

## Catalase mRNA Expression in the Male Rat Reproductive Tract

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**ABSTRACT:** Reactive oxygen species (ROS) have been shown to impair sperm function. The actions of ROS are reduced by antioxidant enzymes, including catalase. Although catalase-like activity has been demonstrated in semen, there has been no localization or characterization of catalase mRNA expression in the male reproductive tract. Catalase mRNA levels were evaluated by northern blot analysis and *in situ* hybridization from the male reproductive organs of normal 60-day-old rats, testes of 10- to 90-day-old rats, and testes of rats subjected to efferent duct ligation. Radioactive DNA probes were synthesized using a Klenow polymerase-based specific primer synthetic procedure with a known published sequence for rat catalase. All tissues demonstrated a single transcript of 2.5 kilobases (kb). Low levels of catalase mRNA were detected in the normal testis, epididymis, vas deferens, and prostate. No expression was detectable with northern analysis in seminal vesicle. The levels of catalase mRNA in reproductive organs were compared with the high levels of expression detectable in rat liver. In the testis, catalase

expression was primarily localized to peritubular and interstitial cells. In the epididymis and prostate, mRNA was detected in the epithelium. The observed decrease in catalase mRNA levels in the maturing rat testis is consistent with its interstitial localization. The increase in testicular catalase mRNA levels seen in parallel with progressive thinning of the germinal epithelium after efferent duct ligation is also in keeping with a peritubular or interstitial cell localization. The relatively low levels of catalase mRNA expression in the normal adult male reproductive tract undermine the role of catalase as a major antioxidant enzyme in these tissues. The low levels of catalase mRNA in the testis, and the undetectable levels in the seminiferous epithelium, however, imply that the germinal epithelium is predisposed to an oxidative state. These findings may help to explain the known susceptibility of the testis to oxidative stress.

**Key words:** Reactive oxygen species, antioxidants, testis, efferent duct ligation, seminiferous epithelium.

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Reactive oxygen species (ROS) such as the superoxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ) and the hydroxyl radical ( $OH$ ) are ubiquitous in aerobic biologic systems (Grisham and McCord, 1986). These are highly active and potentially cytotoxic substances derived from oxygen. Normally there exists a balance between ROS production and ROS degradation or scavenging, so that cells are protected against potential cytotoxicity and normal cellular function is maintained.

The implied importance of ROS in male infertility stems from studies that have shown that ROS are detected in the semen of 40% of unselected infertile patients, whereas none are detected in the semen of either normal or azoospermic men (Iwasaki and Gagnon, 1992). The cell types that generate ROS are primarily abnormal sper-

matozoa. However, neutrophils are also a source of ROS in semen and, furthermore, neutrophils can generate significantly higher levels of ROS than can spermatozoa. As such, neutrophils have a greater potential to induce spermatozoal damage than abnormal spermatozoa (Plante et al, 1994).

It appears that much of the ROS-induced damage is a result of spermatozoal membrane lipid peroxidation (Alvarez et al, 1987; Aitken et al, 1989). It has been shown that spermatozoa have a high content of polyunsaturated fatty acids on their cell membranes that render them especially prone to lipid peroxidation. In addition to peroxidative damage to lipids, ROS-induced axonemal ATP depletion may also contribute to the loss of sperm motility after ROS exposure (de Lamirande and Gagnon, 1992). Another proposed site of ROS-induced injury is at the level of the DNA, with the potential for abnormal sperm function and genetic defects (Fraga et al, 1991).

Although the excessive production of ROS may have detrimental effects on spermatozoa, the controlled release of these same ROS appears to modulate sperm function. At low concentrations,  $H_2O_2$  can promote sperm capacitation *in vitro*, although higher  $H_2O_2$  concentrations are toxic to spermatozoa (Griveau et al, 1994). Similarly, although prolonged exposure to superoxide decreases

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sperm motility, the initial exposure to superoxide causes spermatozoal hyperactivation (de Lamirande and Gagnon, 1993).

Catalase, superoxide dismutase (SOD), and glutathione peroxidase are known to specifically scavenge ROS (Grisham and McCord, 1986). Smaller-molecular-weight non-enzymatic substances can also react as ROS scavengers (Alvarez and Storey, 1983). There is evidence to suggest that catalase, which catalyzes the degradation of  $H_2O_2$  to oxygen and water, is involved both in modulating the levels of ROS to maintain normal sperm function and in protecting spermatozoa against potentially toxic ROS (Jeulin et al, 1989; Griveau et al, 1994). To the best of our knowledge, there is no published study demonstrating the specific localization of catalase mRNA in male reproductive organs.

In this study we examined the expression of catalase, a ROS scavenger, in the male rat reproductive tract tissues, using northern analysis and *in situ* hybridization. We evaluated the relative specific concentration of catalase mRNA in the testis, epididymis, vas deferens, prostate, and seminal vesicles. Testicular catalase was further characterized by studying catalase expression during sexual maturation and after efferent duct ligation.

## Materials and Methods

### Animals

Male Sprague Dawley rats were purchased from Charles River Laboratories (Wilmington, Massachusetts). The animals were housed at the Laboratory Animal Research Center (LARC) at Rockefeller University, and their care and use was in accordance with the NIH guidelines for animal care. Animals were caged under controlled lighting (14 hours light: 10 hours dark) and temperature (24°C). Food and water were provided *ad libitum*. The animals were killed at 10, 20, 40, 60, and 90 days of age by  $CO_2$  asphyxiation. The testes, epididymides, vasa, seminal vesicles, ventral prostates, and liver were removed. These organs were immediately frozen and stored in polypropylene tubes at  $-70^\circ C$  for RNA extraction. Reproductive tract organs were also embedded in Tissue-Tek cryoprotectant (Miles, Elkhart, Indiana) and snap frozen in 2-methyl butane.

### Efferent Duct Ligation

Adult (60-day-old,  $n = 16$ ) male rats were subjected to unilateral efferent duct ligation. After induction of anesthesia with an intraperitoneal (i.p.) injection of pentobarbitol (50 mg/kg body weight; Abbott Laboratories, North Chicago, Illinois), the right testis and epididymis were delivered via a lower abdominal incision. The right efferent ducts were doubly ligated with small metal clips after carefully dissecting away the nearby testicular blood vessels. After 1, 4, 8, 16, and 28 days, animals were sacrificed and testes harvested for RNA extraction and histologic evaluation. Sham-operated rats ( $n = 6$ ) were subjected to a similar lower abdominal incision with delivery of the testis-epidid-

ymis and sacrificed 4 ( $n = 3$ ) and 28 ( $n = 3$ ) days later. The effectiveness of the surgical procedure was evaluated by monitoring testicular weight and histology. At least two testes (separately processed for RNA extraction) were used for each time point.

### Northern Analysis

Total RNA was isolated using the lithium chloride/urea precipitation method (Auffray and Fougéon, 1980), and its concentration was quantified spectrophotometrically at 254 nm. Electrophoresis of extracted total RNA was performed on a 1.3% agarose formaldehyde gel, with 25  $\mu g$  of total RNA loaded per lane. An RNA ladder (Promega, Madison, Wisconsin) was simultaneously run on the same gel to determine the transcript lengths of the detected RNA species. RNA was transferred to the GeneScreen Plus membrane (Dupont, Boston, Massachusetts), followed by fixation using ultraviolet (UV) light, and then kept under vacuum at  $80^\circ C$  for 2 hours. The antisense  $^{32}P$ -labeled DNA probes were synthesized as described below. Hybridization was performed using a hybridization chamber (American Synthesis, Pleasanton, California) at  $42^\circ C$  for 14 hours, followed by high-stringency washing at  $42^\circ C$ . Relative concentrations of mRNA in each sample were determined by laser densitometry and calibrated against an internal control using a polymerase chain reaction (PCR)-amplified antisense  $^{32}P$ -labeled 655-mer glyceraldehyde 3-phosphate dehydrogenase (GAPDH) probe complementary to nucleotides 554 to 1220 (Tso et al, 1985).

### Probe Preparation

The method of probe preparation was modified after a published procedure developed in this laboratory (Schlegel et al, 1992). Antisense probes were prepared from oligonucleotide templates and specific primers as described below, using a known cDNA sequence for rat catalase. The selected sequence was analyzed using the Genbank database to ensure specificity of the probe. The 50-mer template sequence, derived from bases 1227 to 1276 of rat catalase cDNA (Nakashima et al, 1989), was synthesized on a gene assembler (Pharmacia, Piscataway, New Jersey) and was subsequently used for synthesis of the antisense probe. Specific 8-mer oligonucleotide primers, corresponding to the first eight base pairs (bp) in the desired probe sequence, were similarly synthesized. Following purification on reverse-phase columns, the templates and primers (400 ng each) were mixed and annealed by heating to  $95^\circ C$  for 10 minutes, to  $65^\circ C$  for 10 minutes, and maintained at  $42^\circ C$  for 10 minutes. The reaction mixture was brought to a total volume of 10  $\mu l$  with sterile water. This was followed by addition of 1.0  $\mu l$  each of 0.5 mM dATP, dGTP, and dTTP, as well as 3.9  $\mu l$  of  $10\times$  Klenow buffer (Boehringer-Mannheim, Indianapolis, Indiana), 20  $\mu l$  of  $^{32}P$ -labeled or  $^{35}S$ -labeled dCTP (6,000 Ci [110 Tbj]/mmol; Amersham Life Sciences, Arlington Heights, Illinois), and 2  $\mu l$  of Klenow polymerase (2 U/ $\mu l$  in glycerol [50% v/v; Boehringer-Mannheim]), and incubated for 2 hours at  $42^\circ C$ . The reaction was terminated by the addition of 1.6  $\mu l$  of 0.5 M ethylenediaminetetraacetic acid (EDTA). Labeled oligonucleotides were separated from unincorporated dCTP on a Sephadex G-50 column (Pharmacia, Uppsala, Sweden).



FIG. 1. Northern blot (representative of three experiments) showing catalase mRNA levels in 60-day-old rat testis (T), epididymis (E), vas deferens (V), ventral prostate (P), seminal vesicle (SV), and liver (L). The relative expression of the 2.5-kb catalase transcript is much lower in reproductive tissues than in liver.

### In Situ Hybridization

Five-micron sections were cut from the embedded specimens using a cryostat microtome (Bright Instrument, Cambridge, UK) at  $-20^{\circ}\text{C}$  and placed on glass slides precoated with poly-L-lysine (Sigma Chemical Co., St. Louis, Missouri). The sections were immediately fixed for 20 minutes with a freshly prepared 4% paraformaldehyde solution in phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 4.3 mM  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , and 1.4 mM  $\text{KH}_2\text{PO}_4$ , pH 7.4). Following treatment with proteinase K, triethanolamine, and acetic anhydride, tissue sections were covered with prehybridization solution containing 50% deionized formamide. Sections were then hybridized with either sense or antisense  $^{35}\text{S}$ -labeled oligonucleotide probes, prepared as described above. Slides treated with sense and antisense probes had the same total radioactivity. Prehybridization and hybridization were performed at  $42^{\circ}\text{C}$  for 2 and 16 hours, respectively. Following washing and dehydration, the slides were coated with a thin film of autoradiography emulsion NTB2 (Kodak, Rochester, New York). After storage at  $4^{\circ}\text{C}$  for 5 weeks, the slides were developed and stained with hematoxylin and eosin.

### Statistics

Values are expressed as means  $\pm$  standard deviation (SD). An unpaired *t*-test was used to determine the statistical difference between the grain counts on consecutive sections of antisense and sense *in situ* hybridization slides of rat epididymis, to confirm that the visibly weak signal in the epididymis was significant. Twenty antisense and 20 sense epididymal tubules were randomly selected, and the number of grains over analogous  $0.02\text{-mm}^2$  areas on consecutive sections were counted. These counts were adjusted by subtracting the background grain counts. A similar analysis was performed for the testis and prostate sections, although these tissues had visibly higher counts in the antisense compared to sense sections. A one-way analysis of variance (ANOVA) was used to determine the statistical differences between the testicular weights and the relative catalase mRNA levels assessed by northern analysis. *P* values of  $<0.05$  were considered statistically significant.

## Results

### Catalase mRNA Levels in Male Reproductive Organs

A single mRNA transcript of 2.5 kilobases (kb), of low abundance, was identified in the testis, epididymis, vas deferens, and prostate, but not in the seminal vesicle, us-

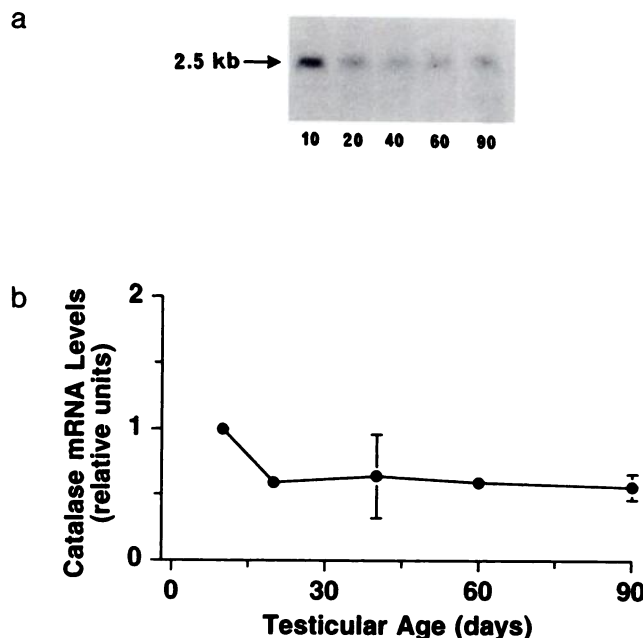


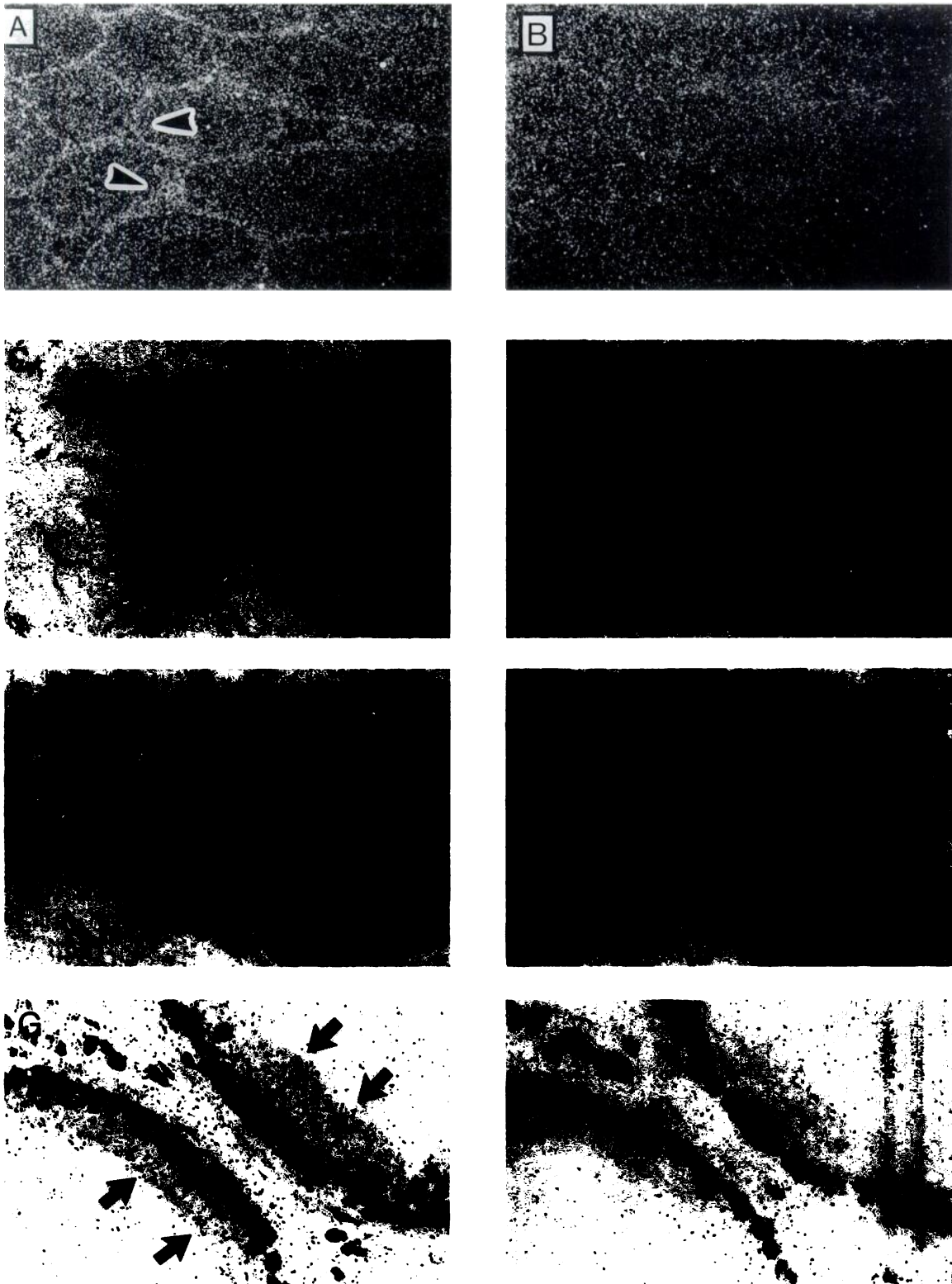
FIG. 2. (a), Northern blot of testicular catalase mRNA levels in the testes of 10- to 90-day-old rats (representative of two experiments). (b), Densitometric analysis of relative catalase mRNA concentrations in the testes of 10- to 90-day-old rats. Catalase mRNA concentrations were highest at 10 days of age, decreasing to about half that level by 20 days, and remaining relatively constant thereafter. The mRNA concentrations were adjusted using an internal control of glyceraldehyde-3-phosphate dehydrogenase expression.

ing the cDNA probe specific to hepatic catalase. In the liver, catalase mRNA concentration was high in comparison to the other tissues. The relative abundance of catalase mRNA, in decreasing order, was: liver (100%), prostate ( $13.7 \pm 10.3\%$ ), testis ( $11.4 \pm 5.9\%$ ), epididymis ( $4.9 \pm 4.1\%$ ), vas deferens ( $3 \pm 0\%$ ), and seminal vesicles ( $0\%$ ) (Fig. 1). These data were obtained from three separate sets of tissues.

The relative abundance of the 2.5-kb transcript was quantified in the testes of 10- to 90-day-old rats. Total RNA was extracted from 20 10-day-old, 10 20-day-old, 5 40-day-old, 5 60-day-old, and 5 90-day-old rat testes and pooled for preparation of RNA membranes. The level of testicular catalase mRNA expression was highest at 10 days of age, decreasing to about half that level by 20 days, and it remained relatively constant thereafter (Figs. 2a,b). The relative testicular catalase mRNA concentrations were significantly lower at 20, 60, and 90 days of age compared to 10 days of age ( $P < 0.05$ ). These data are representative of two experiments (two separate RNA extractions and northern analyses). The amount of RNA loaded per lane was normalized by evaluation of GAPDH expression.

### Localization of Catalase Expression in Rat Testis

In the 60-day-old rat testes, catalase mRNA was primarily interstitial and peritubular (Fig. 3A–D). The grain counts



**FIG. 3.** (A, B), Darkfield photomicrograph of a frozen section of a 60-day-old rat testis. (A), Antisense probe autoradiograph demonstrating mRNA localization in the peritubular and interstitial cell regions (arrows). (B), No specific localization of grains with sense probe. (C, D), Brightfield photomicrograph of a frozen section of a 60-day-old rat testis evaluated with (C) antisense and (D) sense probes, demonstrating catalase mRNA accumulation mainly in the peritubular and interstitial cell compartments (arrows). (E, F), Brightfield photomicrograph of a frozen section of a 60-day-old rat epididymis evaluated with (E) antisense and (F) sense probes, demonstrating catalase mRNA accumulation in the principal and basal cells of the epithelium (arrows). (G, H), Brightfield photomicrograph of a frozen section of a 60-day-old rat prostate evaluated with (G) antisense and (H) sense probes, demonstrating catalase mRNA accumulation in prostate epithelium (arrows). Magnification for A, B  $\times 64$ ; for C, D, G, H,  $\times 500$ ; for E, F,  $\times 640$ .

over the testicular interstitium were significantly greater in the antisense than in the sense sections ( $120.6 \pm 23.8$  vs.  $35 \pm 12.6$  grains per  $0.02 \text{ mm}^2$ , respectively,  $P < 0.05$ ). The resolution of the *in situ* hybridization technique did not permit, however, identification of the individual cells expressing catalase (i.e., Leydig cells, macrophages, and/or peritubular myoid cells). No specific localization of catalase mRNA to the seminiferous epithelium was detectable. These data are representative of two similarly performed *in situ* hybridizations on separate testes.

#### *Localization of Catalase Expression in Rat Epididymis and Prostate*

In the epididymis, catalase mRNA was primarily localized to principal and basal cells of the epithelium (Fig. 3E,F), and the grain counts were confirmed to be significantly greater in the antisense than in the sense sections ( $74.2 \pm 22.0$  vs.  $35.9 \pm 13.2$  grains per  $0.02 \text{ mm}^2$ , respectively,  $P < 0.05$ ). Similarly, in the prostate, catalase mRNA was detected primarily in the epithelium (Fig. 3G,H). The grain counts over the prostate epithelium were significantly greater in the antisense than in the sense sections ( $180 \pm 30.5$  vs.  $47 \pm 14.1$  grains per  $0.02 \text{ mm}^2$ , respectively,  $P < 0.05$ ). These data are representative of two similarly performed *in situ* hybridizations on separate sets of tissues.

#### *Testicular Catalase mRNA Levels After Efferent Duct Ligation*

The weight of efferent duct-ligated testes was significantly increased at 4 days after surgery when compared to age-matched sham-operated animals ( $2.26 \pm 0.23$  vs.  $1.49 \pm 0.01$  g,  $P < 0.05$ ). Sixteen days after efferent duct ligation, the right testicular weight was decreased compared to that of 60-day-old control rats ( $1.03 \pm 0.04$  vs.  $1.38 \pm 0.05$  g,  $P < 0.05$ ), and at 28 days, the reduction in testicular weight was significantly less than that of age-matched sham-operated controls ( $1.07 \pm 0.12$  vs.  $1.69 \pm 0.05$  g,  $P < 0.05$ ). Histologic examination of testes 1, 4, and 8 days after efferent duct ligation revealed marked distension of the interstitial space, as well as that of the lumen of the seminiferous tubules, as previously described by Wang et al (1973). At 16 and 28 days after efferent duct ligation, extensive atrophy of the seminiferous tubules and visibly little effect on the interstitium were observed histologically (data not shown). The relative abundance of the 2.5-kb transcript in rat testes subjected to efferent duct ligation is shown in Figure 4a and b. The level of testicular catalase mRNA at 1 day after efferent duct ligation was significantly less than that in 60-day-old control rats ( $0.50 \pm 0.01$  vs.  $1.0 \pm 0.04$  relative units,  $P < 0.05$ ). At 16 and 28 days after efferent duct ligation, catalase mRNA levels in the surgically li-

gated testes were significantly greater than those in 60-day-old control rat testes ( $2.85 \pm 0.30$  and  $2.60 \pm 0.40$  respectively, vs.  $1.0 \pm 0.04$  relative units,  $P < 0.05$ ). In a separate experiment, the relative catalase mRNA levels in sham testes (28 days after surgery) were compared to those of 60-day-old control testes and found to be similar ( $0.80 \pm 0.02$  vs.  $1.0 \pm 0.11$  relative units,  $P > 0.05$ ). In all of these experiments, the amount of RNA loaded per lane was normalized by evaluation of GAPDH expression.

## **Discussion**

Catalase is a 240-kDa tetrameric enzyme that has been identified in most mammalian tissues, generally occurring in subcellular organelles called peroxisomes (de Duve and Baudhin, 1966). This heme-containing enzyme is highly abundant in liver and specifically catalyzes the degradation of  $\text{H}_2\text{O}_2$  to oxygen and water. In this study we have detected a single 2.5-kb mRNA transcript in the testis, epididymis, vas deferens, prostate, and liver, using a cDNA probe for catalase. The levels of catalase mRNA transcript were significantly lower in male reproductive organs than in the control tissue, liver. This latter finding is in keeping with protein studies showing that in the testis, catalase-like enzymatic activity is about 5% of that measured in the liver (Peltola et al, 1992).

The decreasing specific concentration of catalase mRNA during testicular development is likely due to constant catalase expression in the somatic cell population that is diluted by the rapidly multiplying germ cell population. This is an expected observation for genes that are constitutively expressed by interstitial, peritubular, or Sertoli cells. Clermont and Perry (1957) showed that in the rat, the ratio of supporting cells to germ cells decreases with age, from about 10 to 45 days of age. The northern blot analysis of testes from 10- to 90-day-old rats and the *in situ* hybridization data together show that catalase mRNA is primarily localized to the interstitial and peritubular regions. This is in keeping with reports demonstrating catalase protein and activity in enriched Leydig cell preparations (Chamindrani Mendis-Handagama et al, 1992; Kukucka and Misra, 1993). The increase in testicular catalase mRNA levels seen in parallel with progressive thinning of the germinal epithelium after efferent duct ligation also supports a peritubular or interstitial cell localization (Wang et al, 1973; Risbridger et al, 1981). These data suggest that fluctuations in testicular catalase mRNA levels primarily reflect changes in the ratio of interstitial (and peritubular) cell volume to seminiferous tubule cell volume. However, whether testicular catalase mRNA expression and

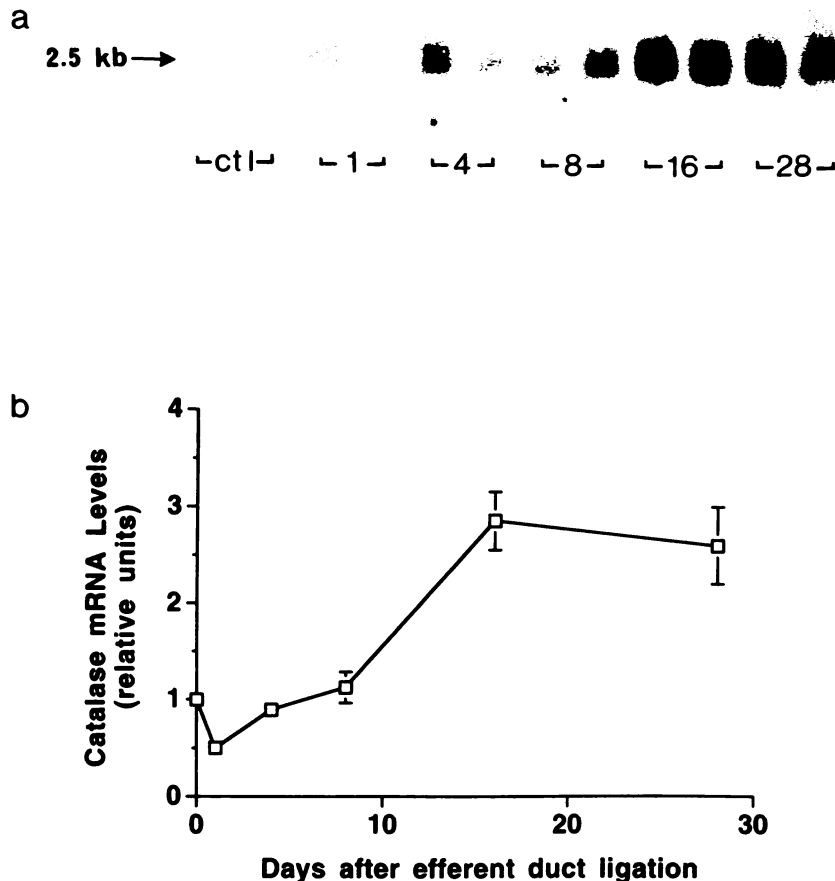


FIG. 4. (a), Northern blot of catalase mRNA levels in control testes and in testes 1, 4, 8, 16, and 28 days after efferent duct ligation. (b), Densitometric analysis of relative testicular catalase mRNA concentrations after efferent duct ligation. Catalase mRNA concentrations were decreased 1 day and increased 16 and 28 days after efferent duct ligation compared to control testes. The mRNA concentrations were adjusted using an internal control of glyceraldehyde-3-phosphate dehydrogenase expression.

mRNA levels are also specifically regulated by intratubular events remains unknown.

Catalase and glutathione peroxidase are the only two enzymes known to specifically and effectively scavenge  $H_2O_2$  (Grisham and McCord, 1986). The relatively low levels of testicular catalase and classical cellular glutathione peroxidase (GSHPx) expression (Zini, unpublished observations) suggest that the testis is ill equipped to adequately scavenge  $H_2O_2$ , and that consequently, production of high levels of  $H_2O_2$  in the testis may initiate significant peroxidative injury. This is substantiated by the finding that the expression of superoxide dismutase (SOD), the enzyme that catalyzes the conversion of the superoxide anion ( $O_2^-$ ) to  $H_2O_2$ , is higher in the testis, where it is primarily localized to the seminiferous epithelium, than in the liver (Jow et al, 1993). It has been demonstrated that the ratio of SOD to the combination of catalase and GSHPx activity in the testis is severalfold higher than in the liver (Peltola et al, 1992). It has also been demonstrated that a high ratio of SOD to catalase plus GSHPx will favor lipid peroxidation (El-

roy-Stein et al, 1986), and importantly, therefore, the undetectable levels of catalase mRNA in the seminiferous epithelium suggest that the germinal epithelium is exposed to oxidizing conditions. As such, it is predisposed to lipid peroxidation. Although excessive lipid peroxidation would have detrimental effects on the germinal epithelium, we speculate that the enzyme phospholipid hydroperoxide glutathione peroxidase (PHGPX), which specifically metabolizes lipid peroxides and is highly expressed in the seminiferous epithelium (Roveri et al, 1992), plays an important role in controlling the levels of lipid peroxidation in the testis. Taken together, these findings suggest that the relatively low levels of testicular catalase favor controlled lipid peroxidation of the germinal epithelium during spermatogenesis.

Low levels of catalase mRNA were detected in the epididymis and in the vas deferens. The functional role of catalase in the epididymal and vasal epithelium is unknown, but it is possible that in this setting catalase contributes to protecting spermatozoa from oxidative in-

jury. Whether catalase is secreted into the lumen of the epididymis and vas deferens or otherwise scavenges  $H_2O_2$  in the medium from the diffusion of  $H_2O_2$  into the epididymal and vasal epithelium is unknown. Epididymal and vasal catalase may also be important in suppressing the development of premature spermatozoal capacitation, which can be induced by  $H_2O_2$  *in vitro* (Bize et al, 1991; Griveau et al, 1994).

In the prostate, catalase mRNA was primarily localized to the epithelium. The physiologic role of catalase in the prostate remains speculative, but, as in the epididymis, prostatic catalase probably contributes to protecting spermatozoa from the induction of capacitation by  $H_2O_2$ . It is recognized that once spermatozoa are ejaculated they are exposed to a higher concentration of oxygen than that present in the lumen of the cauda epididymis (Alvarez and Storey, 1985). It is possible that prostatic catalase is important to protect ejaculated spermatozoa from ongoing oxidative stress. Jeulin et al (1989) demonstrated that human seminal plasma catalase is of prostatic origin, implying that catalase is secreted by the prostatic epithelium. Zini et al (1993) also detected catalase-like activity in seminal plasma, but much of this activity was nonenzymatic, and furthermore, some of the enzyme activity may have been attributable to the enzyme glutathione peroxidase. The findings of this study support the concept that catalase-like activity in semen is largely nonenzymatic and/or due to the activity of enzymes other than catalase.

To the best of our knowledge, this study is the first to demonstrate that male reproductive organs express catalase mRNA. The observed decrease in catalase mRNA in the maturing rat testis and the increase in catalase mRNA with progressive thinning of the seminiferous epithelium, taken together with the *in situ* hybridization data, suggest that catalase is primarily expressed in interstitial and/or peritubular cells. The low levels of catalase mRNA in the testis and the undetectable levels in the seminiferous epithelium imply that the germinal epithelium may be naturally exposed to oxidizing conditions. Although the presence of catalase expression in the testis, epididymis, prostate, and vas deferens suggest that these organs may function to protect spermatozoa from the effects of hydrogen peroxide ( $H_2O_2$ ), the relatively low levels of catalase expression in the adult male reproductive tract undermine the role of catalase as a major antioxidant enzyme in these tissues. Because rat sperm have limited production of ROS, only low levels of catalase expression may be needed to control these ROS. For infertile human males, the need for catalase action may be much greater. Investigation of human reproductive tract tissues are ongoing, and they may shed greater light on the potential role of catalase action or dysfunction on human male infertility.

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