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# Trichloroethylene exposure elicits damage in epididymal epithelium and spermatozoa in mice

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**Summary.** We have investigated the toxic effects of trichloroethylene (TCE) on the epididymis and epididymal sperm in mice. Mice were exposed to TCE (1000 ppm) by inhalation for 6 h/day for 5 days/week for 1 to 4 weeks. Segments of the epididymis (caput, corpus and cauda) were examined by light and electron microscopy. At the light microscopic level, degeneration and sloughing of epithelial cells were evident as early as 1 week after TCE exposure, and were most pronounced after 4 weeks. Such epithelial damage was observed in the caput, corpus and cauda regions of the epididymis. Ultrastructural observations revealed vesiculation in the cytoplasm, disintegration of basolateral cell membranes, and sloughing of epithelial cells. Sperm were found *in situ* in the cytoplasm of degenerated epididymal cells. Additionally, a large number of sperm in the epididymal lumen exhibited abnormalities including malformation of head and tail components. Our results demonstrated that exposure to TCE by inhalation causes damage to the epididymal epithelium and sperm.

**Key words:** Trichloroethylene, Epididymis, Sperm

## Introduction

Trichloroethylene (TCE) is a colorless, volatile liquid that is used widely in the automotive and metal industries for vapor degreasing and cold cleaning of metal parts (ATSDR, 1997). It is also a common intermediate in the production of polyvinylchloride and fluorochemicals, pharmaceuticals, flame-retardant chemicals, and insecticides (ATSDR, 1997). In addition, it is widely used in the textile industry in dyeing and finishing operations as well as for scouring cotton, wool, and other fabrics (ATSDR, 1997). A number of household products including adhesives and aerosol formulations, paint removers and strippers, typewriter correction fluids, spot removers, and rug-cleaning solutions contain TCE as one of their components (ATSDR, 1997). It is estimated that 3.5 million people are exposed occupationally to TCE (NTP, 1988, 1990). Others are exposed through the use of TCE-containing products or due to TCE contamination of the environment (NTP, 1988, 1990). The widespread use of TCE has resulted in its prevalence as an environmental contaminant in groundwater, soil, and air (ATSDR, 1997). It is one of the most common contaminants in landfill sites.

Many studies have been undertaken to elucidate the acute effects of TCE, and these include severe skin and eye irritation, dizziness, nausea, unconsciousness, and sometimes death (ATSDR, 1997). There is also an extensive database concerning sub-acute or chronic toxicity and carcinogenic effects of TCE exposure (see Scott and Cogliano, 2000 and monographs therein for a review). Several studies in humans and rodents indicated that TCE exposure is associated with impairment of male fertility. An early study evaluating fifty-five occupational categories for occupational infertility indicated that men who were involved in degreasing of engine parts appeared to have the highest risk of idiopathic infertility (Rachootin and Olsen, 1983). A later study reported that men exposed occupationally to TCE exhibited a reduction in sperm density (Chia et al., 1996) although the degree of effect in this study was minor. Studies in rodents have also indicated the presence of adverse effects of TCE on the male reproductive tract. Chronic inhalation exposure to TCE increased the incidence of Leydig cell tumors (Maltoni et al., 1988). Inhalation of TCE for 90 days also resulted in male infertility, reduction of testis size, and reduction in both the number of epididymal sperm and serum testosterone levels (Kumar et al., 2000, 2001). In mice,

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increased sperm abnormalities were found in animals exposed to TCE (2000 ppm) by inhalation for 4 h/day for 5 days/week for a total of 4 weeks (Land et al., 1981). In other studies, intraperitoneal injection of chloral hydrate, a primary TCE metabolite, but not TCE, caused an increase in the number of micronuclei in the germ cells of male mice (Allen et al., 1994). However, spermatotoxic effects were not detected in rats treated orally with TCE at concentrations of 10, 100 or 1000 mg/kg for 5 days/week for a total of 6 weeks (Zenick et al., 1984). These findings suggested that susceptibility to TCE-induced reproductive toxicity may be dependent and/or modulated by factors including dose, species and route of exposure as well as whether bioactivation is involved. In more recent studies, a decrease in the percentage of zona-free eggs fertilized by sperm has been reported in rats exposed to TCE in drinking water (DuTeaux et al., 2004). Similarly, exposure of mice to TCE by inhalation (1000 ppm) led to a significant decrease in their ability to fertilize eggs and for their sperm to bind to receptive zona-bearing eggs in vitro (Xu et al., 2004). Another study, using an identical inhalation regimen, showed that TCE exposure of mice for 4 weeks produced cytotoxicity in the epididymis but not in the testis (Forkert et al., 2002). Enhanced levels of CYP2E1, a primary enzyme involved in TCE metabolism, and formation of chloral, a major TCE metabolite, were found in the epididymis, suggesting that the epididymis is a target of the action of TCE (Forkert et al., 2002). In view of these findings, we have undertaken a series of experiments to investigate in greater detail, the histological changes induced by inhalation exposure to TCE in the epididymis and in epididymal sperm, at both the light and electron microscopic levels. Our results showed that epididymal epithelial cells as well as epididymal spermatozoa sustained cytotoxic damage, indicating that TCE elicits severe damage to male reproductive structures.

## Materials and methods

### *Materials*

Trichloroethylene (>99.5% purity) and glutaraldehyde (Grade I - 25%) were obtained from Aldrich Chemical Co, Montreal, Quebec, Canada. An Epon embedding kit was purchased from CANEMCO, Montreal, Quebec, Canada. All other chemicals used were of reagent grade and were purchased from standard commercial suppliers.

### *Animal treatments*

Male CD-1 mice (80-90 days old at the start of TCE exposure) were obtained from Charles River (St-Constant, Quebec, Canada). The animals were individually housed in polycarbonate cages, maintained on a 12 h light/dark cycle and were acclimatized to laboratory conditions for one week prior to TCE exposure. The animals were given free access to standard Purina rodent chow (Ralston-Purina, St. Louis, MO) and water ad libitum. All procedures relating to animal treatment adhered to guidelines stipulated by the Canadian Council on Animal Care and were approved by the Animal Committee of Health Canada prior to initiation of animal studies.

### *Inhalation exposure to TCE*

After acclimatization to laboratory conditions for one week, mice were transferred to inhalation chambers (2.5 $m^3$ volume) and housed individually in suspended stainless-steel wire-mesh cages with an integral food dish and a water spigot. During the exposure period, food containers were removed from the chambers to avoid consumption of any possible TCE-laden food. The chambers received HEPA and activated charcoal filtered air with standardized temperature (23±3°C) and humidity (50±10%) at a flow rate (500 liters/min) that allowed the entire chamber air to be replaced once every 15 min. Mice were exposed by inhalation to atmospheres containing a TCE concentration of 1000 ppm (5.37 mg/l). Five groups of 4 male mice each were exposed to TCE (1000 ppm) for 1 day, 1 week, 2 weeks, 3 weeks and 4 weeks. For the 1-day exposure group, the animals were exposed to TCE for 6 h for only 1 day before they were sacrificed. For each of the other four groups, the duration of TCE exposure was 6 h/day for 5 days/week. To generate the atmospheres, TCE was evaporated through a glass evaporative system with the resulting vapor being carried by an air stream into the chamber inlet and mixed with the incoming air. The concentration of TCE in the chamber was monitored every 10 min throughout the entire period of exposure by inline gas-chromatography (X-Tra Process gas chromatography, Amscor, USA) connected to the chamber through a multi-valve system (Douglas et al., 1999). The mean daily concentration of TCE in the chamber was found to range from 970 to 1,010 ppm. At the end of the intended exposure period, the flow of TCE was terminated without changing the flow rate of the incoming air stream. The animals were sacrificed on the morning after the final inhalation exposure to TCE. For each experiment, control animals were housed in an adjacent chamber and treated identically except that the TCE evaporating system was not connected to the air intake.

### *Tissue preparation*

Mice were sacrificed by cervical dislocation, and epididymides were rapidly excised. The tissues were immediately immersed into a fixative containing 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2. The tissue samples were kept overnight at 4°C in the fixative. After being fixed overnight in glutaraldehyde, epididymides were washed three times with 0.1 M PBS (pH 7.4), trimmed of adipose tissue and then separated into the caput, corpus and caudal segments. From each

segment, small cubes of approximately 1 $mm^3$ in size were prepared, processed and embedded in Epon according to standard procedures.

### *Preparation of tissue sections for light and electron microscopy*

One-micron thick tissue sections were first prepared from Epon blocks containing one of the three segments of the epididymis (i.e. caput, corpus, and cauda). One-micron thick sections were cut on a LKB ultramicrotome using glass knives. The sections were stained with toluidine blue and examined on a light microscope to locate areas of interest (i.e. areas showing signs of damage to the epididymis after exposure to TCE by inhalation). After the areas of interest were selected, ultra-thin sections of pale gold interference color were subsequently prepared with a diamond knife from the same Epon block and mounted on 300-mesh copper grids. All ultra-thin sections were counter-stained with uranyl acetate and lead citrate. Photomicrographs were taken on a Hitachi 7000 electron microscope operated at 75 Kv.

## Results

### *Histopathology of epididymis in mice after TCE exposure*

Light microscopic examination of one-micron thick sections of tissue samples of epididymis prepared from mice after inhalation exposure to TCE revealed evidence of damage to the epithelial cells in comparison to tissue sections prepared from control animals. The morphologic appearance representative of the tubules of the caput, corpus and cauda segments of the epididymis from animals after TCE inhalation exposure are shown in Figures 1-3. Damage to the epididymal epithelium was observed throughout the length of the epididymis as early as one week after inhalation exposure to TCE (Fig. 1a,b). The damage became progressively more severe as the time of inhalation exposure increased (Figs. 2a,b, 3a,b). In general, tubules that were affected by exposure to TCE showed fragments of epithelium sloughing off the tubules (Figs. 1a,b, 2a,b). Occasionally, clusters of epithelial cells were observed in the lumen of the epididymis (Fig. 1a,b). Epithelial cells in different stages of degeneration were also detected along the epithelium that remained attached to the rest of the tubule (Fig. 2a,b). Damage to the epididymis was most pronounced in mice after 4 weeks of TCE exposure. In these animals, cross-sectioned profiles of some epididymal tubules showed a complete absence of epithelium, indicating sloughing of large sheets of epithelium along the length of the epididymis (Fig. 3a,b). Examination of the seminiferous tubules in one-micron tissue sections revealed that, in mice after 4 weeks of TCE exposure, about 15% of the tubules in the caput, 21% in the corpus and 27% in the cauda showed either fragments of epithelial cells in the lumen or signs of disruption of the epithelium. Detailed morphologic changes, however, could not be seen clearly in the luminal sperm under the light microscope due to the inherent limited resolution of light microscopy. Tissue sections prepared from control animals showed healthy tubules with intact epithelial lining and no signs of structural damage. Figure 4 shows a representative photomicrograph from a control animal

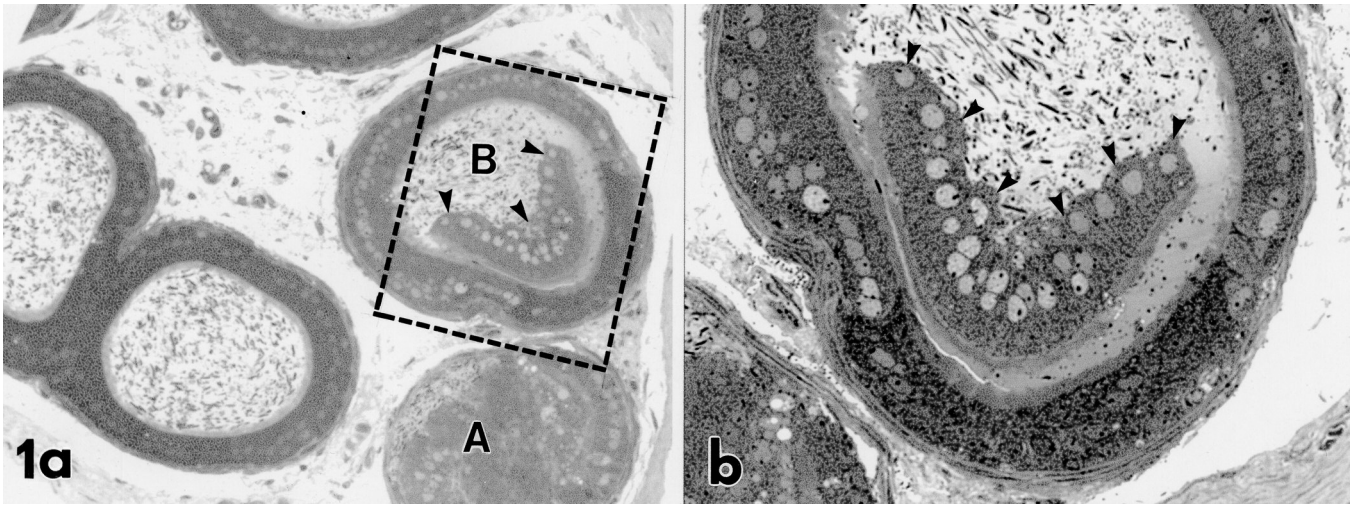


**Fig. 1. a.** Section of the corpus region of epididymis from an animal exposed to TCE for 1 week showing cross-sectioned profiles of the epididymal tubule. Note that in one cross-sectioned profile (A), a large portion of the epithelial lining appears to be separated from the underlying basement membrane. In another cross-sectioned profile (B), a fragment of epithelium (arrowheads) can be seen in the lumen. **b.** Higher magnification of the framed box (a) showing a fragment of the epithelial lining. The point of detachment of epithelial cells (arrowheads) from the tubule appears to be located at the region where the cells are normally in contact with the basement membrane. a, x 260; b, x 460

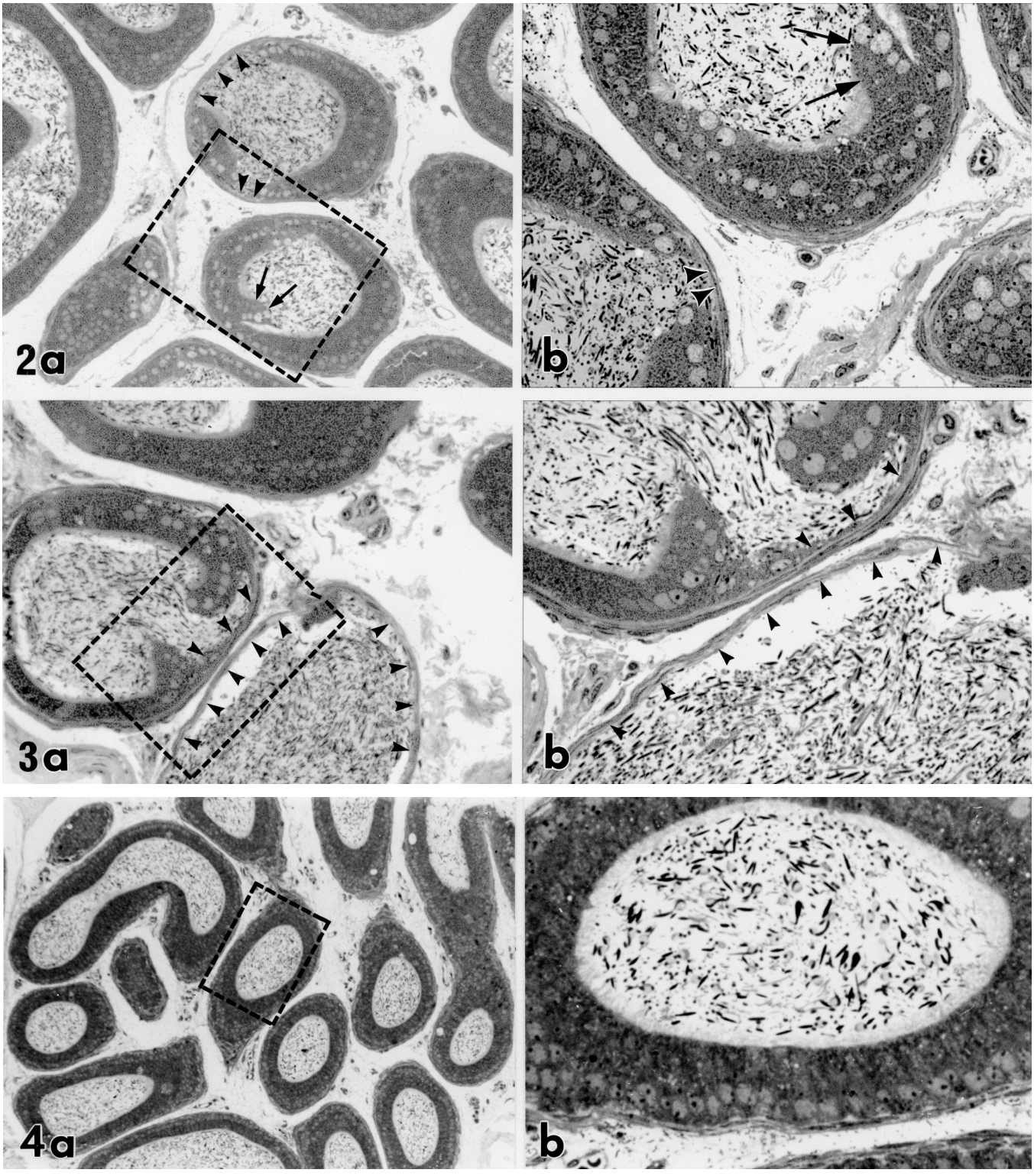


**Fig. 2. a.** Section of the caput region of epididymis from an animal exposed to TCE for 2 weeks showing the epididymal tubule in different planes of sectioning. In a cross-sectioned profile, the epithelial lining (arrows) can be seen sloughing off the tubule. An adjacent tubule also shows signs of disruptions (arrowheads) along the epithelium. **b.** High magnification of the framed box (a) showing fragment of epithelium (arrows) sloughing off the tubule and an adjacent tubule with disrupted epithelium (arrowheads). a, x 260; b, x 500

**Fig. 3. a.** Section of the cauda region of epididymis from an animal exposed to TCE for 3 weeks showing severe damage. Most of the cross-sectioned profiles of the tubule are either partially bare of epithelial lining or are completely devoid of epithelium (arrowheads). **b.** Higher magnification of the framed box (a) showing the bare region (arrowheads) of two epididymal tubules. a, x 260; b, x 450

**Fig. 4. a.** Section of the caput region of epididymis from a control animal showing normal histology of the epididymal tubule. **b.** Higher magnification of the framed box (a) showing a cross-sectioned tubule lined by an intact pseudostratified columnar epithelium with stereocilia. a, x 110; b, x 560

sacrificed after being kept for 4 weeks in the inhalation chamber without the TCE evaporating system connected to the air-intake.

*Electron microscopic examination of epithelial damage and morphologic alterations in sperm*

To assess more detailed morphological changes that occurred in the epididymis and luminal sperm after TCE exposure, ultra-thin sections were utilized. Examination of thin sections of caput, corpus, and cauda segments of epididymides obtained from mice after 1 day, 1, 2, 3, and 4 weeks of TCE exposure confirmed the results observed in one-micron thick sections and further revealed details of cellular damage to the epithelium and luminal sperm. Since various levels of damage to epithelium in the epididymis occurred at all five time-points studied, only representative photomicrographs from mice after 1, 3 and 4 weeks of exposure to TCE are shown here. Damage to the epithelium was evident after 1 week of TCE exposure as fragments of epithelium, mainly principal cells, could be seen separating from the basal lining remaining with the tubule (Fig. 5). Sheets and clusters of epithelial cells were also found in the lumen

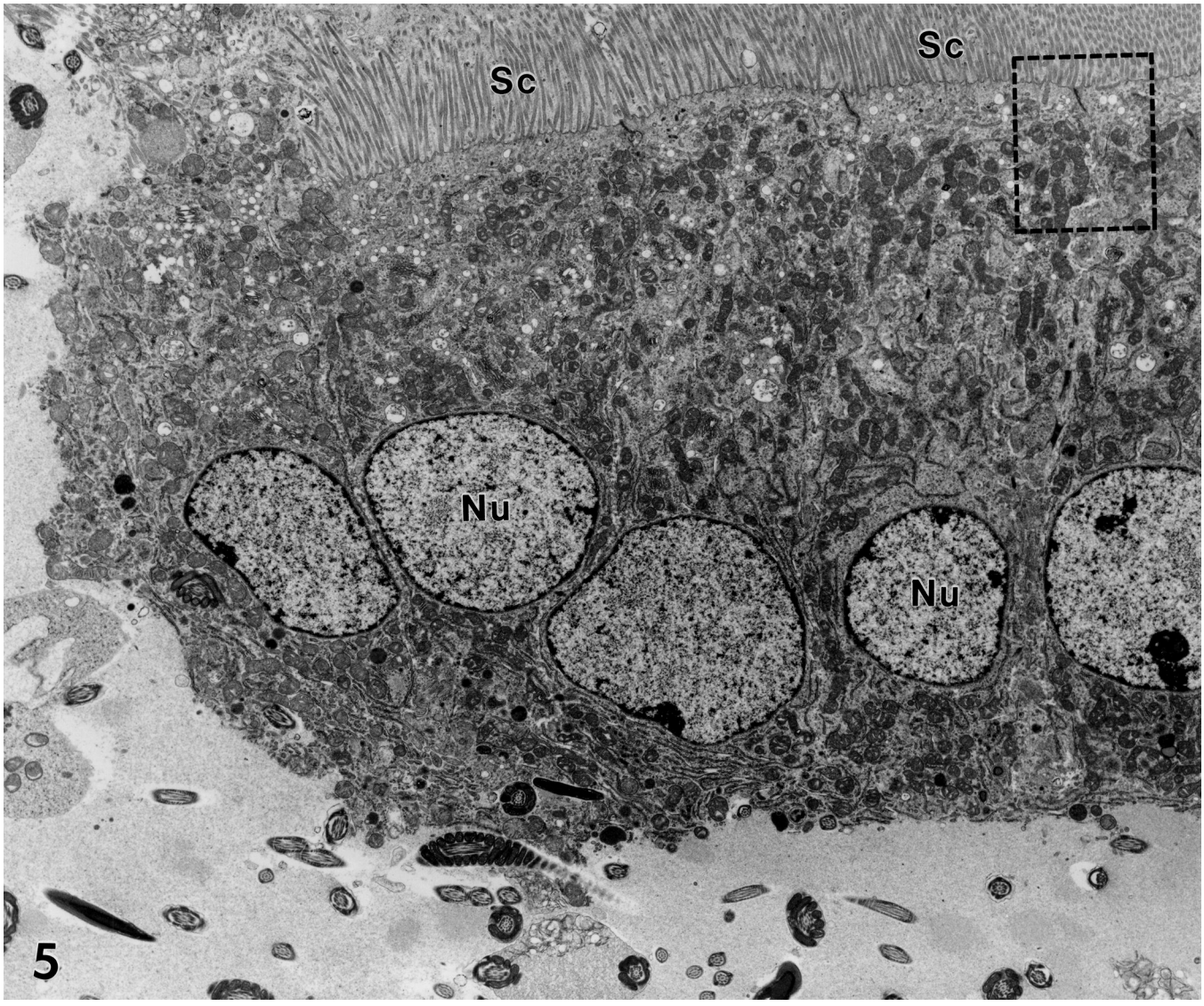


**Fig. 5.** Electron photomicrograph showing a portion of the epithelium sloughing off from an epididymal tubule in the corpus region of the epididymis of an animal exposed to TCE for 1 week. Many spermatozoa in different planes of section can be found in between the basal region of the cells and the underlying basement membrane. Even at this low magnification, disorganization of mitochondria can be seen in some the of cross-sectioned profiles of the mid-piece of the sperm tails. Sc: stereocilia; Nu: nucleus. x 5,300

(not shown). Sloughing of cells from the epithelium appeared to start initially at the level of the basal cell membrane although signs of disintegration were noted along the lateral cell membrane of principal cells. Many epididymal sperm in different planes of section could be seen in the luminal space and many displayed abnormalities in disruption of plasma membrane of the sperm head and disorganization of mitochondria in the sperm tail (Fig. 5). At a higher magnification, foci of disruption could be seen along the lateral cell membranes separating two adjacent principal cells (Fig. 6). The separation of the basal membrane from the underlying tissue and cellular components, and the breakdown of lateral membranes between adjacent cells resulted in the sloughing of isolated cells or large fragments of cells from the epithelium (Fig. 6). The disruption of lateral cell membranes was also observed in animals exposed to TCE for 2 weeks (Fig. 7). Concomitant with the breakdown of the lipid bilayer at the level of the basolateral cell membranes was the

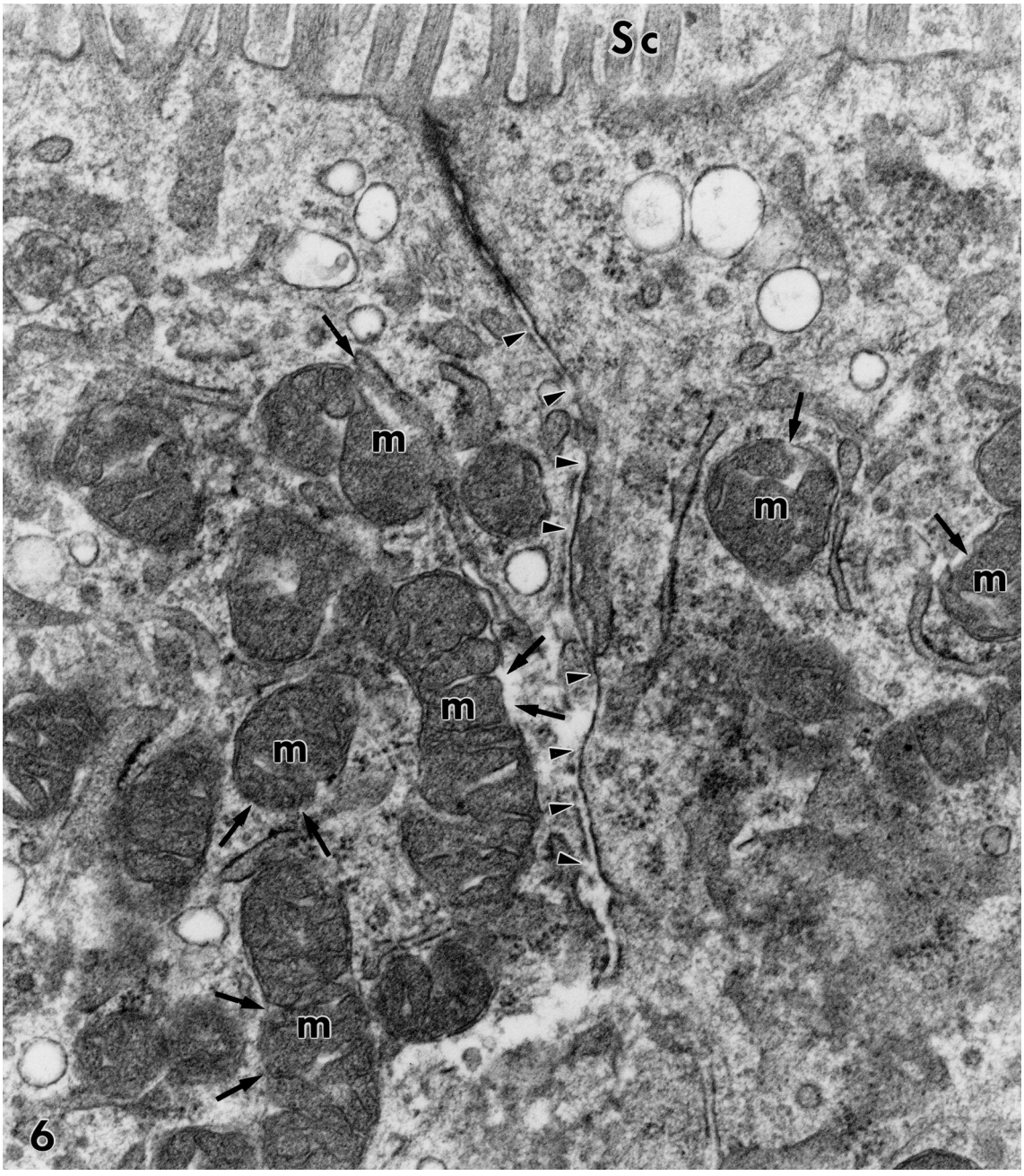


**Fig. 6.** High magnification electron photomicrograph showing the framed box in Fig. 5. Note the breakdown of the lateral cell membranes (arrowheads) demarcating two adjacent epithelial cells. Disintegration of the outer and inner mitochondrial membranes (arrows) is also evident in many mitochondria (m). Sc: stereocilia. x 51,400

occurrence of disorganization and morphologic changes in the cytoplasm. Within the adherent epithelium, some cells showed signs of degeneration (Figs. 7, 8). These degenerated principal cells are characterized by the presence of large vacuoles in the cytoplasm (Figs. 7, 8). Changes observed in the cytoplasm include fragmentation of Golgi saccules (Fig. 7), disruption of mitochondrial membranes (Fig. 8), and formation of large vacuoles (Figs. 7, 8). In the epididymis of animals exposed to TCE for 2 weeks, some epithelial cells exhibited almost complete disintegration (not shown). As a result of these morphologic alterations, epididymal sperm in different planes of the sections were observed in the cytoplasm of the degenerated cells or between fragments of epithelium and the underlying basement membrane and connective tissue. The most severely damaged epididymides were found in animals exposed to TCE for 4 weeks; many tubules were found to be completely devoid of their epithelium (Fig. 9). Electron microscopic examination of tissue thin-sections revealed the adverse effects of TCE exposure on sperm morphology and ultrastructure. Electron photomicrographs show epididymal sperm lying directly above the barren basement membrane and the

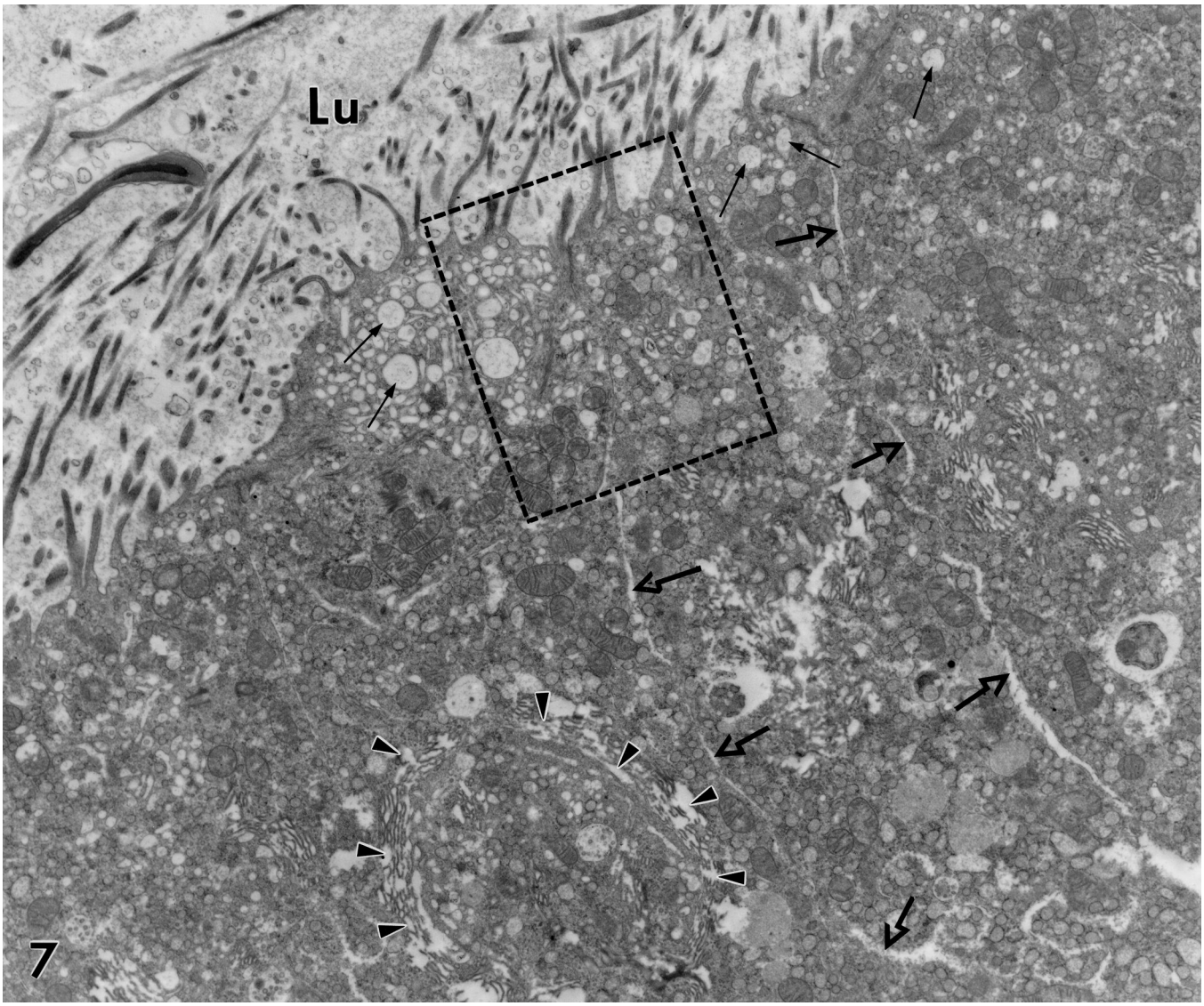


**Fig. 7.** Electron photomicrograph showing morphologic alterations of the principal cells in the caput region of the epididymis following 2 weeks of inhalation exposure to TCE. Dissolution of appositional plasma membranes has created large gaps (open arrows) between adjacent cells. Inside the cells, the appearance of vesiculation and large vacuoles (arrows) as well as swelling of the Golgi saccules (arrowheads) are prominent features indicative of cell damage. Lu: lumen. x 8,400

underlying connective tissue layer (Fig. 9). Pronounced abnormalities of different structural components involving the sperm head and tail were detected in epididymal sperm (Fig. 9: Inset). In sections of epididymides prepared from control animals that were not exposed to TCE, the epithelial cells displayed normal histology with no signs of damage and morphologic alterations were not found in epididymal sperm in any of the control animals (results not shown).

## Discussion

A recent study carried out in mice on the toxicity of TCE in the testis and epididymis following inhalation exposure (1000 ppm) indicated that the epididymis is a specific target of TCE but the testis was not affected (Forkert et al., 2002). However, the extent to which the epididymis is adversely affected by inhalation exposure to TCE and details of morphological changes occurring

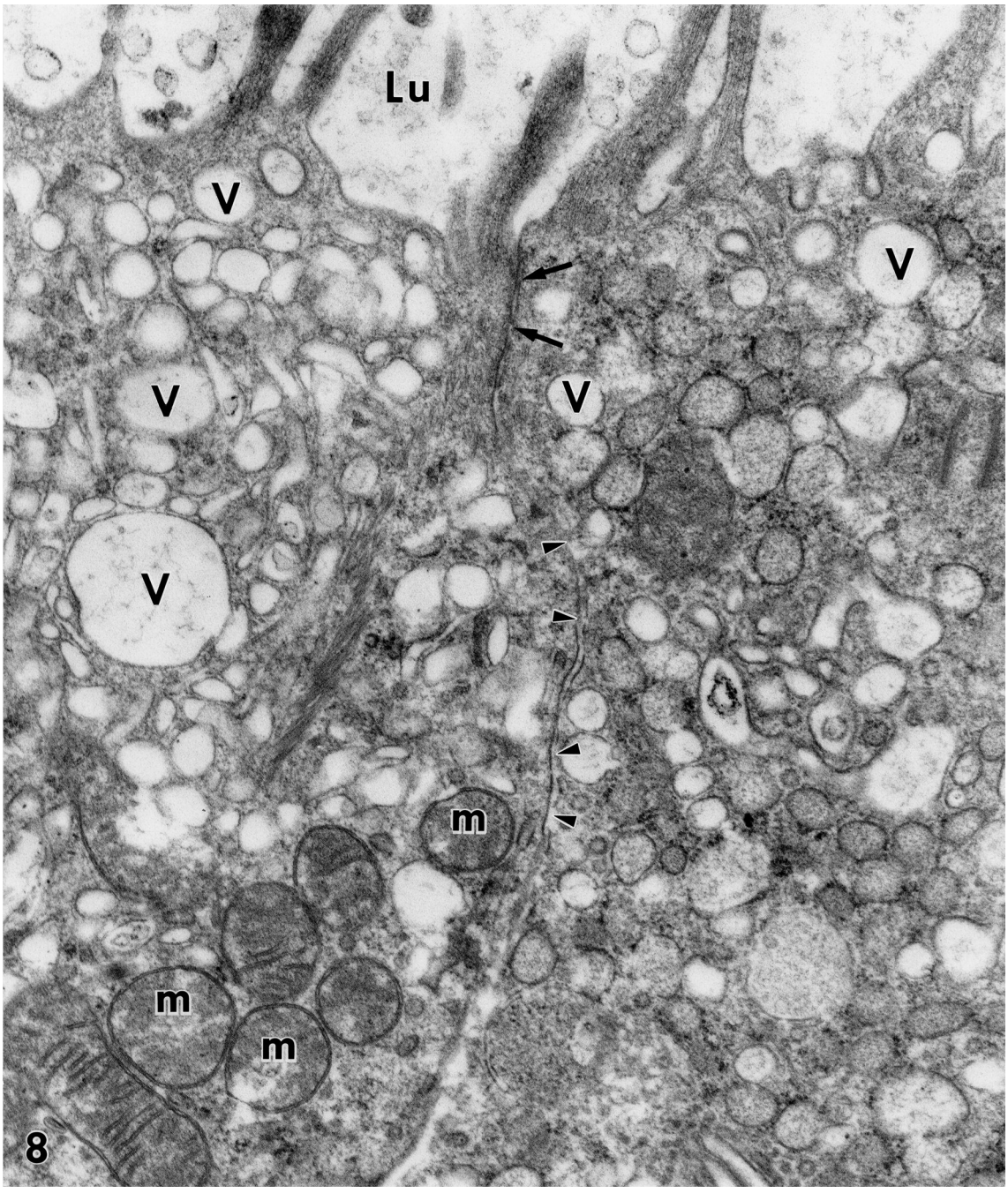


**Fig. 8.** High magnification electron photomicrograph of the famed box in Fig. 7 showing the apical region of two adjacent principal cells. Vesiculation of the apical cytoplasm takes the form of numerous vacuoles of various sizes (V). The tight junction (arrows) between the two cells shows foci of interruptions while disintegration of the lipid bilayer also occurs along the lateral cell membranes (arrowheads). Several mitochondria (m) nearby show signs of degeneration. Lu: lumen. x 38,600

in the tissue and luminal sperm have not been examined in detail. Our earlier preliminary study suggested that TCE did not elicit an effect on the epididymis until after four weeks of exposure (Forkert et al., 2002). The results in the present study showed a different temporal appearance of defects and demonstrated that subacute inhalation exposure to TCE (1000 ppm) causes extensive damage and exfoliation of the epithelial layer in the epididymis. In addition, a large number of morphologically abnormal sperm was detected in the lumen of the epididymis after inhalation exposure to TCE, indicating that epididymal sperm were susceptible to the toxic effect of TCE. These observations represent novel findings of TCE toxicity in the epididymis.

In the present study, light microscopic observations of epididymides from mice exposed to TCE indicate that damage to the epithelial lining of the epididymis by TCE was not restricted to any particular region. Sloughing of epithelium was observed in the caput, corpus and cauda segments throughout the length of the epididymis. Damage to the epithelial lining of the epididymis was evident one week after TCE exposure, and the degree of damage worsened with increased duration of exposure suggesting that TCE-induced insult to the epididymal epithelium was time- and concentration-dependent. No damage was observed in the epididymis and sperm obtained from mice that had not been exposed to TCE. These light microscopic observations were confirmed by electron microscopic examination of thin sections which revealed details, at the ultrastructural level, of TCE-induced damage to the epithelial lining of the epididymis as well as to epididymal sperm. The present electron microscopic study indicates that the plasma membrane and membranous organelles in the cytoplasm of the

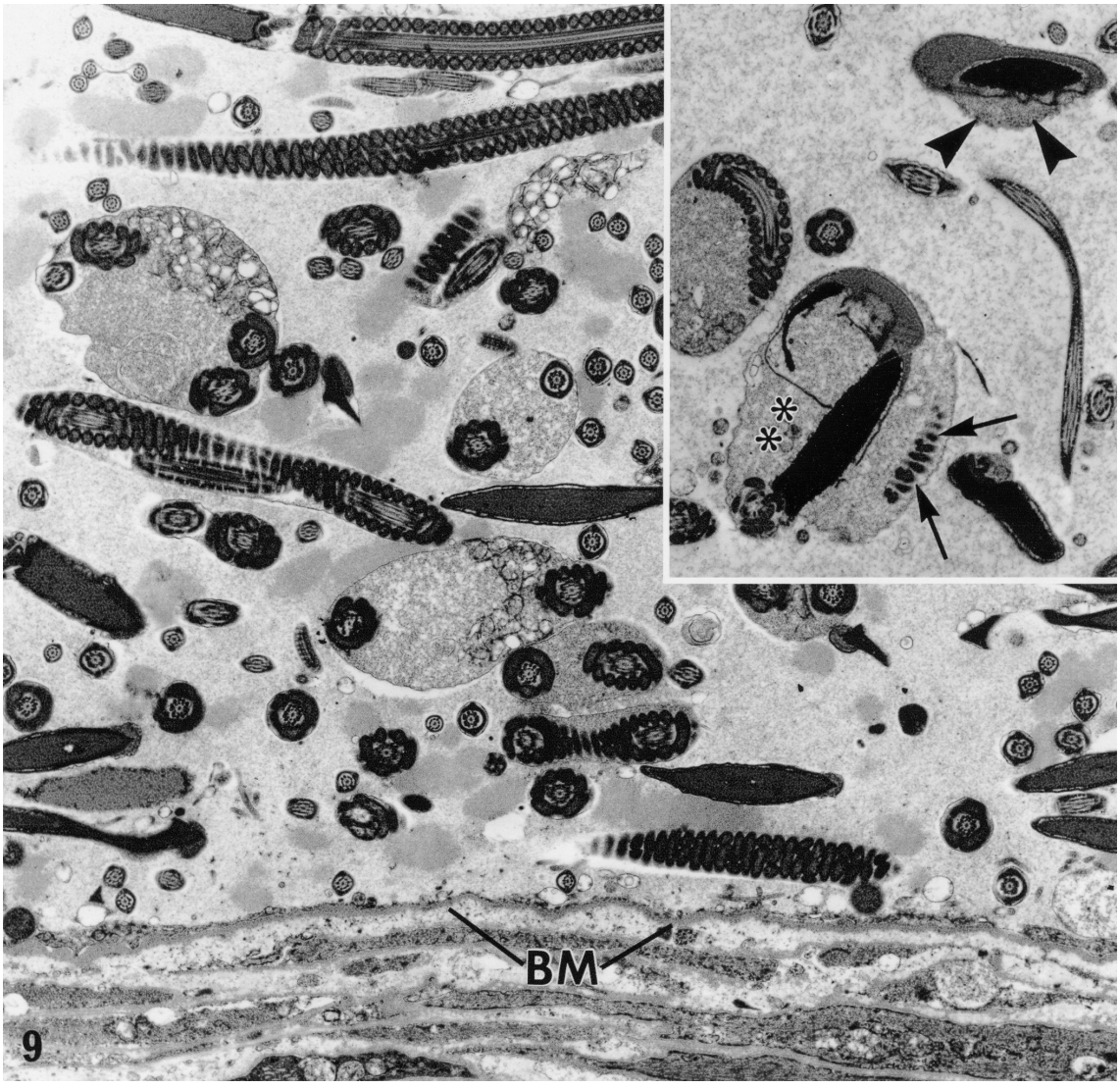


**Fig. 9.** Electron photomicrograph showing a portion of an epididymal tubule in the cauda region of the epididymis prepared from an animal exposed to TCE for 4 weeks. The figure shows the region of the tubule where the epithelium has sloughed off. Numerous spermatozoa in different planes of section can be seen directly above the remnant of the basement membrane (BM) and the underlying connective tissue. In one particular sperm (inset), the entire sperm head with an apparent malformation of the acrosome (**) is enclosed within the cytoplasm, and several mitochondria (arrows) of an incompletely formed tail are seen in its vicinity. Another sperm head (inset: arrowheads) located on the upper right hand corner is shown to be associated with redundant cytoplasm. x 8,800; Inset: x 7,700

epididymal epithelial cells are most susceptible to damage caused by TCE. This toxic effect of TCE is indicated by the disintegration of the basolateral cell membrane, mitochondrial membrane and membrane of the Golgi saccules in principal cells as shown in the electron photomicrographs. The breakdown of the basal membrane of epithelial cells of the epididymis resulted in separation of the epithelial layer from the underlying basement membrane. The breakdown of the lateral cell membranes between adjacent cells is probably responsible for the dismantling of junctional complexes that keep the cells as an intact epithelium. Together, the breakdown of the basolateral cell membranes contributed to the sloughing of cells and epithelial fragments from the epididymal tubules.

The results of the present study are consistent with the findings from a recent study showing that CYP2E1, the enzyme responsible for conversion of TCE to toxic metabolites, is present in the epididymal epithelium of mice (Forkert et al., 2002). In this latter study, epididymal epithelial cells were shown to stain intensely for immunoreactive CYP2E1 and microsomes isolated from mouse epididymides were found to have the capacity to convert TCE to chloral, a major TCE metabolite (Forkert et al., 2002). Furthermore, the results of the present study corroborated and extended the findings of epididymal epithelial damage in mice treated with TCE by inhalation, as recently reported (Forkert et al., 2002).

The toxicity of TCE in rodents is mediated primarily through its metabolism to chloral hydrate, trichloroacetate (TCA) and dichloroacetate (DCA), primarily via the catalytic activity of CYP2E1 (Lash et al., 2000). Recent results have indicated that hepatic tumors are induced by TCA and/or DCA in mice (Herren-Freund et al., 1987; Bull et al., 1990; DeAngelo et al., 1991) while only DCA, but not TCA, causes liver tumors in rats (DeAngelo et al., 1996). It has been suggested that TCA is the major metabolite of TCE leading to liver tumor induction in mice (Elcome et al., 1985; Fisher et al., 1991) and that TCA is much more effective in inducing hepatic peroxisomes in mice than in rats (DeAngelo et al., 1989). Chlorate hydrate, a TCE metabolite from which TCA is derived (Lash et al., 2000), may be involved in epididymal damage as it has also been implicated in inducing Clara cell death in the lungs of TCE-exposed mice (Odum et al., 1992). However, this possibility is not supported by studies in rats where drinking water exposure to chloral hydrate (up to 188 mg/kg/day) for 52 weeks did not cause notable epididymal histopathology (Klinefelter et al., 1995). It is probable that damage to the cell membranes and membranous organelles in the epididymis, noted in the present study, is a consequence of one or more of these metabolites being produced within the target cells through the action of cytochrome P450 including CYP2E1 (Forkert et al., 2002, 2003). It is notable, in this regard, that previous studies of male reproductive toxicity utilizing inhalation exposure, resulting in delivery of unmetabolized TCE to the reproductive tract, have demonstrated adverse effects on epididymis structure, sperm morphology or function (Land et al., 1981; Kumar et al., 2000, 2001; Forkert et al., 2002). However, a similar study employing an oral route of exposure showed minimal impact on reproductive tract function by TCE (Zenick et al., 1984). These findings suggest that the route of exposure is an important consideration for TCE-induced reproductive toxicity where first pass metabolism by the liver may limit TCE available for *in situ* metabolism in the male reproductive tract.

As many functional properties necessary for sperm motility and fertilization are acquired during transit of sperm in the epididymis (Eddy and O'Brien, 1994; Hinton and Palladino, 1995), the sloughing of epithelial cells into the epididymal lumen and indications of epithelial cell death suggested that these animals will sustain reduced fertility. Our morphological results corroborated with findings of a recent study that showed a significant decrease both in the number of sperm bound to egg and in the percentages of eggs fertilized when sperm were retrieved from mice exposed to TCE by inhalation (1000 ppm) for 1 to 6 weeks (Xu et al., 2004).

Another major finding in the present study was the detection of morphological abnormalities of epididymal spermatozoa in TCE-treated animals. Epididymal sperm with undifferentiated sperm head and malformation of the acrosome were the predominant abnormalities revealed by electron microscopy. It is well known that sperm have to traverse through the epididymis in order to attain full fertilizing capacity. Formation of the sperm head including the acrosome is complete once the spermatozoon is released from the testis into the lumen of the epididymis (Eddy and O'Brien, 1994). Therefore, the malformation of the sperm head, and in particular, any mishap in the assembly of various components of the acrosome should have already taken place in the testis. As CYP2E1 activity has been detected in the testis, specifically in the interstitial region (Jiang et al., 1998; Healy et al., 1999; Forkert et al., 2002), the formation of toxic metabolites of TCE within the testis may occur as a consequence of inhalation of TCE and these could plausibly disrupt testicular function. However, an earlier study examining the metabolism and toxicity of TCE in the epididymis and testis of mice carried out under similar conditions failed to detect morphological alterations in the testis (Forkert et al., 2002). This could be due to the fact that alterations might have occurred at the cellular level that cannot be detected macroscopically by light microscopy. These findings indicated that a more detailed analysis of the testis after inhalation exposure to TCE is warranted. It should be pointed out that preliminary studies carried out in our laboratory examining testicular toxicity of TCE exposure failed to reveal any significant influence on sperm chromatin condensation, an endpoint indicative of toxicity during the latter stages of spermatogenesis

(unpublished observations). Furthermore, our preliminary results also showed no change in spermatogenic cell populations or in *ex vivo* function of Leydig cells (unpublished observations).

The significance of the epididymal damage by inhalation TCE observed in mice for predicting reproductive hazards for occupationally exposed men is unclear. The recent demonstration of CYP2E1 immunoreactivity in human epididymal epithelium and the detection of TCE and the corresponding short-lived metabolite, chloral, in seminal fluid of men occupationally exposed to TCE indicate that epididymal sperm in humans are likely exposed to TCE-metabolites (Forkert et al., 2003). Previous studies that failed to demonstrate any major impact of occupational TCE exposure on semen quality in humans (Rasmussen et al., 1988; Chia et al., 1996) may have underestimated the adverse effects of TCE on the fertility of these men. This is very likely given the limited evaluation of sperm function that was carried out in these latter studies.

In summary, the present study showed that subchronic exposure of CD-1 mice to TCE imparted severe damage to the epididymis and resulted in abnormal acrosome and tail formation in epididymal sperm. These results suggest that exposure to TCE by inhalation might lead to infertility in animals.

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