

# Chronic Ethanol Ingestion During Puberty Alters the Transient Increase in Testicular $5\alpha$ -Reductase in the Swiss-Webster Mouse

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Previous experiments with inbred mice showed that chronic ethanol treatment delays male pubertal development. An initial event in sexual maturation in the rat is a transient increase in  $5\alpha$ -reductase. The present study was conducted to determine whether similar ethanol effects occur in outbred mice (Swiss-Webster), to determine the ontological profile of testicular  $5\alpha$ -reductase in the mouse, and to evaluate the effect of ethanol treatment on this enzyme. After 29 days of treatment with a liquid diet (beginning at age 20 days), reductions in the ethanol-treated mice as compared with the controls were noted in testicular weight ( $55.0 \pm 2.0$  vs.  $63.0 \pm 2.4$  mg;  $P < 0.01$ ), epididymal sperm content ( $6.8 \times 10^5$  vs.  $14.4 \times 10^5$ ;  $P < 0.05$ ), and sperm motility (45% vs. 57%;  $P < 0.05$ ). After 43 days, differences no longer existed. In chow-fed mice, a substantial rise in  $5\alpha$ -reductase (1 unit = 1 pmole DHT formed/45 min/mg testis) began at age 24 days. Activity peaked at approximately 65 units at 25 to 30 days and gradually declined to  $6.4 \pm 0.8$  units at 63 days. After 29 days treatment,  $5\alpha$ -reductase of the pair-fed control group was  $26.8 \pm 4.9$  units, which decreased to a baseline value of  $7.0 \pm 2.1$  units after 43 days treatment. In contrast,  $5\alpha$ -reductase of the ethanol-treated group remained at baseline levels after 29 days ( $7.7 \pm 2.3$  units) and 43 days of treatment ( $7.6 \pm 2.3$  units). These data show that chronic ethanol treatment delays male pubertal develop-

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ment in outbred mice, that a transient pubertal increase in  $5\alpha$ -reductase occurs in the mouse, and that ethanol treatment may prevent the puberty-associated rise in  $5\alpha$ -reductase. Prevention of the increase in  $5\alpha$ -reductase may represent at least one mechanism for ethanol-induced delayed sexual maturation.

**Key words:** chronic ethanol ingestion, delayed puberty, pubertal development, testicular  $5\alpha$ -reductase.

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The adverse effects of chronic ethanol ingestion on reproductive function in adult males is well-documented, in both clinical and laboratory animal studies (for reviews, see Van Thiel, 1983; Anderson et al, 1983a; Bannister and Lowosky, 1987). Very little, however, is known regarding the sensitivity of the reproductive tract to insult by ethanol during various periods of development (eg, puberty). Identification of risk factors related to subsequent fertility that are associated with alcohol abuse during puberty would be of significance, in view of the relatively high inci-

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dence of young adolescents who are classified as problem drinkers (Jessor and Jessor, 1975; NIAAA, 1984; Jessor, 1985). A recent clinical investigation has suggested that a reproductive endocrine imbalance is associated with adolescent drug and alcohol abuse (Diamond et al, 1986).

Recent studies by our laboratory (Anderson et al, 1987b) indicated that pubertal development of male C57Bl/6J mice is delayed subsequent to chronic ingestion of a liquid diet containing 5% (v/v) ethanol, beginning at age 20 days. This conclusion was based upon the observation of impairment of several indices of reproductive function following 29 days of treatment, while after a 43-day treatment with the same diet, measurements of reproductive function approached control values. Parameters measured in these studies included reproductive organ weights, testicular morphology, epididymal sperm content, morphology and motility of spermatozoa, and the ability of epididymal spermatozoa to fertilize mouse oocytes *in vitro*. These data were supported by earlier observations by Ramaley (1982), who observed delayed balanopreputial separation in rats given ethanol during puberty. However, it remains to be established whether an ethanol-induced delay in reproductive tract function is specific to the highly inbred C57Bl/6J mouse, or is a more generalized phenomenon.

An early biochemical change associated with pubertal development in the rat and the hamster (Tsuji et al, 1984) is a transient increase in the level of testicular 5 $\alpha$ -reductase (EC 1.3.1.22). Increased circulating levels of dihydrotestosterone (DHT) consequent to increases in the activities of this enzyme may participate in a cascade of events during male puberty that ultimately result in a sexually mature hypothalamic-pituitary-gonadal axis (Nazian and Mahesh, 1980). Decreased levels of the hepatic form of this enzyme in baboons have been reported following chronic ethanol exposure (Gordon et al, 1979). However, it is not known whether a change in testicular 5 $\alpha$ -reductase occurs in the mouse, and, if so, to what extent such a change may be affected by chronic ethanol treatment during pubertal development.

It was therefore the purpose of the present study 1) to confirm previous observations of ethanol-induced delayed sexual maturation using an outbred strain of mice (Swiss-Webster) to minimize possible genetic bias of the results; 2) to follow the ontogeny of testicular 5 $\alpha$ -reductase during pubertal development in the Swiss-Webster mouse; and 3) to determine the effects of ethanol treatment during pubertal

development on the level of this enzyme in the testis. The results obtained with this outbred strain of mouse provide additional evidence that chronic ethanol ingestion delays male sexual maturation, and show that ethanol treatment reduces the level of testicular 5 $\alpha$ -reductase as compared with values obtained in age-matched, pair-fed control mice.

## Materials and Methods

### Materials

Nicotinamide adenine dinucleotide phosphate, reduced form (NADPH), DHT, and testosterone (T) were products of Sigma Chemical Company (St. Louis, MO). Radiolabeled T ([1 $\beta$ , 2 $\beta$ -<sup>3</sup>H(N)], 49 Ci/mmol) was purchased from NEN Research Products (Boston, MA). Ethanol (95%, v/v) was obtained from the University of Illinois Health Sciences Center central supply facility, and chocolate-flavored Carnation Slender™ was purchased from a local grocer. All other chemicals were at least of reagent grade quality.

### Animals

All mice used in this study were born and raised at the central animal care facility at the University of Illinois Health Sciences Center, and were derived from Swiss-Webster (CFW) breeding pairs obtained from Charles-River Laboratories (Wilmington, MA). At age 18 days, all animals were weaned and individually housed. Animals were maintained on a 14 hour/10 hour light/dark cycle (lights on at 0700) at an ambient temperature of 22  $\pm$  1 C. Lab chow and water was provided *ad libitum* until chronic ethanol treatment was initiated. Body weights, tail lengths, and testicular 5 $\alpha$ -reductase were measured in individually housed, chow-fed animals at several ages from 20 to 63 days for purpose of comparison.

### Measurement of Testicular 5 $\alpha$ -reductase

Measurement of testicular 5 $\alpha$ -reductase was based on the method of Altman et al (1972). Animals were killed by cervical dislocation and each testis was removed, decapsulated and weighed. This tissue was homogenized (8.9%, w/v) in assay buffer consisting of 50 mM sucrose, 0.2 mM [<sup>3</sup>H]T (30  $\mu$ Ci/ $\mu$ mole) and 60 mM potassium acetate, pH 5.6. The homogenate was divided into two equal portions and placed in a 37 C water bath for 90 seconds prior to initiating the reaction by addition of 1 mM NADPH (final concentration) to one portion. The other portion served as a blank, and received an equal volume of water rather than NADPH. Incubations were carried out for 45 minutes at 37 C, after which time reactions were terminated and product (DHT) was extracted by the addition of 0.5 ml of 0.5 M potassium acetate (pH 5.6) and 2 ml methylene chloride, followed by vortexing for 1 minute. A small aliquot of the organic phase was taken to determine the recovery of radiolabeled steroids. One milliliter of the methylene chloride was transferred to a clean tube and evaporated to dryness (60 C). The residue was dissolved in 0.1 ml chloroform: ethyl acetate (80:20). A portion of this

material (10  $\mu$ l) was applied to a silica gel G plate (Whatman, Linear k; thickness = 250  $\mu$ m). Plates were developed with chloroform:ethyl acetate (80:20) at ambient temperature. Authentic T and DHT standards were chromatographed on adjacent lanes. These steroids were visualized by spraying with 5% H<sub>2</sub>SO<sub>4</sub> in methanol followed by acetic anhydride. Incubation of the plates at 120 C for 2 minutes resulted in blue-green color development for T and reddish brown for DHT. Areas in lanes containing extracts from incubations corresponding to the migration of T ( $R_f = 0.32 \pm 0.02$  (S.D.; N = 5) and DHT ( $R_f = 0.42 \pm 0.01$ ; N = 5) were scraped into scintillation vials and their radioactivity was quantitated by liquid scintillation spectrometry. Under these conditions, extraction of T and DHT was quantitative. Formation of product was linear in incubations ranging from 20 to 60 minutes, ( $r = 0.992$ ) with 50 to 200 mg equivalents of testicular homogenate ( $r = 0.986$ ).

### Chronic Ethanol Treatment

Starting at age 20 days, male mice were subjected to chronic ethanol treatment via a vitamin-fortified liquid diet by a modification of the method of Willis et al (1983). Previous studies (Anderson et al, 1987b) had employed a liquid diet (chocolate-flavored Carnation Slender™) containing 5% (v/v) ethanol. This diet is consumed uniformly as a function of age in adult mice (Anderson et al, 1980; Willis et al, 1983), resulting in relatively constant daily peak blood ethanol levels. However, variable peak blood ethanol levels, as well as diet consumption, were observed in pubertal animals, particularly when measured at age 30 days (week 2 of treatment). During this time, blood ethanol levels and diet consumption were increased from 44% to 65% and from 32% to 42%, respectively, compared with values observed during other periods of treatment. In an attempt to produce more uniform blood ethanol levels throughout the treatment period, animals were first given access to diets containing 3% (v/v) ethanol (19% of total calories), followed by 4% (v/v) ethanol and, finally, 5% (v/v) ethanol.

Males were weaned at 18 days of age. Littermates were matched by body weight and divided into two groups: experimental and pair-fed control. All animals were given free access to a liquid diet of chocolate-flavored Carnation Slender that contained 3 g/l of vitamin diet fortification mixture (ICN Biomedicals, cat. no. 904654) and sucrose (41.2 g/l). The sucrose concentration was isocaloric to that of 3% (v/v) ethanol, so that both ethanol-treated and control animals received the same number of calories. At age 20 days, the experimental group was given free access to diet containing 3% (v/v) ethanol. Each animal from the control group was given sucrose-containing diet in an amount equal to that consumed by its experimental counterpart on the previous day. Treatment was continued until ages 39 and 44 days, at which times the ethanol content was raised to 4% (v/v) and 5% (v/v), respectively. Corresponding control diets contained 55.0 g sucrose/l and 68.8 g sucrose/l. Body weights and tail lengths were measured every 2 days throughout the treatment as an estimate of somatic growth.

After 29 days of treatment, 10 animals from each group were removed from their diets and were given free access

to laboratory chow and water. Forty-eight hours later, animals were killed, and testes and epididymides were removed and weighed. The testicular tissue was processed for measurement of 5 $\alpha$ -reductase (see above). Several cuts were made into the cauda epididymides of each animal; the cut tissue was placed in medium (consisting of 123 mM NaCl, 4.26 mM KCl, 1.23 mM MgSO<sub>4</sub>, 2 mM glucose, and 16 mM HEPES [(N-2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid], pH 7.4) to allow dispersal of spermatozoa. Sperm content and motility were determined as previously described (Anderson et al, 1980, 1983b). After 43 days treatment, the remainder of the animals in each treatment group were removed from their diets and given laboratory chow and water. Forty-eight hours later, testicular and epididymal weights, testicular 5 $\alpha$ -reductase activity, and epididymal sperm content and motility were measured as described above.

### Measurement of Blood Ethanol

At days 5 to 7, 13, 14, 19, 21, 28, and 40 of treatment, blood ethanol profiles were determined from 1800 to 0400 hours at 2-hour intervals. Tail blood (25  $\mu$ l) was removed from each of five randomly selected animals at each time point. Blood was dispensed into stoppered 25-ml erlenmeyer flasks containing 1 ml of 0.1 N HCl and 25 mM thiourea. Ethanol content of the headspace over the solution was determined by gas chromatography, as previously described (Anderson, et al, 1985). Data were reported as the average peak blood ethanol level for each day.

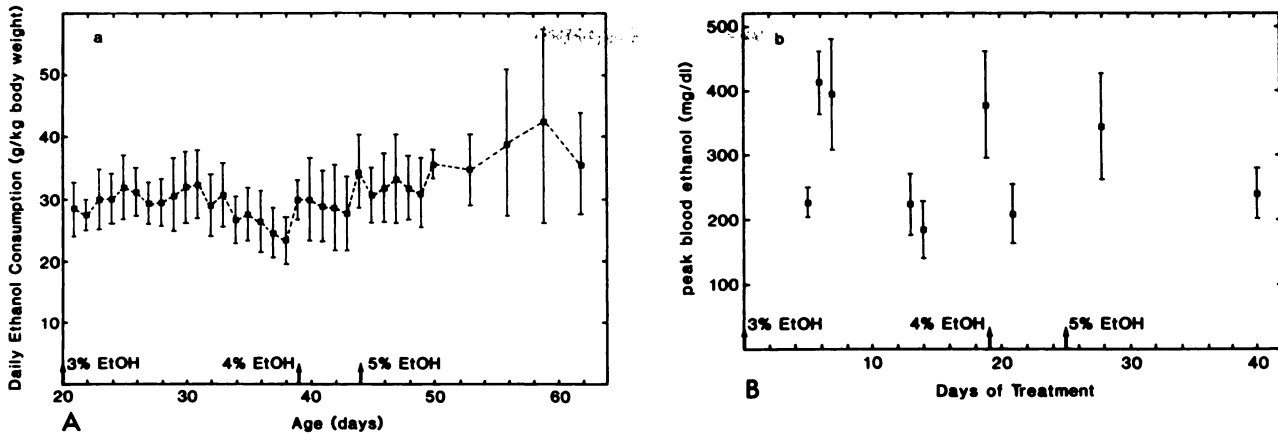
### Statistics

Differences in body weight and tail length between ethanol-treated and control groups were evaluated using the student's *t*-test. Data are expressed as the mean  $\pm$  standard deviation or standard error of the mean. Data on sperm count and motility were first subjected to logarithmic and arcsin transformations, respectively (Sokal and Rohlf, 1981), prior to parametric analysis. Values for these parameters were expressed as the backtransform of the average transformed value, with 90% confidence limits indicated in parentheses. Differences in testicular 5 $\alpha$ -reductase as a function of age and as a result of ethanol treatment were identified by analysis of variance and the Newman-Keuls multiple range test (Woolf, 1968).

### Results

The treatment regimen used in this study resulted in consistent ethanol consumption throughout the treatment period (Fig. 1A), averaging  $30.8 \pm 3.9$  g/kg body weight (SD; N = 45). Peak blood ethanol levels were relatively high on days 6 and 7 of treatment, (approximately 400 mg/dl) despite the lower ethanol content of the diet during this period (3%, v/v) as compared with subsequent treatment. Overall, peak blood ethanol levels throughout the treatment period averaged  $274 \pm 19$  (SEM; N = 45) mg/dl (Fig. 1B).

Body weight and tail length increased as a function

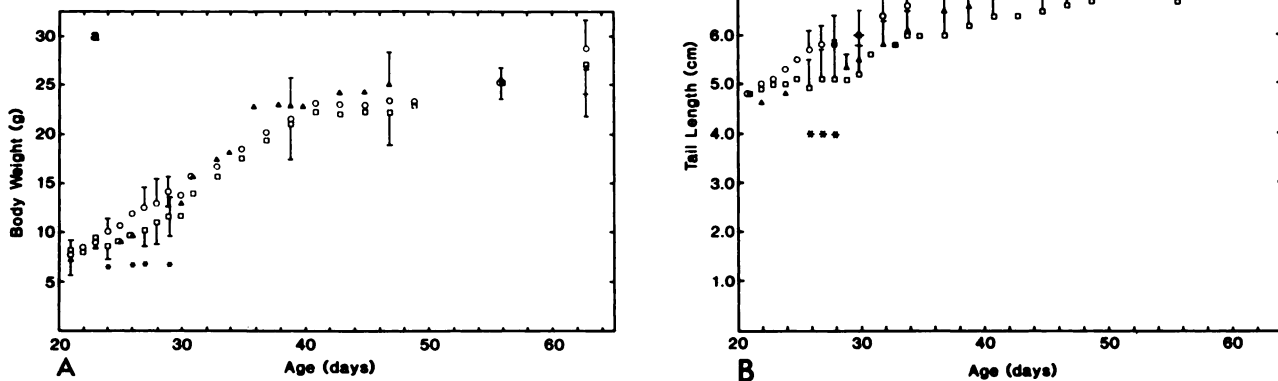


**Fig. 1.A.** Daily ethanol consumption throughout ethanol treatment of male pubertal Swiss-Webster mice. Animals were given free access to ethanol-containing diets as described in Materials and Methods. Transition to diets with increasing ethanol content is indicated by arrows on the abscissa. Values (measured as g ethanol consumed/kg body weight) are expressed as the mean  $\pm$  standard deviation of 20 measurements per day from ages 20 to 49 days and 10 measurements per day from ages 50 to 63 days. **B.** Average peak blood ethanol levels during treatment. On the indicated treatment day, blood ethanol levels were determined from 1800 hours to 0400 hours at 2-hour intervals, as described in Materials and Methods. Five animals were randomly selected per time point. Values represent the highest mean  $\pm$  standard error of the blood ethanol level (mg/dl) obtained for each day. Time of occurrence of the peak of average blood ethanol levels varied from 1900 to 2400 hours. Transitions to diets with different ethanol content are indicated by arrows on the abscissa. Overall average peak blood ethanol level was  $274 \pm 19$  mg/dl ( $N = 45$ ).

of age to a similar extent in ethanol-treated, pair-fed control, and chow-fed control animals (Figs. 2A and 2B). Small, but significant decreases in body weight were observed for ethanol-treated as compared with pair-fed control animals at ages 24 days (14%), 26 days (18%), 27 days (17%), and 29 days (17%), and in tail length at ages 26 days (13%), 27 days (12%), and 28 days (12%). On the other hand, no significant differences in body weights or tail lengths were observed between ethanol-treated and chow-fed

control groups at any time during the treatment period.

Testicular weights were significantly lower in ethanol-treated as compared with pair-fed control animals after treatment for 29 days  $P < 0.01$ , Newman-Keuls multiple range test; Table 1). After 43



**Fig. 2.A.** Body weights of Swiss-Webster male mice throughout ethanol treatment. Values represent the mean ( $\pm$  standard deviation, where indicated; for clarity, all standard deviations have not been included) of 20 measurements for each age from ages 21 to 49 days and 10 measurements for each age from ages 50 to 63 days. Ages marked with asterisks indicate those in which body weights for the ethanol-treated group ( $\square$ ) differ ( $P < 0.05$ ) from those of the pair-fed control group ( $\circ$ ). Data for chow-fed control animals ( $\Delta$ ) are included for comparison. **B.** Tail lengths of Swiss-Webster mice throughout ethanol treatment. Tail length was measured in ethanol-treated ( $\square$ ), pair-fed control ( $\circ$ ), and chow-fed control ( $\Delta$ ) groups from ages 21 to 63 days. Details are given in the legend to Fig. 2A.

TABLE 1. Delayed Testicular, but not Epididymal, Growth by Chronic Ethanol Ingestion During Pubertal Development\*

Treatment Period	Organ Weights (mg)			
	Testis		Epididymis	
	Control	Ethanol-Treated	Control	Ethanol-Treated
29 days	63.0 ± 2.4	55.0 ± 2.4†	24.4 ± 0.7	23.9 ± 0.9
43 days	64.3 ± 3.0	61.2 ± 3.7	26.0 ± 0.8	24.0 ± 1.0

\*Mice were maintained on either ethanol-containing or sucrose-containing (control) diets for either 29 or 43 days, as described in Methods. Forty-eight hours after withdrawal, animals were killed and organs (right, from each animal) were weighed. Values represent means ± standard errors of 10 animals per group.

†Value differs from that of its respective control group ( $P < 0.01$ , Newman-Keuls multiple range test).

days of treatment, a difference in testicular weights between the two groups was no longer evident ( $P > 0.1$ ; Table 1). In contrast, epididymal weights were the same in ethanol-treated and control animals after either 29 or 43 days of treatment (Table 1). Similar to the effect of ethanol treatment on testicular weights, epididymal sperm content and sperm motility were depressed ( $P < 0.05$ ) after 29 days treatment but these effects were no longer evident after 43 days of treatment (Table 2).

In chow-fed mice, testicular  $5\alpha$ -reductase activity varied as a function of age; transient secondary increases in specific activity were noted at ages 16 and 21 days (Fig. 3A). This was followed by a greater and more prolonged increase in activity, which began at approximately 24 days of age, reaching a peak at 25 to 30 days. Thereafter, activity gradually declined to adult (age 63 days) levels of approximately 6 to 7 pmoles DHT formed/45 min/mg testis. The magnitude of this transient peak in activity at age 25 days was approximately 10-fold higher than adult levels of the enzyme. A similar profile was observed with regard to total enzyme activity per testis as a function of age (Fig. 3B).

Testicular  $5\alpha$ -reductase activity was significantly ( $P < 0.05$ ) depressed in ethanol-treated, as compared with pair-fed control animals after 29 days of treatment, both with regard to specific activity and total activity (Table 3). After 43 days, activity of pair-fed control animals decreased to values similar to those seen in age-matched chow-fed controls, whereas no difference in activity was noted in ethanol-treated mice between 29-day and 43-day treatment periods ( $P > 0.1$ , Newman-Keuls multiple range test).

### Discussion

In agreement with previous experiments with C57Bl/6J mice (Anderson et al, 1987b), the present study has demonstrated that chronic treatment of an outbred strain (Swiss-Webster) of male mice with moderate to high levels of ethanol impairs pubertal development, as measured by decreased testicular weights, reduced epididymal sperm content and reduced sperm motility. Testicular morphology was not measured in the present experiments, since all testicular material was required for the measurement of  $5\alpha$ -reductase activity. However, recent work with the Swiss-Webster mouse has demonstrated

TABLE 2. Reduced Epididymal Sperm Content and Motility After 29 Days, but not After 43 Days of Ethanol Ingestion during Pubertal Development\*

Treatment Period	Sperm Content/Epididymal Pair ( $\times 10^{-6}$ )		Sperm Motility (%)	
	Control	Ethanol-Treated	Control	Ethanol-Treated
29 days	14.4 (9.2-22.6)	6.8† (3.5-13.1)	57 (49-65)	45† (37-53)
43 days	67.9 (50.8-90.7)	55.0 (36.3-83.2)	61 (58-64)	58 (56-60)

\*Forty-eight hours after withdrawal from their diets, all animals (10 from each group) were killed; cauda epididymal sperm content and motility were determined (see Materials and Methods). Values represent the means, with 90% confidence limits in parentheses. Data on sperm content and sperm motility were subjected to logarithmic and arcsin transformations, respectively, prior to further statistical analysis.

†Value differs from that of the appropriate control group ( $P < 0.05$ , Newman-Keuls multiple range test).

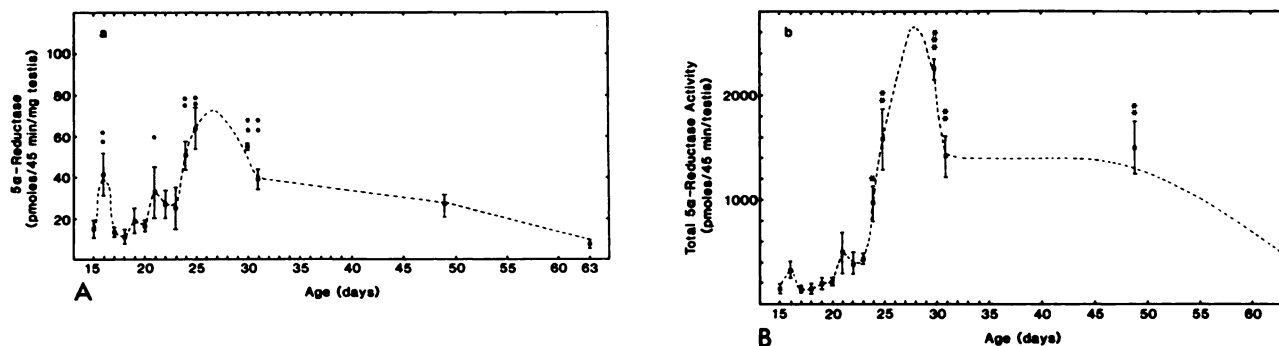


Fig. 3.A. Specific activity of testicular 5 $\alpha$ -reductase as a function of age in chow-fed Swiss-Webster mice. 5 $\alpha$ -reductase activity was measured in testicular homogenates using [ $^3$ H]T as substrate, as described in Materials and Methods. Values represent the mean  $\pm$  standard error of seven to 10 mice for each age. Values designated with one ( $P < 0.05$ , Newman-Keuls multiple range test) or two ( $P < 0.01$ ) asterisks differ from that of adult (age 63 days) values. B. Total testicular 5 $\alpha$ -reductase (per testis) as a function of age. Values represent the mean  $\pm$  standard error of seven to 10 mice for each age. Means designated by zero, one, two, or three asterisks differ ( $P < 0.05$ , Newman-Keuls multiple range test).

biochemical and morphologic impairment of the testis following ethanol treatment during adolescence (Anderson et al, 1987a). These results support the validity of the use of the Swiss-Webster mouse as a model for the study of ethanol's effects on pubertal development, while minimizing the introduction of a genetic bias.

If the ethanol-induced effects seen after 29 days treatment were due to disruption of pre-existing function (ie, if sexual maturation occurred normally, but subsequent reproductive function was compromised by ethanol treatment), they would likely have been exacerbated by an additional 2 weeks of treatment; this was not observed (Tables 1 and 2). Measurements of ethanol's effects were made (age 51 days) shortly after the mice reached reproductive competency (Altman and Dittmer, 1972). Motile spermatozoa are not seen in electroejaculated semen obtained from this strain of mouse prior to age 42 to 44 days (data not shown). Thus, if ethanol were disrupting pre-existing function, it would have to be manifested in a relatively brief period (approximately 5 to 7 days). This is unlikely, since previous studies showed only minimal reproductive impairment resulting from similar treatment of sexually mature mice (age 90 days) for 5 weeks (Willis et al., 1983). Taken together, these results strongly suggest that chronic ethanol ingestion delays the process of sexual maturation in the male.

A transient increase in 5 $\alpha$ -reductase during puberty may be required for sexually mature levels of T, LH, and a mature hypothalamic-pituitary axis. Elevated circulating levels of DHT that result from this increase in enzyme activity may augment FSH

release, which is required for initiation of spermatogenesis and increased Leydig cell responsiveness to LH stimulation (for review, see Nazian and Mahesh, 1980).

Earlier evidence for increased 5 $\alpha$ -reductase during puberty was indirect, based upon an increased *in vitro* accumulation of 5 $\alpha$ -reductase metabolites from radiolabeled steroid precursors in rat testicular preparations (Inano and Tamaoki, 1966; Ficher and Steinberger, 1971; Rivarola et al, 1972). Only recently has a transient increase in 5 $\alpha$ -reductase been shown as a result of direct measurement of either 5 $\alpha$ -reductase (Tsuji et al, 1984) or of circulating levels of DHT (Jean-Faucher et al., 1985). The present data support these findings and provide direct evidence of a similar transient increase in testicular 5 $\alpha$ -reductase in the mouse.

Formation of [ $^3$ H]DHT from [ $^3$ H]T was used as an estimate of 5 $\alpha$ -reductase activity. Other 5 $\alpha$ -reduced steroids include 5 $\alpha$ -androstan-3 $\alpha$ , 17 $\beta$ -diol, and 5 $\alpha$ -androstan-3 $\beta$ , 17 $\beta$ -diol. These steroids are formed in relatively large quantities in the sexually immature rat (Ficher and Steinberger, 1971; Purvis et al, 1978; Eckstein et al, 1987), although not in the immature rabbit (Chubb and Ewing, 1981). While the chromatography system used in the present study was able to separate T, DHT, and androstanediol(s) ( $R_f = 0.26 \pm 0.02$ ) standards, separation was not sufficient to quantify the appearance of small amounts of radiolabeled androstanediol, due to its proximity to the high concentration of [ $^3$ H]T. Incubations were therefore carried out under the conditions of the 5 $\alpha$ -reductase assay (see Methods) in the presence of [ $^3$ H]DHT at concentrations ranging from 15 to 75  $\mu$ M to deter-

mine the extent to which DHT may be further metabolized. Assays were carried out with testicular homogenates from mice at ages 26, 40, and 98 days. A fractional conversion of DHT to androstenediol of  $30 \pm 4\%$  ( $N = 9$ ) was observed, which was independent of age or DHT concentration. Thus, although the  $5\alpha$ -reductase activities reported in the present study may be somewhat underestimated, the relative age-related profiles are valid.

Enzyme activity in pair-fed control animals after 29 days of treatment was similar to that measured in chow-fed control animals of the same age (compare Fig. 3A and Table 3). Enzyme levels in ethanol-treated animals at age 51 days (Table 3) were similar to those found in prepubertal chow-fed controls at ages 15 to 19 days (Fig. 3A). At ages 63 to 65 days, enzyme activity in the pair-fed and chow-fed control groups returned to baseline values; a similar value was observed for the ethanol-treated animals. Ethanol treatment may have caused a premature increase in  $5\alpha$ -reductase, which had returned to baseline prior to age 51 days. Also, a transient increase in activity in ethanol-treated animals from ages 51 to 65 days would have gone undetected. However, a more tenable explanation of the results is that ethanol prevented the increase in testicular  $5\alpha$ -reductase. This is based upon enzyme levels in ethanol-treated animals, which did not deviate from baseline values at either ages 51 or 65 days. Enzyme activity in chow-fed control mice is elevated for more than 30 days, a period far exceeding the interval of measurements in the present investigation. Undetected increases would have required not only a shift in time, but also a change in its duration. Confirmation of these speculations, however, is subject to empirical verification.

An ethanol-induced reduction in testicular weight (Table 1) and reduction in spermatogenesis (as reflected by decreased sperm content; Table 2), in the absence of an effect on accessory sex gland weight, suggest that the tubular compartment of the testis may be more severely affected than that of the interstitial (Leydig) cells. In contrast, the previous study (Anderson et al, 1987b) showed a small (15%) ethanol-related decrease in epididymal weights, although no effect on seminal vesicles weights was observed. A larger number of animals was examined in the present study; the lack of change in epididymal weights due to ethanol treatment may represent a more reliable estimate. Weight and secretory activity of the accessory glands reflect long-term androgenic status of the animal rather than transient fluctuations (Mann and Lutwak-Mann, 1981). Acid phosphatase

TABLE 3. Prevention of Pubertal Rise in Testicular  $5\alpha$ -Reductase by Chronic Ethanol Treatment\*

Treatment Group	$5\alpha$ -Reductase Activity	
	pmoles DHT formed/mg testis	nmoles DHT formed/testis
Pair-fed control, 29 days	$26.8 \pm 4.9$	$1.58 \pm 0.32$
Ethanol-treated 29 days	$7.7 \pm 2.3^\dagger$	$0.38 \pm 0.10^\dagger$
Pair-fed control, 43 days	$7.0 \pm 2.1$	$0.42 \pm 0.11$
Ethanol-treated, 43 days	$7.6 \pm 2.3$	$0.46 \pm 0.15$

\*Starting at age 20 days, male mice were given free access to liquid diet containing ethanol or pair-fed diet containing isocaloric amounts of sucrose for either 29 or 43 days, as described in Materials and Methods. Forty-eight hours after withdrawal from their diets, animals (10 from each group) were killed by cervical dislocation and the  $5\alpha$ -reductase content of the testicular tissue was assessed as described in Materials and Methods. Incubations were carried out for 45 min at 37 C. Results are expressed as means  $\pm$  standard error.

$^\dagger$ Value differs from control ( $P < 0.05$ , Newman-Keuls multiple range test).

and fructose levels (indices of prostatic and seminal vesicular secretory activities, respectively) of semen collected by electroejaculation (Anderson et al, 1987b) were unaffected following ethanol ingestion throughout pubertal development (43-days treatment), suggesting that those animals were not androgen-deficient. Evidence for a direct effect of ethanol on the Sertoli cell has been provided by recent studies in which ethanol inhibited tissue-type plasminogen activator release by Sertoli cells in primary culture (Mueller et al, 1986).

When mice were treated from ages 20 to 49 days with a 5% (v/v) ethanol diet (Anderson et al, 1987b), diet consumption, as well as blood ethanol levels, were approximately 2- to 3-fold higher during the 2nd week as compared with the rest of the treatment period. This was not unexpected in view of the rapid growth seen in mice at this age (Altman and Dittmer, 1972). In this study the ethanol content of the diet was decreased in the early part of the treatment period in an attempt to prevent greatly increased blood ethanol levels during that time. This approach, however, was only partially successful. Although blood ethanol levels were somewhat more consistent throughout the treatment, they were, with the exception of week 2 of treatment, higher than in the previous study. Despite the different ethanol content of the diets (3%, 4%, 5%), the daily ethanol consump-

tion in g/kg body weight was remarkably consistent throughout the treatment period (Fig. 1A). The treatment regimen used in the present study has provided a means of relatively uniform ethanol exposure of the mouse throughout puberty.

A small, though significant impairment of somatic growth in ethanol-treated as compared with pair-fed control animals was observed near the end of the 1st week of treatment (Fig. 2A and 2B). Although previous studies (Anderson et al, 1980; 1987b; Willis et al, 1983) have not detected growth impairment resulting from similar ethanol treatments, decreased body growth may have been secondary to decreased nutrient availability. Peak blood ethanol levels were relatively high on days 6 and 7 (Fig. 1B). Ethanol, at high intraluminal concentrations, can impair gastrointestinal function, including nutrient absorption (Beck and Dinda, 1981). At any rate, the effect was transient; no difference in either body weight or tail length was seen prior to age 24 days or after age 29 days as a result of treatment.

A nutritional component is not likely a primary factor in ethanol-induced delayed sexual maturation since growth deficiency associated with impaired sexual maturation is considerably greater (Sisk and Bronson, 1985; Glass and Anderson, 1986; Piacsek et al, 1986) than that observed in the present study. Data from pubertal rats (Widdowson et al, 1964; Glass and Anderson, 1986) suggest that steroidogenesis is more adversely affected by underfeeding than is spermatogenesis. This pattern of response is not similar to that seen in the present and in the previous study (Anderson et al, 1987b). The previous study showed that out of five series of measurements of plasma T made on days 24 and 25 of treatment, only one showed significant depression (50%) of T in the ethanol-treated compared with the control group. At no time during the treatment period were either body weight or tail length reduced in ethanol-treated compared with chow-fed control animals (Figs. 2A and 2B). Relative to the untreated male mouse, ethanol treatment did not create a state of impaired growth.

The data in Table 3 clearly indicate differences in testicular 5 $\alpha$ -reductase activity due to ethanol treatment. Prevention of the transient increase in 5 $\alpha$ -reductase (and, presumably, the increase in circulating levels of DHT) could offer at least a partial explanation for the ethanol-induced delay in sexual maturation. This mechanism would imply that an increase in enzyme activity facilitates, but is not an absolute requirement for, pubertal development. Further

studies regarding the ability of this enzyme or its product, DHT, to modulate pubertal development are clearly warranted.

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### References

- Altman K, Gordon GG, Southren AL, Vittek J, Wilker S. Induction of hepatic testosterone A-ring reductase by medroxyprogesterone acetate. *Endocrinology* 1972; 90:1252-1260.
- Altman PL, Dittmer DS, eds. *Biology data book*. Vol 1. Bethesda, MD: Federation of American Societies for Experimental Biology, 1972; 138; 208.
- Anderson RA, Phillips JF, Berryman SH, Russell LD. Biochemical and morphological evaluation of ethanol-induced delay in male pubertal development. *Biol Reprod* 1987a; 36(suppl 1):127.
- Anderson RA, Willis BR, Phillips JF, Oswald C, Zaneveld LJD. Delayed pubertal development of the male reproductive tract associated with chronic ethanol ingestion. *Biochem Pharmacol* 1987b; 36:2157-2167.
- Anderson RA, Quigg JM, Oswald C, Zaneveld LJD. Demonstration of a functional blood-testis barrier to acetaldehyde: evidence for lack of effect of acetaldehyde on ethanol-induced depression of testosterone in vivo. *Biochem Pharmacol* 1985; 34:685-695.
- Anderson RA, Willis BR, Oswald C, Reddy JM, Beyler SA, Zaneveld LJD. Hormonal imbalance and alterations in testicular morphology induced by chronic ingestion of ethanol. *Biochem Pharmacol* 1980; 29:1409-1419.
- Anderson RA, Willis BR, Oswald C, Zaneveld LJD. Male reproductive tract sensitivity to ethanol: a critical overview. *Pharmacol Biochem Behav* 1983a; 18(suppl 1):305-310.
- Anderson RA, Willis BR, Oswald C, Zaneveld LJD. Ethanol-induced male infertility: spermatozoal impairment. *J Pharmacol Exp Ther* 1983b; 225:479-486.
- Bannister P, Lowosky MS. Ethanol and hypogonadism. *Alcohol* 1987; 22:213-218.
- Beck IT, Dinda PK. Acute exposure of small intestine to ethanol: effects on morphology and function. *Dig Dis Sci* 1981; 26:817-838.
- Chubb C, Ewing LL. Steroid secretion by sexually immature rat and rabbit testes perfused in vitro. *Endocrinology* 1981; 109:1999-2003.
- Diamond F, Ringenberg L, Macdonald D, Barnes J, Hu CS, Duckett G, Sweetland M, Root A. Effects of drug and alcohol abuse upon pituitary-testicular function in adolescent males. *J Adolesc Health Care* 1986; 7:28-33.
- Eckstein B, Borut A, Cohen S. Metabolic pathways for androstenediol formation in immature rat testis microsomes. *Biochem Biophys Acta* 1987; 924:1-6.
- Ficher M, Steinberger E. In vitro progesterone metabolism by rat testicular tissue at different stages of development. *Acta Endocrinol* 1971; 68:285-292.
- Glass AR, Anderson J. Dissociation of steroidogenic and spermatogenic development during puberty in underfed male rats. *J Androl* 1986; [abstr]7:P16.
- Gordon GG, Vittek J, Ho R, Rosenthal WS, Southren AL. Effect of chronic alcohol use on hepatic testosterone 5 $\alpha$ -A-ring reductase in the baboon and in the human being. *Gastroenterology* 1979; 77:110-114.
- Inano H, Tamaoki B-I. Conversion of steroids in immature rat testes in vitro. *Endocrinology* 1966; 79:579-590.

- Jean-Faucher C, Berger M, de Turckheim M, Veyssiere G, Jean C. Testosterone and dihydrotestosterone levels in the epididymis, vas deferens and preputial gland of mice during sexual maturation. *Int J Androl* 1985; 8:44-57.
- Jessor RJ. Adolescent problem drinking: psychosocial aspects and developmental outcomes. In: Towle LH, ed. *Proceedings: NIAAA-WHO collaborating center designation meeting and alcohol research seminar*. Washington: US Government Printing Office, 1985; 104-143.
- Jessor R, Jessor SL. Adolescent development and the onset of drinking. *J Stud Alcohol* 1975; 36:27-51.
- Mann T, Lutwak-Mann C. *Male reproductive function and semen*. New York: Springer, 1981; 174.
- Mueller PL, Downey C, Anderson RA. Alcohol and dihydrotestosterone inhibit insulin-enhanced basal plasminogen activator production by Sertoli cell-enriched testicular preparations. *Biol Reprod* 1986; [Abstr] 34(suppl 1):58.
- Nazian SJ, Mahesh VB. Hypothalamic, pituitary, testicular and secondary organ functions and interactions during the sexual maturation of the male rat. *Arch Androl* 1980; 4:283-303.
- NIAAA. *Fifth special report to the US Congress on alcohol and health*. Washington: US Government Printing Office, 1984; 1.
- Piacsek BE, Bonifer TM, Tan RC. Altered testosterone feedback in pubertal male rats raised on reduced caloric intake. *J Androl* 1986; 7:292-297.
- Purvis K, Clausen OPF, Hansson V. Age-related changes in responsiveness of rat Leydig cells to hCG. *J Reprod Fertil* 1978; 52:379-386.
- Ramaley JA. The regulation of gonadotropin secretion in immature ethanol-treated male rats. *J Androl* 1982; 3:248-255.
- Rivarola MA, Podesta EJ, Chemes HE. In vitro testosterone-<sup>14</sup>C metabolism by rat seminiferous tubules at different stages of development: formation of 5 $\alpha$ -androstenediol at meiosis. *Endocrinology* 1972; 91:537-542.
- Sisk C, Bronson FH. Effect of food restriction and restoration on gonadotropin and growth hormone secretion in immature male rats. *Biol Reprod* 1985; [Abstr] 32(suppl 1):209.
- Sokal RR, Rohlf FJ. *Biometry*. San Francisco: WH Freeman, 1981; 417-428.
- Tsuji M, Takeyama M, Takasuka D, Yabumoto H, Terada N, Matsumoto K. 5 $\alpha$ - and 5 $\beta$ -reductases for 4-ene-3-ketosteroids and 17 $\beta$ -ol-dehydrogenase in epididymis and testis of golden hamster during sexual development. *J Steroid Biochem* 1984; 21:179-183.
- Van Thiel DH. Ethanol: its adverse effects upon the hypothalamic-pituitary-gonadal axis. *J Lab Clin Med* 1983; 101:21-33.
- Widdowson EM, Mavor WO, McCance RA. The effect of undernutrition and rehabilitation on the development of the reproductive organs: rats. *J Endocrinol* 1964; 29:119-126.
- Willis BR, Anderson RA, Oswald C, Zaneveld LJD. Ethanol induced male reproductive tract pathology as a function of ethanol dose and exposure. *J Pharmacol Exp Ther* 1983; 225:470-478.
- Woolf CM. *Principles of biometry*. Princeton, NJ: WB Saunders, 1968; 101-109.

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