarbitone (50 mg/kg) and put to sleep with hexobarbitone (125 mg/kg).
* $P<0.001$: different from males of similar treatment.
† $P<0.001$; different from castrates of the same sex.

Administration of testosterone to castrated mice did not initiate a reappearance of sexual dimorphic sleeping times. Instead, males and females injected with testosterone had sleeping times similar to those found in intact males. While androgen treatment of castrated *Tfm* mice produced a significant elevation in sleeping times when compared to intact or castrated *Tfm* mice, the magnitude of response was smaller than that found in males ($P<0.001$) and females ($P<0.01$) treated with testosterone.

Phenobarbitone treatment resulted in a dramatic decline in hexobarbitone-induced sleeping times in all castrated mice. In fact, mice injected with phenobarbitone slept only 50% as long as intact females and *Tfm* mice.

Effects of castration, testosterone and phenobarbitone on hepatic microsomal protein levels

There was a small (20%) but statistically significant sexual difference in the concentration of hepatic microsomal protein in intact male and female mice (Table 2). The concentration of hepatic microsomal protein found in intact *Tfm* mice was similar to that found in intact males, but significantly greater ($P<0.01$) than that found in intact females.

Following castration, males, females and *Tfm* mice had similar levels of hepatic microsomal protein. The administration of testosterone caused an elevation in the concentration of hepatic microsomal protein in castrated males and females. Although treatment of castrated *Tfm* mice with testosterone did not result in a significantly higher level of microsomal protein when compared to untreated castrated *Tfm* animals, the levels were higher ($P<0.001$) than those found in intact *Tfm* mice. Administration of phenobarbitone produced a 55–60% increase in the concentration of microsomal protein in all castrated groups.

Effects of castration, testosterone and phenobarbitone on hepatic hexobarbitone hydroxylase

Activities of hepatic hexobarbitone hydroxylase reflected the sexual dimorphism found in sleeping times for intact males and females (Table 1). Intact males had significantly lower levels of the enzyme than did intact females (Table 3). The genetically male *Tfm* mice exhibited levels similar to females.

Again, as reflected in hexobarbitone-induced sleeping times, castration abolished the sexual differences in hexobarbitone hydroxylase activities. Following orchidectomy, the enzyme levels in males were elevated to female levels which were unchanged by ovariectomy. Removal of the testes in the *Tfm* mouse had no effect on the activity of hexobarbitone hydroxylase which remained feminine.

Administration of testosterone to castrated mice did not result in a reappearance of sexually dimorphic enzyme activities. Instead, males and females injected with testosterone had the reduced levels of hexobarbitone hydroxylase found in intact males. While androgen treatment of castrated *Tfm* mice did not produce a statistically significant reduction of the activity of hexobarbitone hydroxylase when compared to untreated *Tfm* castrates, the enzyme level was significantly ($P<0.01$) lower when compared to intact *Tfm* mice. Administration of phenobarbitone resulted in a 5 fold increase in hepatic hexobarbitone hydroxylase activity in castrated males and an 8 fold increase in castrated females and *Tfm* mice.

Effects of bedding on male, female and Tfm *mice*

In agreement with Table 1, on hardwood bedding males slept longer than females when injected with an equal dose of hexobarbitone (Table 4). *Tfm* mice slept as long as females. Males continued to sleep longer than females or *Tfm* mice when the animals were on softwood bedding. However, female and *Tfm* mice on softwood bedding had shorter sleeping times than when maintained on hardwood bedding. The type of bedding had no effect on sleeping times in males. The sexual dimorphism in hexobarbitone-induced sleeping times was reflected in the differences in activities of hepatic microsomal hexobarbitone hydroxylase. On hardwood bedding the V_{max}

Table 2 Microsomal protein concentrations (mg/g liver) of male, female and *Tfm* mice

	Sham castrate	*Castrate*	*Castrate + testosterone*	*Castrate + phenobarbitone*
Male	17.5 ± 2.8	16.1 ± 2.1	23.8 ± 4.0†	27.0 ± 4.4†
Female	14.1 ± 1.8*	16.3 ± 3.4	27.1 ± 4.1†	29.8 ± 4.5†
Tfm	16.5 ± 1.2	18.1 ± 3.0	20.7 ± 2.9	33.6 ± 5.8†

Results are expressed as means ± s.d. of 10 mice per group. Mice were treated daily with either diluent, testosterone (100 mg/kg) or sodium phenobarbitone (50 mg/kg).
* $P<0.005$; different from males of similar treatment.
† $P<0.005$; different from castrates of the same sex.

Table 3 Hexobarbitone hydroxylase (nmol min^{-1} mg^{-1} protein) in livers from male, female and *Tfm* mice

	Sham castrate	*Castrate*	*Castrate + testosterone*	*Castrate + phenobarbitone*
Male	1.9±0.2†	2.4±0.1	1.6±0.1†	11.6±1.5†
Female	2.6±0.4*	2.3±0.3	1.6±0.3†	18.1±5.3*†
Tfm	2.5±0.3*	2.4±0.4	2.1±0.3*	18.6±3.1*†

Results are expressed as means ± s.d. of 10 mice per group. Mice were treated daily with either diluent, testosterone (100 mg/kg) or sodium phenobarbitone (50 mg/kg).
* $P < 0.001$; different from males of similar treatment.
† $P < 0.001$; different from castrates of the same sex.

of the enzyme was 40% greater in females than in males, while the K_m of the enzyme was significantly greater in the males. The data indicate that the *Tfm* mice had enzyme kinetics similar to females. When placed on softwood bedding, there was a significant increase in the V_{max} and K_m for hexobarbitone hydroxylase in females and *Tfm* mice. Consistent with the sleeping time results, softwood bedding had no effect on enzyme kinetics in the males.

Discussion

Sexual dimorphism

Unlike the rat where there is a 200 to 400% difference in the activities of drug metabolizing enzymes between the sexes (Kato, 1974; Sonawane *et al.*, 1979), we have found, in agreement with others (Noordhoek, 1972; Brown *et al.*, 1978a), that there is only a 20 to 40% sex difference in mice. While these comparisons indicate that studies of sexual dimorphism in mice may require more animals in an experiment and more exact analytical and statistical evaluations than with rats, it should not be concluded that the mouse model is less useful than the rat. On the contrary, the mouse model may be more representative of the vast majority of outbred species in which few or no statistically significant sex differences have been found in drug metabolism (Holck, Munir, Mills & Smith, 1937; Quinn, Axelrod & Brodie, 1958; Kato, 1974). Furthermore, we have found that these 20 to 40% sex differences in *in vitro* drug metabolism in the mouse are pharmacologically significant as they reflect a similar magnitude of change in the biological measure of hexobarbitone-induced sleeping time.

Sexual dimorphism in hepatic drug metabolism in mice has been shown to be strain-dependent (Vesell, 1968; Noordhoek, 1972; Brown *et al.*, 1978a). In some strains of mice (e.g., CPB-SE, Crl:CD-1 and AL/N) the females have greater drug metabolizing enzyme activities than males, while in other strains (e.g., BALB/cJ and DBA/2J) the males have greater enzyme activity and in some strains (C3H/HeJ, C57BL/10J, CFW and CPB-FT) no sex differences have been found. Ethylmorphine *N*-demethylase activity has been reported (Brown *et al.*, 1978b) to be greater in normal females of the *Tfm* strain than in the males. However, castration had no effect on the enzyme levels in either sex, while testosterone administration reduced hepatic ethylmorphine *N*-demethylase activity in both sexes. In addition, there were no sex differences in the concentration of hepatic microsomal protein: castration had no effect, and treatment with testosterone increased microsomal

Table 4 Hexobarbitone-induced sleeping times and hepatic hexobarbitone hydroxylase of male, female and *Tfm* mice housed on hardwood or softwood bedding

	Sleeping time (min)		*Hexobarbitone hydroxylase* V_{max} *(nmol* min^{-1} mg^{-1} *protein)*		*Hexobarbitone hydroxylase* K_m *(*$\times 10^{-4}$ *M)*	
	Hardwood	*Softwood*	*Hardwood*	*Softwood*	*Hardwood*	*Softwood*
Male	61.7±10.7	62.0±9.8	1.9±0.5	2.2±0.5	1.60±0.18	1.48±0.15
Female	52.0± 6.2*	41.0±5.3*†	3.0±0.5*	5.2±0.9*†	1.19±0.19*	1.51±0.09†
Tfm	47.4± 4.5*	30.2±3.7*†	3.3±0.7*	5.8±0.8*†	1.18±0.07*	1.42±0.11†

Results are expressed as means ± s.d. of 10 mice per group.
* $P < 0.01$; different from males on same bedding.
† $P < 0.001$; different from hardwood of the same sex.

protein concentration in both sexes. We have found a similar sexual dimorphism in hepatic hexobarbitone hydroxylase in the *Tfm* mouse strain. Testosterone administration reduced the activity of the enzyme in both sexes and as might be expected from the repressive effects of androgens, orchidectomy resulted in an elevation of hepatic hexobarbitone hydroxylase activity to female levels. The changes in hexobarbitone-induced sleeping times did reflect the activities of hepatic hexobarbitone hydroxylase. Although we found sex difference in the concentration of hepatic microsomal protein in the *Tfm* strain, castration had no statistical effect but treatment with androgen did increase the protein concentration in both sexes. Thus, like the Crl:CD-1 and CPB-SE mice, normal male mice of the *Tfm* strain have lower activities of hepatic mono-oxygenase enzymes than females. Our results with the *Tfm* strain and those with CPB-SE mice (Noordhoek, 1972) indicate that the sexual dimorphism in drug metabolizing enzymes in these mice is due to the repressive effects of gonadal androgens in the male.

Tfm *mice*

Studies using the *Tfm* mouse (Brown *et al.*, 1978b) have shown that androgen-induced changes in ethylmorphine *N*-demethylase activity require a functional androgen receptor in the liver but that androgen stimulates hepatic microsomal protein content by a mechanism independent of the androgen receptor. In agreement with these findings, we have found that in the *Tfm* mouse the V_{max} and K_m for hexobarbitone hydroxylase were similar to those of the female, but significantly different from those of the male. Hepatic microsomal protein concentration was similar in the *Tfm* and male and androgen administration increased microsomal protein in castrated *Tfm* mice when compared to the intact *Tfm*. Thus, the deficiency in androgen receptors in the *Tfm* mouse prevents the animal's endogenous androgens (Bardin *et al.*, 1973) from masculinizing (depressing) the mono-oxygenase enzymes but does not prevent androgen stimulation of hepatic microsomal protein.

Studies using *Tfm* rats (Bullock *et al.*, 1971) and *Tfm* mice (Brown *et al.*, 1978b) have indicated that neither endogenous nor exogenous androgens are capable of affecting the activity of hepatic ethylmorphine *N*-demethylase. In contrast, we have found that drug metabolism and action in the mouse can be affected by treatment with androgen. Testosterone prolonged hexobarbitone-induced sleeping times and reduced the activities of hexobarbitone hydroxylase. Although we have found that the *Tfm* mouse responds to treatment with testosterone, the magnitude of response was significantly greater in androgen-treated males and females. Furthermore, the depressed levels of hexobarbitone hydroxylase found in the castrated *Tfm* mouse treated with androgen was only statistically significant when compared to intact *Tfm* mice and not castrates. It is possible that in the *Tfm* mouse the activities of hexobarbitone hydroxylase are more sensitive than ethylmorphine *N*-demethylase to androgens, although this is not the case in CPB-SE mice (Noordhoek, 1972). Perhaps, we were able to produce an androgenic response in the *Tfm* mouse because we administered testosterone for 14 days as compared to 6 days by Brown *et al.*, (1978b). It has been reported (Fouts, 1971) that two to four weeks of treatment with anabolic steriods seems to be required to produce maximal stimulation of hepatic microsomal enzymes in some rodents. In this regard, the activity of hepatic microsomal ethylmorphine demethylation was increased in *Tfm* rats treated with testosterone for 33 days (Bullock *et al.*, 1971). Like the *Tfm* rat (Naess, Haug, Attramadal, Aakvaag, Hansson & French, 1976), the *Tfm* mouse has some cytosolic androgen receptors (Attardi & Ohno, 1978), and like the *Tfm* rat (Bardin *et al.*, 1973; Goldman & Klingele, 1974), the *Tfm* mouse (Schenkein, Levy, Bueker & Wilson, 1974), may be responsive to the long treatment period with large doses of androgen administered by us. Finally, it is possible that a sufficient amount of the injected androgens were aromatized peripherally to oestrogens (Baird, Horton, Longcope & Tait, 1969). As oestrogens and androgens have similar effects on ethylmorphine *N*-demethylase in the *Tfm* strain of mice (Brown *et al.*, 1978b), our androgenic response might actually have been an oestrogenic response. Nevertheless, our results do agree with the conclusion that the cytosolic androgen receptor is required to mediate the normal response of mono-oxygenases to testosterone. The ability of the *Tfm* mouse to respond like normal males and females to the enzyme-inductive effects of phenobarbitone and softwood bedding indicates that these inducers do not act through the androgen receptor.

Bedding effects

It has been previously reported that the type of bedding upon which mice are maintained can alter sleeping times and that the effects are reversible (Ferguson, 1966; Vesell, 1968; Fujii, Jaffe & Epstein, 1968). Keeping mice on softwood bedding shortened sleeping time and elevated hepatic hexobarbitone hydroxylase activity without changing the brain level of hexobarbitone on awakening. Induction by softwood bedding of aniline hydroxylase and morphine *N*-demethylase in hepatic microsomes of rats and mice was also reported (Vesell, 1968). We have found that when females of the *Tfm* strain were transferred from hardwood to softwood bedding

there was a significant decrease in hexobarbitone-induced sleeping time with a concomitant increase in the V_{max} and K_m of hepatic hexobarbitone hydroxylase. Males, however, were unaffected by the type of bedding. In previous studies using only male mice, the effects of softwood bedding on drug metabolism and action were dramatic (Ferguson, 1966; Vesell, 1968). Although we cannot explain these contradictory findings, there are major differences between our study and previous ones (Ferguson, 1966; Vesell, 1968; Fujii *et al.*, 1968). Firstly, each investigation used a different strain of mice. Secondly, we used white pine for our softwood bedding while the other researchers used red cedar. Thirdly, in our investigation, drug metabolism and action was studied in mice that were housed on softwood bedding for 2 months, while in previous studies, mice were kept on softwood bedding for only 1 to 7 days.

The inability of softwood to decrease sleeping time and increase hexobarbitone hydroxylase in the males might be due to the repressive effects of endogenous androgens on drug metabolism. This possibility is strengthened by the fact that the androgen-insensitive, genetically male *Tfm* mouse responds to the inductive effects of softwood like a female.

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