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Assessment of Environmental Factors Affecting Male Fertility

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Exposure to drinking water containing as much as 500 ppm aluminum chloride for periods of 30, 60, and 90 days had no apparent effect on male reproductive processes.

In an attempt to correlate enzyme activity with particular spermatogenic cell types, postnatal development of testicular enzymes was studied. Eight enzymes were selected: hyaluronidase (H), lactate dehydrogenase isoenzyme-X (LDH-X), dehydrogenases of sorbitol (SDH), α-glycerophosphate (GPDH), glucose-6-phosphate (G6PDH), malate (MDH), glyceraldehyde-3-phosphate (G3PDH), and isocitrate (ICDH). Enzyme specific activities in testicular homogenates were determined. Two types of enzyme developmental patterns were observed. One was represented by H, LDH-X, SDH, and GPDH; and the other by G6PDH, MDH, G3PDH, and ICDH. The former was characterized by a change in enzyme activities from low in newborn to high in adult while in the latter this pattern was reversed. The two complementary enzyme systems crossed each other at puberty. Prior to puberty, only spermatogonial cells are present; sperm differentiation initiated at puberty adds spermatocytes and spermatids to the testicular cell population.

Male rats were exposed to borax in their diet for periods of 30 and 60 days. Concentrations of boron were 0, 500, 1000, and 2000 ppm. At the end of each experimental period, the specific activities of the selected enzymes were determined in the testis and prostate. Correlations of enzyme activity with testicular histology and androgen activities of the male accessory organs were sought. In addition, plasma FSH, LH, and testosterone levels were measured to assess pituitary-testicular interaction. Plasma and testicular boron concentrations were determined and a minimum boron concentration which induced germinal aplasia and male infertility was estimated.

In both 30 and 60 day feeding studies, male rats receiving 500 ppm failed to demonstrate any significant adverse effects. In contrast, male rats receiving 100 and 2000 ppm boron displayed a significant loss of germinal elements, although most of the Leydig and Sertoli cells appeared normal. Testicular atrophy was associated with a decrease in seminiferous tubular diameter and a marked reduction of spermatocytes and spermatogenic cells. These morphologic alterations were associated with a concomitant reduction of H, SDH, and LDH-X specific activities. In contrast, the specific activities of G3PDH and MDH were significantly elevated above control. The increase in these enzyme activities can be attributed to the relative enrichment of spermatogonial cells during the loss of spermatocytes and spermiogenic cells. Boron-induced male germinal aplasia was also associated with significantly elevated plasma FSH while plasma LH and testosterone levels were not significantly altered. Plasma testosterone levels were unaltered.

Male fertility studies demonstrated that at the 500 ppm boron level, fertility was unaffected. However, at 1000 and 2000 ppm boron, male fertility was significantly reduced. Most effects were reversible within 5 weeks. However, the male group receiving 2000 ppm boron for 60 days remained sterile. There was no dose-related decrease in litter size or fetal death *in utero*. Therefore, the boron-induced infertility was apparently not due to a dominant lethal effect but rather to germinal aplasia. Boron appears toxic to spermatogenic cells at testicular concentrations of 6–8 ppm.

Introduction

The effect of trace elements in the environment is an important environmental health concern, and considerable literature pertaining to the general toxicology of these elements is available. However, very little attention has been focused on the adverse effects of trace elements on male reproduction. Mutagenic and cytotoxic effects on spermatogenic cells, as well as actions on the pituitary-hypothalamic axis and accessory organs, need further study. At the present time, in male infertility clinics, the etiology of nearly half of the cases of male germinal aplasia is not only unknown but the

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incidence of male germinal aplasia is apparently increasing. Increasing evidence suggests that some environmental trace metals might play an important role. Subfertility and male germinal aplasia might be associated with a subtle and chronic exposure to environmental chemicals in air, water, soil, and foodstuffs.

The objectives of our continuing U.S.S.R.–U.S. cooperative laboratory efforts continue to be the development, improvement, and validation of toxicologic test methods and the assessment of selected environmental factors for male reproductive toxicity. At the last symposium, we reported studies which attempted to evaluate the reproductive toxicity of boron (borax) and cadmium chloride and determine the mechanisms of toxicity in the rat following acute and subchronic oral exposure. Cadmium (*1*), methylmercury, and mercury (*2*) far surpass other elements for toxic effects on the male gonads following parental administration. Boron, titanium, and lead have been reported to rank next in toxicity (*3*).

Recently, Soviet health scientists have expressed concern regarding the toxic effects of long-term human exposure to drinking water with high concentrations of boron. A higher incidence of male infertility was postulated to be associated with high levels of boron in the drinking water (*4*). Boron levels of water in the Alaverdi, Martuni, and Yekhegnadzor regions were reported to be more than 0.40 mg/l.; the concentration of boron in artesian wells was considerably higher (1.2 mg/l.) (*5*).

Much less concern surrounds the possible health hazards associated with boron compounds in the United States. The United States Public Health Service does not list boron in its 1962 chemical standards for drinking water and the element is not thought to be highly toxic. Borax has been used as a cleaning agent and as a food preservative. Boron has also been used in medicine as sodium borate and boric acid. Accidental poisoning is usually due to the oral ingestion of boric acid or systemic absorption when boric acid solution was used to irrigate body cavities, the gastrointestinal, or urinary tracts (*6, 7*). Intravenous administration of borax up to 20 g (2.13 g boron equivalent) was without dangerous toxic effects in 10 patients who were receiving treatment for brain tumors by neutron capture therapy, utilizing B^{10} as a capture element (*8*). Weir and Fisher (*9*) have studied the chronic toxicity of borax and boric acids in laboratory animals. They report a severe degree of testicular atrophy and spermatogenic arrest at a treatment level of 140 mg/kg for 90 days. Bouissou and Castaghol (*10*) also reported a marked testicular atrophy with almost complete degeneration of the seminiferous tubular epithelium following chronic administration of boric acid (25 mmole/kg). Similar effects were also observed following long-term administration of a boron-containing tranquilizer, methyl-5-*n*-propyl-5-*p*-tolyl-2-dioxaborane (*11*). The relatively low concentration of boron found in the testes of intoxicated males was in marked contrast to the serious toxic effects observed.

Acute oral doses, expressed as boron, were 45, 150, and 450 mg/kg while doses for cadmium equivalent were 6.25, 12.5, and 25 mg/kg. Rats were also allowed free access to drinking water containing either boron (0.3, 1.0, and 6.0 mg/l.) or cadmium (0.001, 0.01, and 0.1 mg/l.) for 90 days. Randomly selected animals were studied following 30, 60, and 90 days of treatment. These initial studies, utilizing a variety of methods to assess the reproductive toxicity of environmental substances in male animals, suggested that boron and cadmium at the concentrations and dose regimens tested were without significant reproductive toxicity in male rats (*12*).

The studies to be described here seek to assess the reproductive toxicity of aluminum chloride and lead acetate administered in drinking water for 90 days using an experimental design similar to that previously reported. The metals for study were suggested by our Soviet counterparts. In addition, further studies were undertaken to assess the effects of higher concentrations of boron on male reproductive function.

Aluminum is one of the IIIa elements of the periodic table which also includes boron, gallium, indium, and thalium. Hart and Adamson reported that these elements are increasingly cytotoxic, e.g., boron is least and thalium most toxic (*13*). Few studies have considered the possible reproductive effects of aluminum. Kamboj and Kar (*14*) reported that daily subcutaneous injection of aluminum sulfate for 30 days (2.67 μmole/kg-day; total 0.08 mmole) produced shrinkage of testes and some degree of spermatogenic arrest without any damage to the Leydig cells. Furthermore, acute treatment of aluminum by either the subcutaneous or intratesticular route caused testicular atrophy after 30 days. No assessment of the toxicity associated with oral exposure of animals nor occupational exposure of men is available.

It has been reported that feeding male rats with 200 μg of lead per day results in hyperplasia of the prostate and seminal vesicle while the weight of the testes were decreased (*15*). Transplantable interstitial tumors have been induced with lead acetate (*16*). Epidemiologic studies concerning male reproductive function following occupational exposure to lead (*17*) indicate that workers with blood concentrations of lead between 0.23 and 0.75 ppm exhib-

ited infertility associated with asthenospermia, oligospermia, and teratospermia. Leydig cell function was apparently not altered based on both testosterone level and histopathology; infertility was most likely due to a direct effect on the male gonads.

In an effort to improve techniques to assess male reproductive function, studies were initiated to determine enzyme activity in different spermatogenic cell types and their usefulness as "biochemical markers" for assessing toxicity of environmental agents on spermatogenesis. Developing animals were especially appropriate for these studies.

Localization of enzymes in rodent testes at different stages of development has been studied histochemically and biochemically. In general, two main categories have been described. Hyaluronidase (H), sorbitol dehydrogenase (SDH), lactate dehydrogenase isoenzyme-X (LDH-X), hexose kinase "sperm type," cyclic nucleotide phosphodiesterase, 5′-nucleotidase, *S*-adenosyl-L-methionine decarboxylase (*18*), carnitine acetyltransferase (*19*), and α-glycerophosphate dehydrogenase (GPDH) (*20*) appear first in spermatocytes or spermatids. Activity increases rapidly and reaches maximum levels with complete spermatogenesis. In contrast, activity of γ-glutamyl transpeptidase (*21*), β-glucuronidase, uridine diphosphatase, acid phosphatase isoenzymes I and II, ornithine decarboxylase, glucose-6-phosphate dehydrogenase (G6PDH) (*18*), and malate dehydrogenase (MDH) (*22*) are highest in Sertoli cells, interstitial cells, or spermatogonia. Their levels of activity appears to be highest before puberty and then decreases as spermatocytes, spermatids, and spermatozoa began to appear sequentially in the seminiferous epithelium. Eight enzymes were selected and their activities determined quantitatively: H, SDH, LDH-X, GPDH, MDH, G6PDH, isocitrate dehydrogenase (ICDH), and glyceraldehyde-3-phosphate dehydrogenase (G3PDH). Changes in these enzyme levels were correlated with testicular toxicity produced by high levels of boron.

In summary, this paper reports the findings of our routine assessment of aluminum and lead on male reproductive function, the association of various testicular enzymes with spermatogenic cell types, and further studies of the reproductive toxicity of boron employing testicular "marker enzymes" to aid in assessment.

Materials and Methods

Reproductive Toxicity Studies of Aluminum Chloride and Lead Acetate

This study was designed to assess the effects of aluminum and lead on the reproductive capacity of male albino rats when administered continuously in the drinking water for up to 90 days prior to breeding. Aluminum chloride ($AlCl_3 \cdot 6H_2O$) had a molecular weight of 241 and an aluminum equivalent weight of 11%. Lead acetate ($PbAc \cdot 3H_2O$) had a molecular weight of 379 and a lead equivalent of 54.6%. Appropriate amounts of the test materials were dissolved in the drinking water (tap water) of the treated rats on a weight-per-volume basis and was available *ad libitum* for up to 90 days. The treated animals had no other source of water. The control animals received untreated tap water *ad libitum*.

For each study, 124 male Sprague-Dawley albino rats were obtained from Charles River Laboratories. For the breeding phases which were conducted following the 90-day exposure period, 400 female rats of the same strain were received in three different shipments for each. Animals were housed individually (except during breeding) with access to food (Purina Laboratory Rat Chow) and water *ad libitum* throughout the study.

The 124 male rats were assigned to the groups listed in Tables 1 and 2 based on individual initial body weights so that a homogeneous distribution of mean weight ranges was obtained between groups.

All animals were observed daily throughout the study for mortality, general appearance and behavior, and signs of toxic and pharmacologic effect. Water intake and body weights were recorded initially and at weekly intervals thereafter for each male rat until the animal was either sacrificed or utilized for the serial mating portion of the study.

Seven males from each group were sacrificed by exsanguination under barbiturate anesthesia on days 30, 60, and 90. The remaining ten males of each group were taken off the test water on day 90, placed on tap water, and used immediately for the serial mating phase of this study.

At the time of sacrifice, a minimum of 7 ml of heparinized blood was collected from each rat, special care being taken to prevent hemolysis. The blood was immediately centrifuged, the plasma labeled, frozen at −20°C, and saved. Plasma levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were determined. Each animal was then subjected to a gross pathological examination. The liver, lungs, spleen, kidneys, brain, and heart were removed, weighed, and fixed in 10% neutral buffered formalin. The testes were weighed, preserved in Bouin's fixative for 12-18 hr, thoroughly rinsed in tap water, and stored in 70% ethanol. Histopathologic examinations were performed on all tissues.

At the conclusion of the 90-day treatment period, one virgin female rat was placed in the cage of each

Table 1. Experimental groups for aluminum study.

No.	Group	Number of males[a]	Aluminum chloride, mg/l.	Aluminum equivalent, mg/l.	Anticipated absorbed dose mg/kg
1	Control[b]	31	0.0	0	0.000
2	Low	31	44.8	5	0.005
3	Mid	31	447.6	50	0.050
4	High	31	4476.0	500	0.500

[a] Seven males/group were sacrificed on days 30, 60, and 90 of study. The other ten were removed from treatment and used for breeding.
[b] Received vehicle of tap water.

Table 2. Experimental groups for lead study.

No.	Group	Number of males[a]	Lead acetate, mg/l.	Lead equivalent, mg/l. (ppm)	Anticipated absorbed dose, mg/kg
1	Control[b]	31	0	0	0.000
2	Low	31	1.83	1	0.001
3	Mid	31	18.3	10	0.010
4	High	31	183.3	100	0.100

[a] Seven males/group were sacrificed on days 30, 60, and 90 of study. The other ten were removed from treatment and used for breeding.
[b] Received vehicle of tap water.

male rat and housed for a period of 7 days. At the end of the 7-day mating period, the female was removed, housed separately, and replaced by another virgin female. This procedure was repeated every 7 days for a total of 70 days. During the weekly exposures to the male animals, the females were observed and records maintained of evidence of mating (presence of vaginal plugs or sperm in smear). The female rats were sacrificed by chloroform overdoses 9 days after removal from the males. The uterus and ovaries were removed and examined. The numbers of implantation sites, corpora lutea, resorption sites, and live and dead implants in each uterine horn were recorded.

The reproduction indices (pregnancy rate, implantation efficiency, incidence of dead implants, incidence of live implants) of the treated groups were compared to the control indices by the chi-square method.

Testicular Enzymes

Animals. Male CD-1 mice 1 to 120 days of age were used for all experiments. After weaning, animals were housed five per cage with food and water available *ad libitum*.

Preparation of Testicular Homogenates. Three groups of mice, each consisted of approximately 35 neonatal or three adult mice, were killed either by decapitation or by cervical dislocation. The testes were promptly dissected out, chilled on ice, combined, and weighed. Testes were then homogenized (1:9 *w/v*) in ice-cold $10^{-3}M$ Tris buffer, pH 7.0, containing 0.1% cetyltrimethylammonium bromide in a glass homogenizer fitted with a motor-driven Teflon pestle. The crude homogenate was centrifuged at 9000*g* for 30 min at 4°C and the supernatant filtered through a Millipore filter. The resultant solution was used for enzyme assays. Beginning at 20 days of age, the epididymis and vas deferens were also removed and crude enzyme preparations obtained as described above.

Enzyme Assays. Activities of hyaluronidase, lactate dehydrogenase isoenzyme-X, sorbitol dehydrogenase, malate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, α-glycerophosphate dehydrogenase, glucose-6-phosphate dehydrogenase, and isocitrate dehydrogenase (*23*) were assayed spectrophotometrically according to methods described previously (*24*). Enzyme activities were expressed as μmole of product formed per hour per milligram protein.

Protein Determination. The total protein concentration of each filtrate was determined by the method of Lowry et al. (*25*).

Histology. Testicular tissue samples were fixed in Bouin's solution for 24 hr, imbedded in paraffin, and 5 μm sections were cut and stained with hematoxylin and eosin. Photomicrographs were taken with a Leitz microscope.

Boron Study

Animals and Chemicals. Male and female rats (Sprague-Dawley) weighing 200 to 250 g were used. Borax ($Na_2B_4O_7 \cdot 10H_2O$) of ACS grade was used from Fisher Scientific Company, Silver Spring, Md. FSH and LH-antisera were obtained from NIAMDD, National Institutes of Health, Bethesda, Maryland.

Subchronic Oral Toxicity of Boron. For each experimental group, 18 male rats were placed for a period of 30 or 60 days on dietary concentrations of borax of 0, 500, 1000, and 2000 ppm as boron equivalent. Borax was added to the basal diet on a weight to weight basis and mixed thoroughly. Throughout the study animals were housed two per cage and provided free access to food and water. Body weights and food consumption were determined at weekly intervals. At the end of each experimental period, five male rats from each experimental group were bred serially to test the functional capacity of sperm. To assay for plasma FSH, LH, and testo-

sterone, 5 to 6 ml of heparinized whole venous blood from jugular vein was collected from each of nine or ten rats per experimental group. Three animals were sacrificed from each experimental group for histology and to determine the weights of testis, epididymis, prostate, liver, spleen, kidney, heart, and lung.

Preparation and Assay of Selected Enzymes of Testes and Prostate Gland. At the end of each treatment period, three-male rats from each group were sacrificed by cervical dislocation. The testes were removed, weighed, and immediately placed in a prechilled Petri dish (0–4°C). To determine the enzyme activities in spermatogenic cells, the seminiferous tubules were separated from the Leydig cells according to Hall et al. (*26*). Methods used to determine enzyme activity have been previously described in this paper.

The prostate gland was also immediately dissected into an ice-cold 15 ml centrifuge tube and weighed, homogenized in five volumes of 10^{-3} *M* Tris buffer (pH 7.0), and centrifuged at 1000*g* for 30 min. The supernatant was filtered through a Millipore filter (0.45 μm), and the filtrate was used to assay for acid phosphatase activity (*27*).

Boron Determinations in Plasma and Testes. Boron levels in both plasma and testes were determined by ashing the tissue samples in the presence of sodium carbonate. After the fusion of the ash, the boron was determined directly in the residue by reaction with curcumin. Optical density was measured at 555 nm with a Spectronic-20 spectrophotometer. The limit of detection was 0.05 μg.

Radioimmunoassay of Plasma FSH, LH, and Testosterone. Eight to ten heparinized plasma samples from each treatment group were used to determine the plasma gonodotropin levels. Anti-FSH and anti-LH were supplied by the National Institute of Arthritis and Metabolic Disease, Rat Pituitary Program. NIAMDD-Rat FSH-RP-1 and NIAMDD-Rat LH-RP-1 were used as reference preparations. The sensitivity of the assay is 4 ng/ml for both FSH and LH with an intraassay variation of 10%. The details of assay for FSH, LH, and testosterone have been previously published (*28*).

In vivo *Assessment of Fertility.* At the end of each treatment period, groups of five male rats were maintained in individual cages and placed on the normal laboratory diet with free access to drinking water, and serial mating studies were initiated. Each male rat was housed singly with a virgin female for a period of 7 days. During each 7-day period, the female animals were examined daily for vaginal plugs to ensure that the treatment did not interfere with libido and mating capabilities. After the 7-day mating period, female rats were removed from the males and replaced with other virgin females. These breeding studies were usually terminated at the end of 12 weeks. Serial mating assesses the biological functionality of sperm cells and produces fertility patterns which are inversely related in time to the phase to spermatogenesis damaged by the chemicals tested (*29*). Pregnant rats were allowed to litter, the offspring assessed for any abnormalities, and development evaluated through weaning.

Histology of Testes and Epididymis. For histological studies of testes and epididymis, three male rats from each experimental group were sacrificed at the end of each experimental period (30 and 60 days). Testes and epididymis were removed and fixed in Bouin's solution, washed, dehydrated, and embedded in paraffin for sectioning. Slides were stained with periodic acid-Schiff and counterstained with Harris' hematoxylin as a nuclear stain. Measurements of seminiferous tubular diameters were carried out with a Leitz ocular micrometer attachment and microphotographs were taken. All other organs were fixed in 10% neutral formalin and the prepared tissue slides were stained with hematoxylin and eosin.

Statistical Analysis. Fisher's nonparametric test (*30*) was used to analyze effects of boron treatment on male fertility. The differences between control and boron-treated animals in other experiments were calculated by using the Student's *t*-test (*31*). The level of significance used was $p < 0.05$.

Results

Aluminum Study

The inclusion of aluminum chloride ($AlCl_3 \cdot 6H_2O$) in the drinking water of young adult male Sprague-Dawley rats at levels as high as 4476 mg/l. for a period up to 90 days did not result in any compound-induced abnormalities in the reproductive capacity of the males measured by histopathologic evaluation, plasma gonadotropin levels (Table 3) and serial mating of the males to untreated virgin females over a 70-day post-treatment breeding period (Table 4). The experimental data are contained in Hazleton Laboratories Report No. 421-112 (Dominant Lethality Study in Rats).

Lead Study

Data of the lead study are currently being evaluated.

Table 3. Plasma concentration of FSH and LH.[a]

Aluminum mg/l.	Duration of treatment, days	FSH, ng/ml plasma[b]	LH, ng/ml plasma[b]
Control	30	478 ± 145	15.3 ± 11.4
	60	375 ± 97	17.5 ± 9.5
	90	348 ± 102	17.4 ± 5.6
5	30	399 ± 100	10.5 ± 6.4
	60	326 ± 52	16.3 ± 7.8
	90	406 ± 98	11.7 ± 6.2
50	30	415 ± 65	15.1 ± 8.3
	60	357 ± 75	17.8 ± 8.0
	90	398 ± 111	9.8 ± 7.1
500	30	406 ± 121	13.6 ± 8.0
	60	363 ± 76	18.3 ± 8.7
	90	339 ± 72	10.6 ± 5.7

[a] *Treated groups were not significantly different from control.*
[b] Each value is the mean ± SD for seven rats.

Table 4. Summary of fertility data.[a]

Week	Aluminum, mg/l.	No. of implantation sites[b]	Average litter size[b]
4	Control	11.1 ± 4.3	10.3 ± 4.9
	5	12.7 ± 2.4	11.9 ± 2.5
	50	14.7 ± 1.2	13.7 ± 1.6
	500	13.8 ± 2.5	12.9 ± 2.8
8	Control	12.5 ± 0.8	11.8 ± 1.3
	5	13.3 ± 1.6	13.0 ± 1.6
	50	12.6 ± 1.4	12.4 ± 1.7
	500	12.4 ± 1.6	12.1 ± 4.0
10	Control	11.5 ± 3.2	9.9 ± 3.2
	5	12.4 ± 3.8	12.1 ± 1.3
	50	11.3 ± 3.2	10.9 ± 3.2
	500	11.7 ± 2.3	11.2 ± 2.7

[a] Treated groups were not significantly different from the controls.
[b] Each value is the mean ± SD of eight to ten rats.

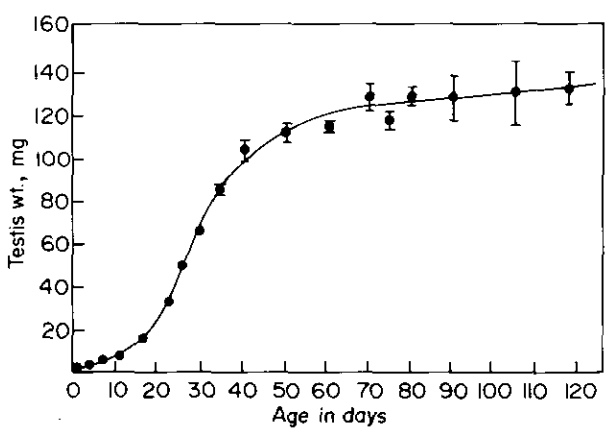

FIGURE 1. Postnatal development of mouse testis are measured by gain in gross weight. Results are expressed as means ± S.D. of three determinations. Points without bars indicate S. D. less than 2.70.

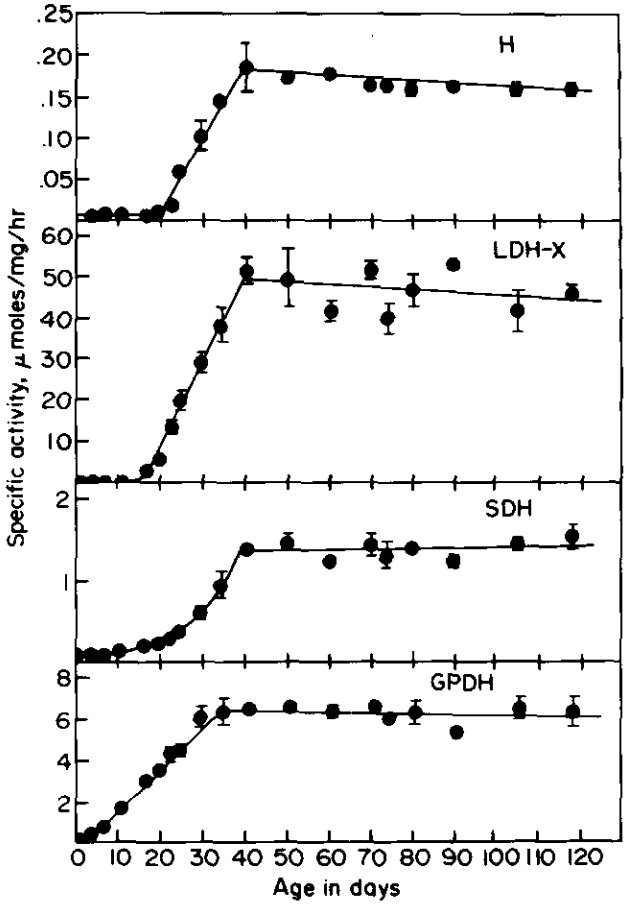

FIGURE 2. Enzyme developmental patterns of hyaluronidase (H), lactate dehydrogenase isoenzyme-X (LDH-X), sorbitol dehydrogenase (SDH), and α-glycerophosphate dehydrogenase (GPDH) in mouse testis. Results are expressed as means ± S.D. of three determinations.

Testicular Enzymes

Testis Weight. The development of testis as measured by increase in net weight is shown in Figure 1. Testicular weights increased slowly from 1 to 15 days of age. Maximum growth rates occurred between 20 and 40 days of age, followed by a slower rate of growth thereafter.

Changes in Activities of H, SDH, LDH-X, and GPDH during Development of the Testis. The developmental patterns of H, SDH, LDH-X, and GPDH in mouse testis are shown in Figure 2. In newborn mice activities of H and LDH-X were nearly nondetectable until 20 and 17 days of age, respectively. However, during the ensuing 20 days,

the specific activities of H and LDH-X rose markedly. After 40 days of age, the activities of H and LDH-X were changed little during the next 80 days.

Newborn mouse testis showed detectable levels of SDH and GPDH at 1 day of age. The activities of these two enzymes also increased rapidly until 35-40 days of age. Activities of these two enzymes then appeared to remain at constant levels for the period measured.

Changes in Activities of G6PDH, MDH, G3PDH, and ICDH during Development of the Testis. Figure 3 represents the developmental patterns of G6PDH, MDH, G3PDH, and ICDH in mouse testis. Enzyme activities were relatively high in the newborn and declined rapidly between 20 and 30 days (G6PDH, MDH, and G3PDH) and from birth to 20 days (ICDH). Activities of G6PDH and MDH rose to their highest levels within five days after birth, and these levels were maintained during the prepubertal period until 19 days of age. Similarly, the activity of G3PDH was highest in the newborn and prepubertal mice between 1 and 19 days of age. Figure 3 also shows that the activity of ICDH was highest in newborn mice although no plateau level was observed during the prepubertal period. Adult levels of G6PDH and MDH have a tendency to decline whereas those of G3PDH and ICDH are maintained at relatively constant levels. The adult steady-state levels of these four enzymes are reached at least 10 days earlier than those of H, LDH-X, SDH, and GPDH.

SDH Activities in Testis, Epididymis, and Vas Deferens. Spermatogenesis is a continuous process in which mature spermatozoa pass from seminiferous tubules into the epididymis and travel through the vas deferens. Figure 4 shows the specific activities of SDH in the testis, epididymis and vas deferens at various ages. Epididymal SDH activity was nondetectable until 35 days of age but increased rapidly thereafter. At the time when mature spermatozoa began to pass to the epididymis, the activity of SDH had increased dramatically to a maximal value at 49 days of age, remaining at that plateau until at least 76 days of age. Maximum levels of epididymal SDH activity were noted approximately eight days after testicular activity reached adult level. This period coincides with the time required for the spermatozoa to pass from the seminiferous tubules into the epididymis. However, epididymal SDH activity soon exceeded the testicular SDH activity by 3 to 4-fold, due to the high concentration of sperm in the epididymis.

Similarly, SDH activity in the vas deferens was not detectable until 41 days of age. Its activity then increased rapidly to a maximal value at approximately 56 days of age and subsequently remained unchanged. Again, the maximum increase in SDH activity lagged behind that of epididymis by seven days, as the first wave of sperms traversed through the vas deferens, and was lower than that of the testis. The specific activities of SDH in testis, epididymis, and vas deferens at steady-state were 1.17, 3.92, and 0.87 μmole/mg-hr, respectively.

G6PDH Activities in Testis, Epididymis, and Vas Deferens. Figure 5 shows the distribution patterns of G6PDH in mouse testis, epididymis, and vas deferens as a function of age. The development of testicular G6PDH activities is essentially the same as presented in Figure 3. The developmental patterns of G6PDH in epididymis and vas deferens before 20 days of age are not shown due to the difficulty in obtaining enough tissue for enzyme assays. However, after 20 days of age, epididymal G6PDH activities were seen to divide into two distinct stages of development similar to those in testis. The transition point was approximately at the age of 38 days. Thus, epididymal G6PDH activity was high and decreased rapidly from 20 days of age and was subsequently unchanged until 81 days of

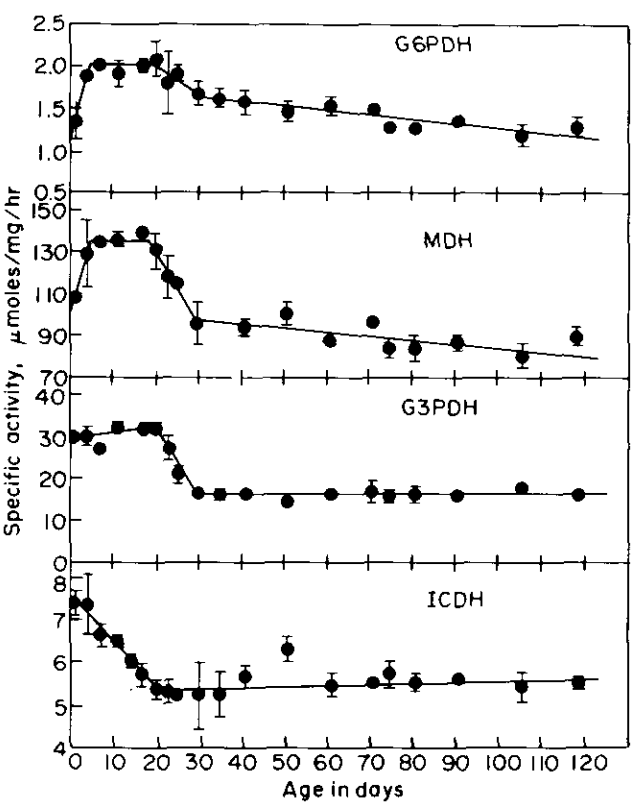

FIGURE 3. Enzyme developmental patterns of glucose-6-phosphate (G6PDH), malate (MDH), glyceraldehyde-3-phosphate (G3PDH), and isocitrate (ICDH) dehydrogenases in mouse testis. Results are expressed as means ± S. D. of three determinations.

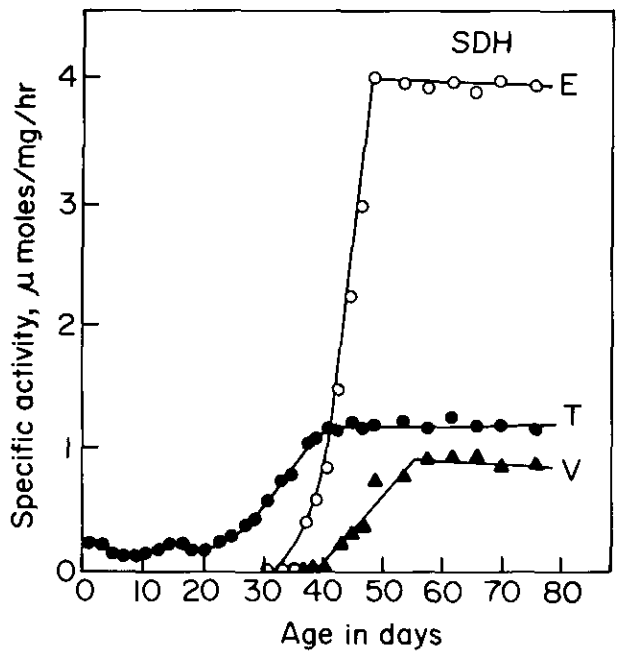

FIGURE 4. Distribution patterns of sorbitol dehydrogenase (SDH) in testis (T), epididymis (E), and vas deferens (V) of mouse. Results are expressed as means of triplicate determinations.

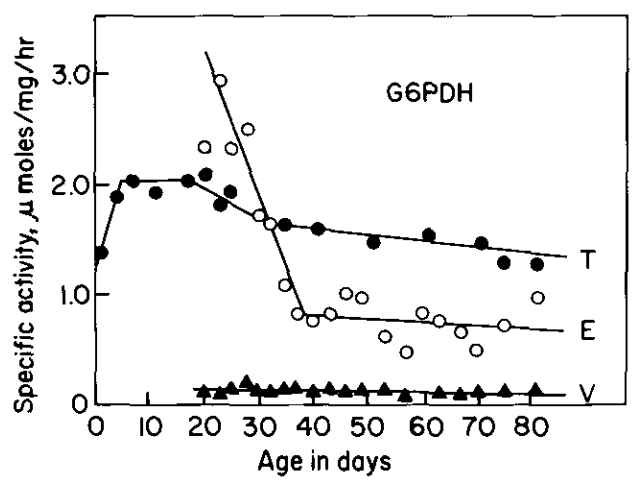

FIGURE 5. Distribution pattern of glucose-6-phosphate dehydrogenase (G6PDH) in testis (T), epididymis (E), and vas deferens (V) of mouse. Results are expressed as means of triplicate determinations.

age. In contrast, the activity of G6PDH in vas deferens was low and unchanged from 20 and 81 days of age. G6PDH activities in testis, epididymis, and vas deferens at 23 days of age were 1.89, 2.95, and 0.12 μmole/mg-hr, respectively; and at 40 days of age were 1.58, 0.75, and 0.12 μmole/mg-hr, respectively.

Histological Differentiation of Germ Cell Maturation. The histological changes in mouse testes from days 1 to 60 are illustrated in Figure 6. From 1 to 9 days of age, there appeared to be little spermatogenic activity in the germinal epithelium (*A-C*) while at 13 days of age there was an increase in both tubular size and the number of cells per tubule (*D*). An increase in the mitotic activity of spermatogonia is apparent. There was a progressive increase in tubular diameter from days 17 to 60 and a concomitant increase in the cellular content of the germinal epithelium (*E-I*). Numerous advanced premeiotic spermatocytes were present at 17 and 21 days of age (*E, F*) while spermatids progressed from the cap phase at 27 days (*G*) to the acrosome phase at 32 days of age (*H*). By 60 days of age, spermatogenesis was complete (*I*).

Boron Study

Subacute Oral Toxicity of Boron Compounds. Animals receiving boron in their diet at 500, 1000, and 2000 ppm for 30 days failed to show any significant differences in either rate of growth or the amount of food consumption when compared to control. Neither were selected organ weights of the treated and control group significantly different, except the weight of the epididymis. The epididymal weights of all treated groups were significantly lower than those of the control group (Table 5).

At the end of 60-day treatment period, in addition to significant decrease in the epididymal weights, the weights of the testes and liver were also decreased in the treatment groups receiving dietary boron at 1000 and 2000 ppm levels (Table 5). Organ weights in those treatment groups as compared to the control groups were 37.7 and 34.9% for the testis, 89 and 81% for the liver, and 62.6 and 70.7% for the epididymis, respectively. The weights of other organs including the prostate glands were not different from control.

Boron-Induced Testicular Atrophy. Decrease in testicular weight is attributed to extensive loss of the germinal elements as revealed by microscopic examinations. Atrophy of the testes is also reflected in a decrease in the mean diameter of seminiferous tubules (Table 6). Although mean diameter of seminiferous tubules from the 30-day treatment was not statistically significant, tubular diameter was significantly reduced in the 60-day treatment groups.

Effects of Boron on Selected Enzyme Activities of Testes. The subchronic effects of boron of the specific activity of three enzymes (H, SDH, LDH-X) associated with postmeiotic spermatogenic cell are presented in Table 7. A dose-response relationship was apparent, as boron exposure resulted

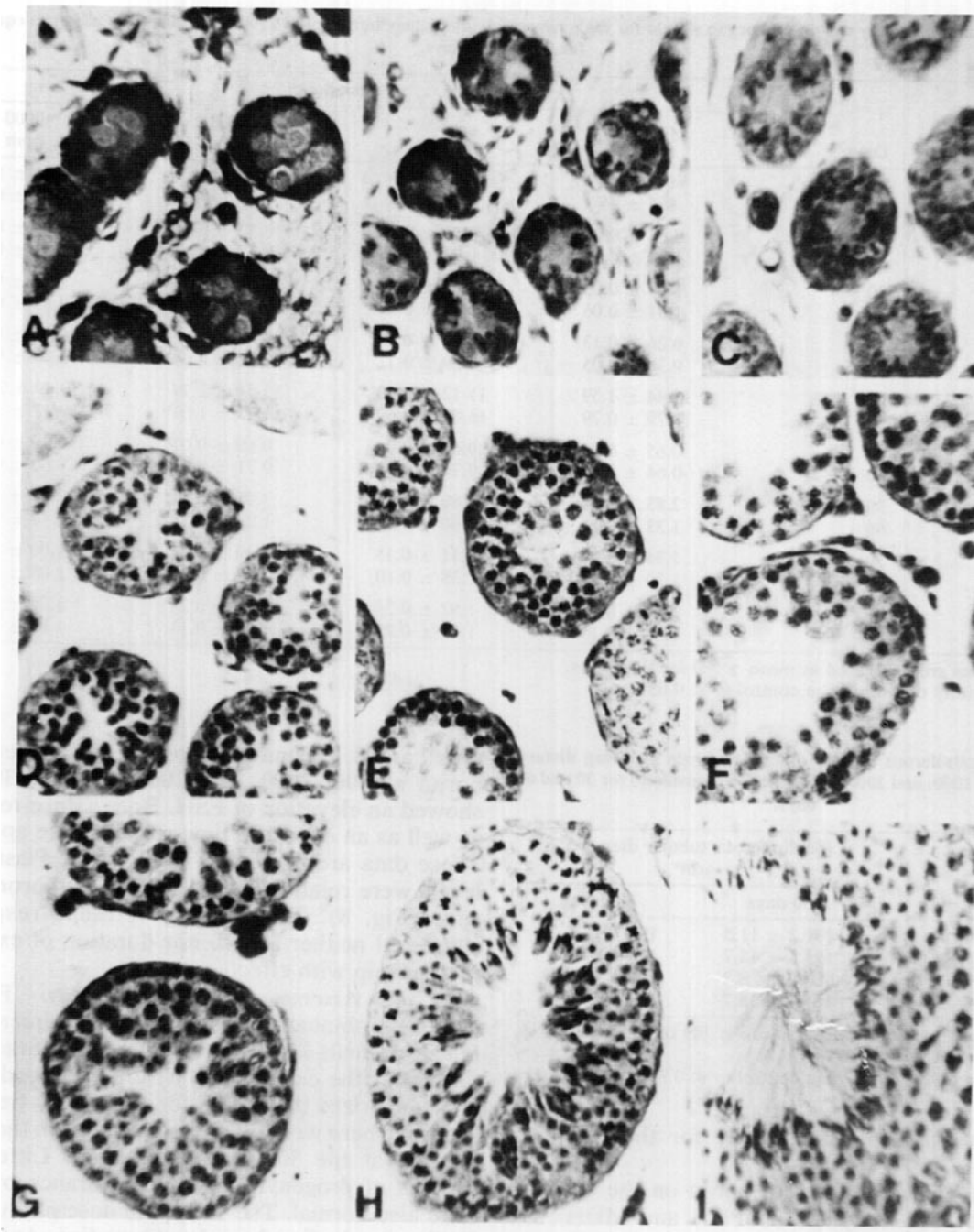

FIGURE 6. Changes in mouse testes. (A) At birth, seminiferous tubules are small and surrounded by an abundance of interstitial cells. Gonocytes containing giant nuclei occupy the lumen of the tubules, while supporting cells are located parietally along the basement membrane of the seminiferous tubules. (B) At 5 days of age, interstitial cells appear reduced in size, and most of the gonocytes have differentiated to immature type A spermatogonia. (C) At 9 days of age, the number of immature type A spermatogonial cells is markedly increased. (D) At 13 days of age, seminiferous tubules continue to enlarge. Sertoli cells become distinguishable, and most cells are still at the spermatogonial stage. A few tubules begin to exhibit early premeiotic cells. (E) At 17 days of age, spermatocytes become abundant within the seminiferous tubules. (F) At 21 days of age, the tubules are markedly enlarged coincident with increased number of spermatogonia and spermatocytes. (G) At 27 days of age, spermatids in cap phase are seen in the lumen. Spermatogonia are staged at periphery of the tubule in a single layer; spermatocytes are distributed in the second and third layer. (H) At 32 days of age, spermatids in acrosome phase fill the lumen. Spermatogenic cells differentiate in an orderly pattern; the more advanced cell types move progressively toward the tubular lumen. (I) At 60 days of age, complete spermatogenesis is achieved, with all cell types actively involved in differentiation (complete spermatogenesis is actually by day 35 in the mouse).

Table 5. Changes of body weight and organ weight for male rats receiving dietary borax (500, 1000, and 2000 ppm as boron equivalent) for 30 and 60 days.

		Weight, g[a]			
	Days	Control	500 ppm	1000 ppm	2000 ppm
Body weight	30	405 ± 28	416 ± 37	431 ± 28	381 ± 55
	60	464 ± 45	439 ± 42	394 ± 40	401 ± 63
Epididymis	30	0.64 ± 0.03	0.55 ± 0.04	0.52 ± 0.05[b]	0.45 ± 0.04[b]
	60	1.23 ± 0.06	1.23 ± 0.09	0.77 ± 0.41[b]	0.81 ± 0.34[b]
Testis	30	1.64 ± 0.05	1.73 ± 0.18	1.54 ± 0.18	1.54 ± 0.19
	60	1.81 ± 0.06	1.76 ± 0.19	0.68 ± 0.16[b]	0.63 ± 0.01[b]
Prostate	30	0.26 ± 0.13	0.31 ± 0.08	0.38 ± 0.06	0.25 ± 0.14
	60	0.36 ± 0.05	0.59 ± 0.12	0.42 ± 0.34	0.47 ± 0.39
Liver	30	13.44 ± 1.59	11.12 ± 1.00	13.51 ± 2.14	10.49 ± 2.60
	60	13.79 ± 0.79	16.09 ± 0.69	11.15 ± 1.03[b]	10.41 ± 1.28[b]
Spleen	30	0.65 ± 0.04	0.69 ± 0.04	0.69 ± 0.07	0.70 ± 0.13
	60	0.64 ± 0.04	0.74 ± 0.05	0.71 ± 0.02	0.60 ± 0.06
Kidney	30	2.83 ± 0.39	2.80 ± 0.10	3.05 ± 0.31	2.77 ± 0.69
	60	1.33 ± 0.11	1.60 ± 0.18	1.23 ± 0.18	1.40 ± 0.18
Heart	30	1.34 ± 0.07	1.11 ± 0.13	1.10 ± 0.05	1.00 ± 0.15
	60	1.20 ± 0.04	1.38 ± 0.10	1.07 ± 0.07	1.08 ± 0.13
Lung	30	2.04 ± 0.12	1.92 ± 0.25	2.1 ± 0.24	2.06 ± 0.47
	60	1.56 ± 0.15	1.73 ± 0.25	1.86 ± 0.13	1.89 ± 0.22

[a] All values are expressed as mean ± SD for three rats.
[b] Significantly different from control ($p < 0.05$).

Table 6. Seminiferous tubular diameter of rats receiving dietary borax (500, 1000, and 2000 ppm as boron equivalent) for 30 and 60 days.

	Seminiferous tubular diameter, μm[a]	
Boron, ppm	30 days	60 days
Control	181.2 ± 11.5	190.7 ± 5.2
500	193.3 ± 15.7	160.2 ± 10.4[b]
1000	180.0 ± 8.2	125.8 ± 8.0[b]
2000	164.1 ± 18.7	119.6 ± 12.4[b]

[a] All values are expressed as mean ± SD of 60 seminiferous tubules from each of three rats.
[b] Significantly different than control ($p < 0.05$).

in a decreased specific activity for the enzymes studied.

The subchronic effects of boron on the specific activity of two enzymes (G3PDH and MDH) associated with premeiotic spermatogenic cells are presented in Table 8. The specific activity of these enzymes was increased by boron treatment and correlated well with dose and duration of exposure.

Effects of Boron Exposure on Prostatic Acid Phosphatase Activity. Acid phosphatase activity in the prostate glands of boron treated groups was not different from that of the control groups (Table 9).

Plasma Gonadotropin and Testosterone Concentrations. Plasma FSH and LH measurements from control and experimental rats are presented in Figures 7 and 8. Plasma levels of FSH in rats receiving boron at 500, 1000, and 2000 ppm for 30 days showed an elevation of FSH. Both a dose-response as well as an exposure time-response are apparent. These data are presented in Figure 7. Plasma LH levels were relatively less affected by boron exposure (Fig. 8). The pattern of these responses suggested neither a dose nor duration of exposure relationship with effect.

In Vivo *Assessment of Male Fertility.* Fertility data were obtained by calculating the percentage of female animals defined as pregnant as a function of days after the end of each treatment period. Table 10 summarizes these data for the 30-day treatment groups. There were no adverse effects on the reproduction at the 500 ppm dose level. Litter size, weights of progeny, and the appearance of litters were also normal. The 1000 ppm dose produced infertility for the first 3 weeks of breeding. At the 2000 ppm dose, fertility was significantly reduced for 8 weeks with only a partial recovery observed after 7-9 weeks. Boron treatment had little effect on litter size.

Table 11 summarizes the fertility data for the 60-day treatment groups. As with the 30-day data, male fertility was not affected at the 500 ppm boron dose. At the 1000 ppm dose level, males were infertile for 4 to 5 weeks and subsequently returned to near normal. At the 2000 ppm boron levels, the fertility

Table 7. Testicular enzyme activities for rats receiving dietary borax (500, 1000, 2000 ppm as boron equivalent).

Boron, ppm	Duration of exposure, days[c]	Enzyme specific activity, μmole/mg-hr[b] H	SDH	LDH-X
Control	30	0.0188 ± 0.0002	1.07 ± 0.10	5.76 ± 0.16
	60	0.0276 ± 0.0022	1.43 ± 0.08	7.33 ± 0.04
500	30	0.0153 ± 0.0009[a]	1.02 ± 0.04	6.20 ± 0.30
	60	0.0223 ± 0.0015[a]	0.97 ± 0.04[a]	6.91 ± 0.36
1000	30	0.0153 ± 0.0006[a]	0.78 ± 0.03[a]	5.46 ± 0.18[a]
	60	0.0143 ± 0.0047[a]	0.18 ± 0.06[a]	0.80 ± 0.73[a]
2000	30	0.0128 ± 0.0010[a]	0.73 ± 0.03[a]	5.50 ± 0.41[a]
	60	0.0005 ± 0.0001[a]	0.12 ± 0.00[a]	0.06 ± 0.01[a]

[a] Significantly different from control ($p < 0.05$).
[b] All values are mean ± SD from three rats.
[c] Animals were fed food containing borax (boron) for 30 and 60 days.

Table 8. Testicular enzyme activities for rats receiving dietary borax (500, 1000, 2000 ppm as boron equivalent).

Boron, ppm	Duration of exposure, days[a]	Enzyme specific activity, μmole/mg-hr[b] G3PDH	MDH
Control	30	17.39 ± 1.07	53.75 ± 3.99
	60	25.82 ± 2.70	57.29 ± 2.34
500	30	21.81 ± 0.39[c]	61.99 ± 6.95
	60	24.02 ± 0.74	57.38 ± 5.01
1000	30	20.61 ± 0.86[c]	64.44 ± 2.28[c]
	60	31.74 ± 3.19	83.15 ± 4.68[c]
2000	30	21.86 ± 0.86[c]	63.47 ± 0.34[c]
	60	33.44 ± 3.00[c]	81.46 ± 7.96[c]

[a] Animals were fed food containing borax (boron) for 30 and 60 days.
[b] All values are mean ± SD from three rats.
[c] Significantly different from control ($p < 0.05$).

Table 9. Prostate acid phosphatase activities for rats receiving dietary borax for 30 and 60 days.

Boron equivalent, ppm	Acid phosphatase activity, μg/g-hr[a] 30 days	60 days
Control	1014.4 ± 287.1	1640.0 ± 628.5
500	1346.7 ± 368.2	1180.5 ± 294.6
1000	1053.5 ± 200.6	1718.3 ± 249.9
2000	1213.9 ± 216.1	996.9 ± 104.8

[a] All values are expressed as mean ± SD for three rats. Treated groups were not significantly different from control.

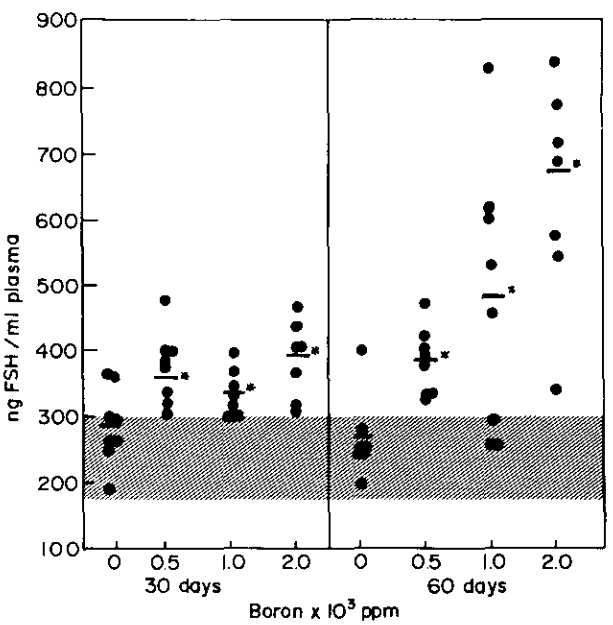

FIGURE 7. Plasma FSH concentration of rats treated with 500, 1000, and 2000 ppm boron for 30 and 60 days. Each solid circle (•) represents a single plasma FSH value for one rat. The mean values are indicated by the solid bar (—). The normal ranges of FSH values are depicted by the shaded bar.

pattern of the 60-day test suggested that treated males were sterile. The 60-day treatment group failed to show any evidence of recovery even 20 weeks after animals were placed on normal diets. Litter size throughout the 12-week period did not vary where more than one female of the group was pregnant.

Boron Levels in Plasma and Testes. The boron levels in plasma and testes of rats receiving dietary boron at 500, 1000, and 2000 ppm for 30 and 60 days are presented in Table 12. Boron levels in plasma and testes of control animals were found to vary between 0.1 and 0.3 ppm. Boron levels in the plasma and testes of treated rats increased with both dose and duration of exposure. Male rats receiving 1000 and 2000 ppm boron for 60 days exhibited higher boron concentration in testes than the plasma suggesting boron accumulation. From the fertility data, it can be estimated that a boron concentration of 6 to 8 ppm is associated with severe

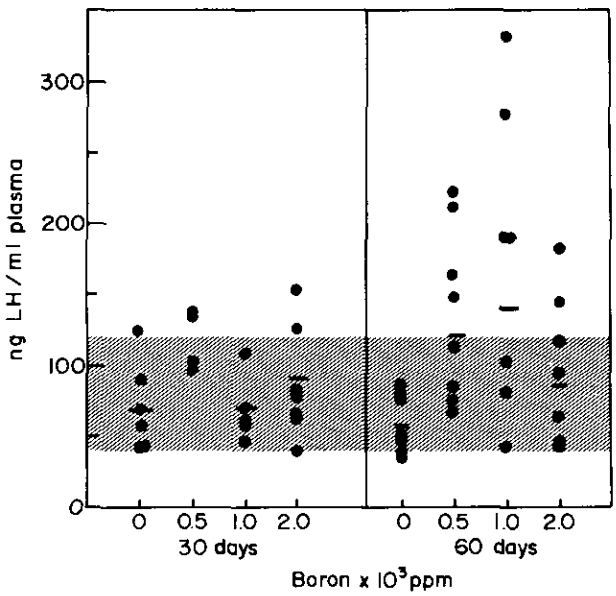

FIGURE 8. Plasma LH concentration of rats treated with 500, 1000, and 2000 ppm boron for 30 and 60 days. Each solid circle (•) represents a single plasma LH value for one rat. The mean values are indicated by the solid bar (—). The normal ranges of LH values are depicted by the shaded bar.

Table 10. Effects of borax (boron) treatment on male fertility (30-day treatment).

Exposure, weeks	Pregnancy, % 0	500 ppm	1000 ppm	2000 ppm	Average litter size 0	500 ppm	1000 ppm	2000 ppm
1	100	100	20[b]	0[b]	10.4	9.6	9.0	—
2	80	100	20[b]	0[b]	10.8	9.6	2.0	—
3	80	80	0[b]	0[b]	11.0	10.5	—	—
4	100	80	100	0[b]	10.6	12.5	9.8	—
5	100	100	60	0[b]	8.0	10.4	11.3	—
6	100	100	100	0[b]	10.5	9.2	9.8	—
7	100	100	100	25[b]	10.4	10.6	9.8	—
8	40	100	80	0	11.0	12.4	12.0	—
9	100	100	80	50	10.8	10.6	11.2	12.5
10	100	100	80	50	13.0	12.5	10.8	6.0
Overall	88	94	66	10	10.6	10.7	10.3	9.2

[a] Each treatment group was composed of five males with the exception of the 2000 ppm dose, which had four.
[b] Significantly different from control ($p < 0.05$).

germinal aplasia and concomitant infertility.

Histology. The test group receiving 2000 ppm boron for 30 and 60 days showed moderate to marked degeneration of germinal elements in the seminiferous tubules (Fig. 9). The testicular histology of the 30-day treatment group demonstrated a marked reduction in the seminiferous tubular diameter accompanied by a significant reduction in the numbers of pre- and postmeiotic spermatogenic cells. In the 60-day treatment group, the seminiferous tubular elements were extensively damaged.

Table 11. Effects of borax (boron) treatment on male fertility (60-day treatment).[a]

Exposure, weeks	Pregnancy, % 0	500 ppm	1000 ppm	2000 ppm	Average litter size 0	500 ppm	1000 ppm	2000 ppm
1	80	60	20	0[b]	11.0	12.3	4.0[c]	—
2	20	60	0	0	12.0[c]	9.0	—	—
3	100	100	0[b]	0[b]	10.2	9.2	—	—
4	100	80	20[b]	0[b]	11.8	13.0	3.0[c]	—
5	80	100	60	0[b]	12.0	10.8	13.0	—
6	100	100	80	0[b]	12.0	10.8	12.0	—
7	100	100	80	0[b]	10.2	11.4	11.2	—
8	100	100	80	0[b]	9.6	10.0	10.8	—
9	100	100	80	0[b]	11.4	9.2	11.8	—
10	100	100	80	0[b]	10.2	10.4	11.5	
11	80	—	80	0[b]	10.8	—	10.2	—
12	80	—	60	0[b]	12.5	—	11.3	—
Overall	87	90	52	0	11.0	10.6	10.9	—

[a] Each treatment group was composed of five males.
[b] Significantly different from control ($p < 0.05$).
[c] Based on one animal.

Table 12. Boron levels in plasma and testes of male rats receiving dietary borax (500, 1000, and 2000 ppm as boron equivalent) for 30 and 60 days.

	Exposure, days	Control	Boron, ppm[a] 500 ppm	1000 ppm	2000 ppm
Plasma	30	0.2	0.9	3.8	5.5
Testes	30	0.3	0.9	—	—
Plasma	60	0.1	2.6	4.3	6.8
Testes	60	0.3	2.1	6.1	8.5

[a] All boron values are the mean of two samples.

Most of the tubules were completely devoid of any normal spermatogenic cells. In spite of the extensive tubular damage, the Leydig cells appear normal. The extensive degenerative changes in the testes of this treatment group appeared to be irreversible.

Discussion

Exposure to drinking water containing as much as 500 ppm aluminum chloride for periods of 30, 60, and 90 days had no apparent effect on male reproductive processes. Indices of fertility as well as plasma gonadotropin levels were within normal ranges. Histopathologic evaluation of tissues also failed to reveal any treatment related lesions.

These results were similar to those obtained after exposure of male rats to boron in their drinking water at concentrations as high as 6 ppm for periods as long as 90 days. None of the reproductive pro-

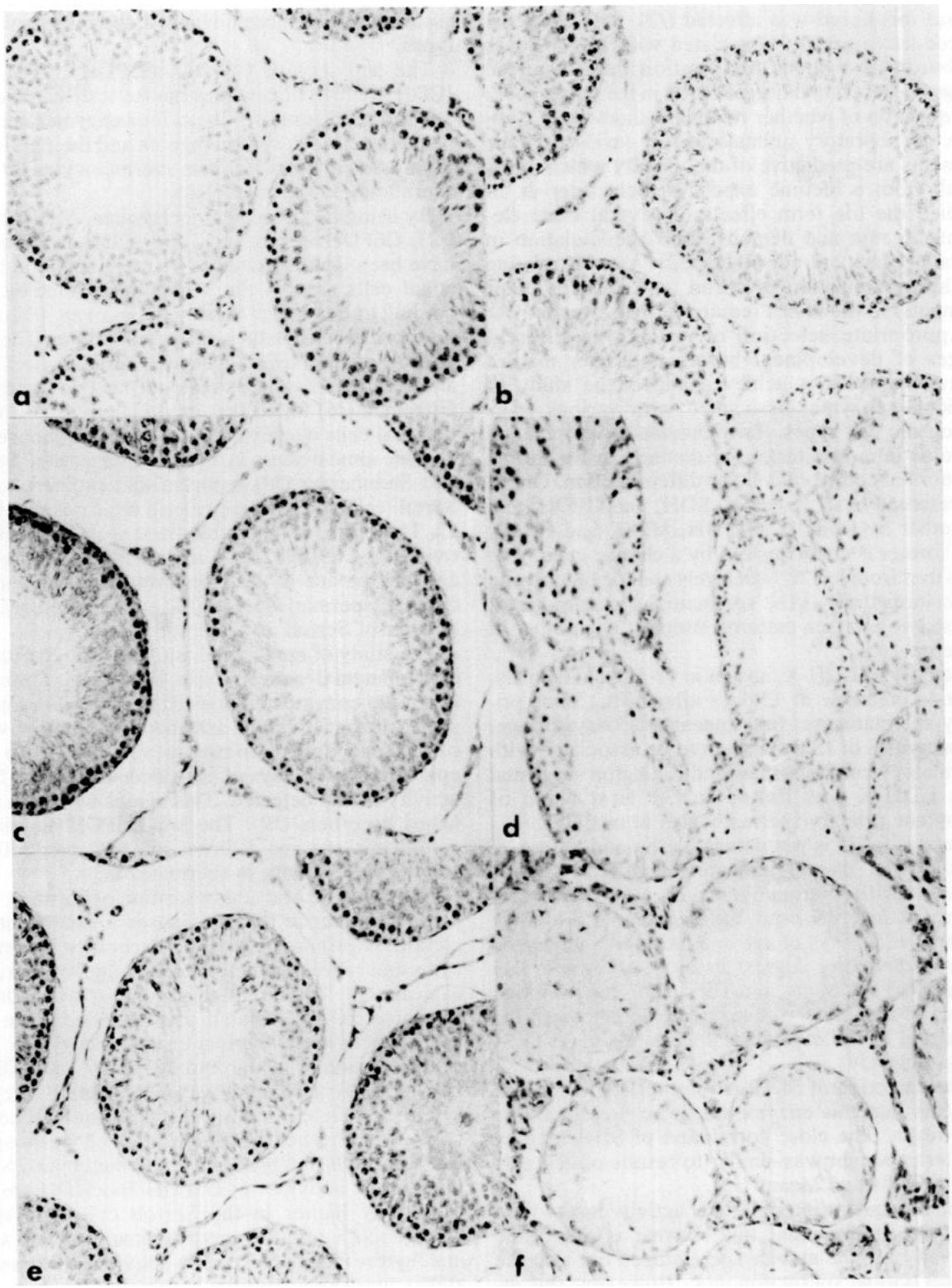

FIGURE 9. Testicular histology of normal and boron-treated rats (hematoxylin and eosin, x 160: (a) 500 ppm boron, 30 days; (b) 500 ppm boron, 60 days; (c) 1000 ppm boron, 30 days; (d) 1000 ppm boron, 60 days; (e) 2000 ppm boron, 30 days; (f) 2000 ppm boron, 60 days.

cesses monitored was affected (*12*). However, the reproductive toxicity associated with higher doses of boron as well as distribution data reported suggest an accumulation of boron in the testes poses the question of whether relatively short-term exposure of laboratory animals to low levels of trace elements are predictive of the toxicity which might result from a lifetime exposure. Schroeder et al. studied the life term effects of several trace elements in rats and demonstrated accumulation in certain organs but not others (*32*). Aspects relating to the accumulation of boron (and perhaps other elements) in the testes require further attention.

Appropriate selection of rodents at different stages of development before and after mature spermatogenesis is achieved allows the study of testicular enzymes associated with various spermatogenic cell types. Two enzyme developmental patterns in mouse testes are distinguishable during the normal course of cellular differentiation. One is represented by H, LDH-X, SDH, and GPDH; and the other by G6PDH, G3PDH, MDH, and ICDH. The former is characterized by a change in enzyme activities from low to high levels and the latter by an opposite pattern as the spermatozoa are produced. These two enzyme patterns intersect each other at puberty.

Activity of LDH-X, absent in 11-day-old animals, is first detectable at 17 days after birth. Since primary spermatocytes first appear at 15 days of age, the activities of LDH-X seem to be associated with spermatocytes. Blackshaw and Elkington suggested that LDH-X was linked to the first wave of pachytene primary spermatocytes in rats (*33*).

Activity of H is not detectable on and before 20 days of age, clearly indicating that H is not associated with spermatogonia or early spermatocytes. On the other hand, the detection of H activity as early as 23 days of age and the rapid increase in activity thereafter suggest its association with late spermatocytes. Males and Turkington reported that in rats H activity first became detectable with the formation of the cap phase spermatids at 33 to 34 days of age (*34*).

The detection of SDH activity in 1-day-old mice suggests that this enzyme might be present in the fetal testis. The close correlation of SDH activity and testis weight was similar to results obtained in rats by Mills and Means (*35*).

The demonstration of GPDH activity in newborn mice also implies that this enzyme is associated with not only the mature spermatozoa but also the differentiating spermatogonia. However, the increase in GPDH activity from 1 to 35 days of age, as other spermatogenic cells appear in the seminiferous tubules, seems to indicate that GPDH is also associated with more advanced spermatogenic cell types.

The activities of G6PDH, G3PDH, MDH, and ICDH are high in newborn mouse testes and low in adult mice. Therefore, these four enzymes seem to be more active in spermatogonia and their activities progressively decrease from spermatocytes through spermatids to spermatozoa.

By using histochemical techniques, MDH in rats (*22*), G6PDH in rats (*33*), and ICDH in mice (*36*) have been demonstrated to be more active in interstitial cells than in the seminiferous tubules. According to Baillie's (*37*) calculation, over 70% of the newborn mouse testis is interstitial tissue. The proportion of interstitial material decreases to 17, 6, and 4% at 1, 2, and 3 weeks of age, respectively. Hitzeman (*36*) found that mitotic activity of the interstitial cells decreased in the neonatal mouse and became almost static at 15 days of age. Steinberger and Steinberger (*38*) reported that proliferation of Sertoli cells in rat tissue ceased after postnatal day 15. Therefore, it is possible that the decrease in activities of G6PDH, MDH, and G3PDH at 20 days of age may be due to the transformation of spermatocytes to spermatids as well as a decreased relative number of Sertoli and interstitial cells.

The study of epididymal and vas deferen enzyme developmental patterns also provides information about the enzyme activities associated with mature spermatozoa. It can be assumed that the first wave of mature spermatozoa is released into the epididymis at 35 days of age since at that time SDH activity is first detected. This agrees with data published by others (*39*). The low G6PDH activity in epididymis and vas deferens in adult mice reflects low enzyme activity in spermatozoa.

The subacute oral administration of boron at 1000 ppm or greater in the diet causes a significant depletion of germinal elements, especially spermatocytes and spermiogenic cells following 30 or 60 days of treatment. The extent of germinal aplasia induced by boron is dose-related and is reflected in the specific activities of certain testicular enzymes. The near depletion of hyaluronidase, SDH, and LDH-X enzyme activities while G3PDH and MDH enzyme activities were elevated in the same animals corresponds with the disappearance of both spermiogenic cells and premeiotic spermatocytes. Since the specific activities of G3PDH and MDH are differentially higher in the Sertoli cells and spermatogonia than in the spermiogenic cells and spermatocytes, increased specific activities of these enzymes apparently reflects a depletion of more differentiated cells and the relative increase of spermatogonia. The extent of changes in specific activities of these enzymes and cellular changes was

dose dependent.

The depletion of spermiogenic cells was accompanied by a concomitant elevation of plasma FSH while neither plasma LH nor plasma testosterone levels were affected significantly. A selective elevation of plasma FSH without an alteration of plasma LH or testosterone level has also been reported associated with spontaneously occurring germinal aplasia (*40*) as well as chemotherapeutic agent-induced germinal aplasia (*41*).

From the *in vivo* fertility data, it is apparent that no dominant lethal effects were observable. The boron-induced infertility, therefore, is attributable to an antifertility effect rather than lethal mutations. The degree of tubular germinal aplasia is dose-dependent and reversible at all doses except the highest and longest exposure.

The minimum boron concentration in the testes of rats which induced complete germinal aplasia appears to be in the order of 6-8 ppm. Testicular boron concentrations were higher than that of plasma. Since boron absorption from the gastrointestinal tract is extremely low (1–3% of total daily intake) and the penetration to male germ cells is impeded by membrane barriers, relatively low concentration of boron in the plasma and testes would be expected. However, the possible accumulation of boron in testicular tissue needs to be investigated further.

Neither the exact localization of boron in the seminiferous epithelium nor the molecular mechanism by which boron induces germinal aplasia can be elucidated from the present studies. However, the boron-induced germinal aplasia can be attributed directly to its selective cytotoxic effects. It has been reported that elements of group IIIa of the periodic table, boron (B), aluminum (Al), gallium (Ga), indium (In), and thalium (Th) are cytotoxic in increasing order. These elements also exhibited a varying degree of antitumor effect against both Walker 256 carcinosarcoma and leukemia (*13*).

Further studies are necessary to document whether a certain trace element accumulates in male gonads during chronic exposure. It is also important to determine whether certain trace elements can potentiate the uptake of others. The minimum effective concentration and duration of exposure necessary for trace elements to affect male reproductive function need to be defined. Growing circumstantial evidence suggests that the unknown etiology of nearly half of the cases of male infertility might be attributed to chronic exposures to various environmental and occupational chemicals. Trace elements might be an important contributing factor.

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