

## Effects of environmental toxicants on the efferent ducts, epididymis and fertility

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Many of the reproductive toxicants have primary effects on the testis, which potentially overshadow effects downstream on the efferent ducts and epididymis. The specific target of these effects depends upon the dosage and time response. It is often necessary to design experiments that separate testosterone-dependent responses arising in the testis from direct effects on epididymal tissues and spermatozoa, to uncover the mechanisms of toxicity in excurrent ducts. Recent studies have confirmed that chemicals can also alter the time required for sperm transport through the epididymis. Currently there are approximately twenty chemicals that can be classified as epididymal toxicants. There are fewer toxicants reported for the efferent ducts, but a few overlap with epididymal effects. The benzimidazole carbamates, like many efferent ductal toxicants, induce occlusions and subsequent testicular atrophy. The mechanisms appear to be related to fluid reabsorption, sperm stasis, followed by leukocyte chemotaxis, sperm granulomas, fibrosis and often the formation of abnormal microcanals. Disruption of oestrogen receptor function in the efferent ducts also interferes with fluid reabsorption and results in testicular swelling and seminiferous tubular atrophy. Thus, studies in which testicular atrophy occurs after chronic or subchronic exposures should be examined for lesions in efferent ducts and head of the epididymis. Such lesions can lead to permanent infertility.

### Introduction

Toxicology of the epididymis has received less attention than other regions of the male reproductive system. A search of the literature for the period 1995-1997 shows that the testis has been the major emphasis in toxicology (nearly 400 papers), while the number of manuscripts focused on toxicity associated with the epididymis is fewer than 90. One problem has been the difficulty in separating direct versus indirect effects on the excurrent ducts, because altered testicular function indirectly alters function of the ductal epithelium downstream. The epididymis is dependent upon androgen stimulation; therefore, any compound that decreases Leydig cell function will decrease androgenic concentration in blood and rete testis fluid, which will have subsequent effects on the epididymis, sperm maturation and fertility. One complication is the fact that a primary effect on the testis may become overwhelming and define the long-term effects on fertility~ regardless Of effects on the epididymis. If there is a decline in spermatogenesis or testicular atrophy ensues, it is of little consequence that clear cells in the epididymis also disappear. However, it is possible that short-term toxicity targets only the epididymis or the spermatozoa in transit. For example, (a-chlorohydrin appears to have direct effects on epididymal spermatozoa without testicular effects, if the dosage is low and exposure time is short (Slott *et al.*, 1997).

If we are to begin separating direct versus indirect effects of toxicants on the male reproductive tract, it is important that experimental design be given top priority. Examining long-term exposures and moderate to high dosages will often provide only primary testicular effects that overshadow effects on the epididymis or spermatozoa. In this review, the ductuli efferentes (efferent ducts) and

**Table 1. Pathways** for direct *effects of* toxicants on the epididymis

1. Sperm target
  - Enzyme activity
  - Sperm proteins
  - Sperm motility
2. Epithelial target
  - Secretory proteins, ions, etc.
  - Cellular degeneration
  - Metabolism, ion flux, organelles
3. Connective tissue target
  - Blood and lymphatic flow
  - Leukocyte chemotaxis
  - Inflammation, granulomas
  - Smooth muscle
4. Mixed targets
  - Leukocyte chemotaxis
  - Inflammation
  - Indirect action on spermatozoa

epididymis will be considered independently but referred to at times collectively as epididymis. Epididymal toxicants and their known mechanisms of action will be listed. However, the primary focus will be on a compound that targets the *efferent ducts* and seminiferous epithelium. This compound is benomyl, a benzimidazole carbamate fungicide. We have studied both this parent compound and its metabolite, carbendazim, because the metabolite is responsible for the primary effects on male reproduction (Lim and Miller, 1997). An understanding of the short-term, as well as the long-term, effects is essential if the causes of infertility and testicular atrophy are to be *revealed*.

### Toxicants That Affect the Epididymis

#### *Direct versus indirect effects*

The use of two different *experimental methods* has improved the recognition of effects on epididymal epithelium or spermatozoa in transit. A good *example is* cyclophosphamide, which induces lethal mutations in males. Only when short-term studies *were performed or* experimental ligation of the *efferent ducts* was used did the investigators determine direct effects on epididymal spermatozoa or the epididymal epithelium (Qiu *et al.*, 1995). Methyl chloride is another example of a toxicant that acts directly on the epididymis (Chellman *et al.*, 1987). However, its mechanism of action is different, as it works through secondary damage to spermatozoa following an inflammatory *response to* treatment and the formation of granulomas. Thus, there are many pathways by which toxicants can affect the epididymis (Table 1) and we have only just begun to understand these mechanisms in a limited number of chemicals. *There are* approximately twenty chemicals that can be classified as epididymal toxicants, *either as* having direct or indirect effects on the epididymal *epithelium or effects* on epididymal spermatozoa (Table 2).

#### *Effects independent of testosterone*

Ethane dimethanesulfonate (EDS) and chloroethylmethanesulfonate (CMS) are alkylating antitumour agents that destroy Leydig cells and thus cause a reduction in testosterone (Klinefelter *et al.*, 1992, 1994a,b). *However, both* compounds also produce epididymal lesions. To determine whether the epididymal effects are independent of testicular *effects*, Klinefelter *et al.* (1992, 1994b) used testosterone implants to maintain androgen stimulation of spermatogenesis and the epididymal *epithelium*. *Both* chemicals caused specific disappearance of clear cells in the cauda epididymis, decreased cauda sperm counts and *altered two-dimensional* gel patterns of proteins in cauda spermatozoa, all independent of *testosterone*.

#### *Direct effects on spermatozoa*

TO *test-the potential* direct effects of EDS on epididymal spermatozoa, Klinefelter *et al.* (1992) *treated spermatozoa* or co-cultured spermatozoa and epididymal epithelium. Using this novel in

Table 2. Effects of toxicants on the epididymis

Toxicants <sup>1</sup>	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s
Granulomas; inflammation	x	x						x					x						x
Spermatocoele; cysts	x	x	x	x	x														x
Loss of clear cells													x	x					
Desquamation of epithelium					x										x				
Fibrosis	x																		
Decreases cauda spermatozoa													x	x	x			x	x
Increase sperm concentration																x			
Decrease sperm motility	x								x										
Decrease sperm transit time	x	x											x			x			
Increase sperm transit time								x						x			x		
Alter sperm protein profile	x												x	x	x				
Epididymal necrosis																			x
Post-implantation loss								x											
Seminiferous tubule dilation	x	x												x	x				
Testicular atrophy	x	x						x						x	x			x	x
Direct effects on spermatozoa	x														x				

## Chemicals:

- a, -Chlorohydrin; b, Epichlorohydrin; c, cc-Bromohydrin; d, glycidol (Cooper *et al.*, 1974; Ericsson, 1975; Slott *et al.*, 1997; Tsang *et al.*, 1981);  
e, 6-Chloro-6-deoxyglucose (Tsang *et al.*, 1981; Wong *et al.*, 1980);  
f, Cyclophosphamide (Qiu *et al.*, 1995);  
g, Methyl chloride (Chellman *et al.*, 1986; Working *et al.*, 1985);  
h, Guanethidine (Evans *et al.*, 1972);  
i, Ornidazole (Oberlander *et al.*, 1994; Cooper *et al.*, 1997);  
j, Methoxychlor (Linder *et al.*, 1992);  
k, Anti-androgens (hydroxyflutamide (Klinefelter and Suarez, 1997), cyproterone acetate (Din-Udom *et al.*, 1985; Tsang *et al.*, 1981), vinclozolin (Kelce *et al.*, 1997));  
l, Ethane dimethanesulfonate (Klinefelter *et al.*, 1990; 1994b);  
m, Chloroethylmethanesulfonate (Klinefelter *et al.*, 1994a; 1997; Klinefelter and Suarez, 1997);  
n, 1,2-Dibromo-3-chloropropane;  
o, Reserpine (Wen and Wong, 1988);  
p, 2,3,7,8-Tetrachlorodibenzo-p-dioxin (Cray *et al.*, 1997);  
q, 2,3-Dihydro-2-1-naphthyl -41H-quinazolinone (Ericsson, 1971);  
r, PCB169 (Gray *et al.*, 1995);  
s, Cadmium (Nagy, 1985).  
<sup>2</sup> See also review by Klinefelter and Hess (1998).

*vitro* method, they found that the effects of EDS on spermatozoa were mediated through the epididymal-epithelium and not directly on the spermatozoa. There are other examples of toxicants having direct effects on spermatozoa within the epididymal lumen. However, these effects are dose dependent; direct effects on spermatozoa occur only at the lower dosages, while higher doses of the

same chemical can cause many pathological changes in the epididymis. In this category, *a*-chlorohydrin is the best example. At lower dosages, dose-dependent effects on sperm velocity and fertility were significant in the absence of epididymal granulomas (Slott *et al.*, 1997), possibly mediated by the inhibition of glycolysis in the spermatozoa. Spermatocoeles and granulomas are typical responses in both efferent ducts and the caput epididymis after treatments at higher dosages (Cooper *et al.*, 1974).

#### *Alterations in sperm transit time*

Another mechanism by which chemicals may interfere with fertility is an alteration in sperm transit time through the epididymis. Oestrogen treatment accelerates the rate of sperm transport in the epididymis (Meistrich *et al.*, 1975). Other experiments have also demonstrated that sperm transport can be altered by disruption of sympathetic or adrenergic innervation of the epididymis (Evans *et al.*, 1972), and thereby alter ion flux, particularly Cl<sup>-</sup> secretion (Wen and Wong, 1988; Wong, 1990). More recent studies have shown that chloroethyl-methanesulphonate and hydroxyflutamide accelerate sperm transit through the epididymis (Klinefelter and Suarez, 1997), supporting the findings that castration or cyproterone acetate treatment enhance sperm transport. Interestingly, *in utero* exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin also results in altered sperm transport without obvious testicular effects in adult life (Gray *et al.*, 1997). In the light of these studies, future efforts should be devoted to uncover the physiological mechanisms responsible for sperm transport that are the target of exposure to environmental chemicals.

#### **Toxicants That Affect Efferent Ducts**

Reports indicate that smoking and ten reported compounds induce some type of toxic response in the efferent ducts (Table 3). Unfortunately, a clear understanding of their mechanisms of toxicity is lacking. However, for some chemicals there are biochemical data suggesting routes of induced injury. For example, *a*-chlorohydrin (or a metabolite) and 6-chloro-6-deoxyglucose (CDG) cause reductions in glucose transport and glucose metabolism (Hinton *et al.*, 1983), which could indirectly interfere with energy-dependent processes such as ion transport, in addition to their direct effects on spermatozoa. It is interesting that *a*-chlorohydrin and epi-chlorohydrin are metabolites of 1,2-dibromo-3chloropropane (DBCP), a chemical responsible for the first well known case of infertility in humans caused by chemical exposure (Whorton *et al.*, 1979). All three compounds cause kidney dysfunction as well as epididymal lesions (Kluwe *et al.*, 1983). Although the literature does not indicate that DBCP affects the efferent ducts, Kluwe *et al.* (1983) did report 'dilatation of the seminiferous tubules' and long-term tubular atrophy, which are common responses to occlusion of these ducts and seen following exposure to *a*-chlorohydrin and epichlorohydrin (Cooper and Jackson, 1972; Cooper and Jackson, 1973; Jones, 1978; Kluwe *et al.*, 1983). Thus, it appears that the general mechanism of toxicity for these chemicals is an alteration of fluid reabsorption followed by sperm stasis and ductal occlusion. In general, there are at least six major responses of the efferent ducts to environmental toxins, which can lead to reduced fertility and even testicular atrophy (Table 4).

#### *Ductal occlusions*

An examination of the chemicals listed in Table 3 indicates that many toxicants of the efferent ducts induce occlusions. This would suggest an interference with fluid reabsorption, or that other factors such as granulomas or inflammation cause the luminal fluid to become stagnant. In 1943, Wakeley stated that most epididymal cysts in man arise from dysfunction of the efferent ducts. As more chemical toxicants are examined, this early prediction appears to be correct for animal models, too. Several mechanisms could be involved in the pathophysiology of ductal occlusions. One possibility is direct or indirect disruption of Cl<sup>-</sup> secretions, which appear to be important for normal flow of spermatozoa in the proximal epididymis (Chan *et al.*, 1995). mRNA encoding cystic fibrosis

## Epididymal effects of environmental toxins

**Table 3.** Effects of toxicants on the efferent ducts

Toxicants <sup>1</sup>	a	b	c	d	e	f	g	h	i	j	k
Ciliary loss										x	x
Phagocytosis	x	x	x							x	
Desquamation			x							x	
Epithelial hyperplasia				x						x	
Recanalization	x	x		x							
Spermatocoele	x	x	x	x	x	x			x	x	
Sperm granuloma	x	x	x	x		x					
Inflammation	x	x			x						
Ductal occlusion	x	x	x	x		x	x			x	
Fibrosis	x	x	x				x				
Testicular swelling	x	x	x		x	x		x			
Seminiferous atrophy	x	x	x	x	x	x				x	

a, Benomyl<sup>®</sup> (Hess *et al.*, 1991);

b, Carbendazim (Carter *et al.*, 1987; Gray *et al.*, 1990; Nakai *et al.*, 1992; Nakai *et al.*, 1993; Rehnberg *et al.*, 1989);

c, a- and epi-Chlorohydrin (Cooper *et al.*, 1974; Ford and Wailes, 1981; Hoffer *et al.*, 1975; Jones 1978);

d, Ethylenedimethane sulfonate (Cooper and Jackson, 1972; Cooper and Jackson, 1973);

e, 6-Chloro-6-deoxyglucose (Ford and Waites, 1981);

f, Quinazolinone (Ericsson, 1975);

g, 1,3-Dinitrobenzene (Linder *et al.*, 1988a);

h, Uranyl nitrate (Mason and Young, 1967);

i, Glycidol (Jones and O'Brien, 1980);

j, Cadmium (Nagy, 1985);

k, Fluoride (Susheela and Kumar, 1991).

<sup>2</sup> See also reviews by Illo and Hess, 1994; and Klinefelter and Hess, 1998.

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transmembrane conductance regulator (CFTR) is more prevalent in the proximal regions of the male tract (Trezise *et al.*, 1993) and adult cystic fibrosis patients are infertile, often due to epididymal obstruction (Gaillard *et al.*, 1997).

## Effects of Benzimidazole Carbamate Fungicides on Efferent Ducts and Fertility

### Effects on fertility

Benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate) and its metabolite carbendazim (methyl 2-benzimidazole carbamate) are both highly effective fungicides and nematocides. Their fungicidal action is due to their ability to disrupt microtubule formation, an effect that is also extended to mammalian cells (see Hess *et al.*, 1991). Regulation of these chemicals by the US Environmental Protection Agency was based partially on their reproductive toxicity, which is associated with testicular degeneration (Carter and Laskey, 1982) and decreased fertility (Carter *et al.*, 1987; Gray *et al.*, 1990; Linder *et al.*, 1988b; Torchinskiy *et al.*, 1976). However, the early studies of benzimidazole compounds did not establish mechanisms of testicular atrophy. Infertility occurred in 50% of the males after the first week of exposure to a single dose of carbendazim (400 mg kg<sup>-1</sup>), but histopathological examination of the testes revealed large variation in tubular atrophy (Carter, 1987). At first, atrophy was assumed to be the result of action of carbendazim as a microtubule poison, which has been shown subsequently to cause necrosis of mitotic and meiotic cells in the testis (Nakai and Hess, 1997) and the formation of abnormal spermatids (Nakai *et al.*, 1997). Other workers found effects on hormones in serum and testicular fluids and attempted to relate the long-term effects on sperm production to an inhibition of the testicular

**Table 4. Major** responses of the efferent ductules to environmental toxins and experimental conditions

Suggested responses	Associated toxicants or experimental conditions	End results
Increased reabsorption of luminal fluid	Benomyl <sup>1</sup> ; carbendazim <sup>2</sup>	Increased sperm concentration/ luminal stagnation/ Spermatocoeles/ductal occlusion
Decreased reabsorption of luminal fluid	Oestrogen receptor dysfunction <sup>1</sup>	Decreased sperm concentration/ dilution/ luminal dilation
Phagocytosis of spermatozoa	Benomyl; carbendazim <sup>1</sup>	Epithelial hyperplasia/ recanalization
Epithelial desquamation	a-chlorohydrin <sup>3</sup> Cadmium <sup>1</sup>	Testicular swelling
Inflammation	Benomyl <sup>1</sup> ; carbendazim <sup>2</sup> ; a-chlorohydrin <sup>1</sup>	Testicular atrophy
Fibrosis	Benomyl <sup>1</sup> ; carbendazim <sup>2</sup> ; a-chlorohydrin <sup>3</sup> ; 1,3-dinitrobenzene <sup>5</sup>	Decreased fertility

<sup>1</sup>(Hess et al., 1991).<sup>2</sup>(Nakai et al., 1992).<sup>3</sup>(Hoffer et al., 1975).

(Nagy, 1985).

<sup>5</sup>(Linder et al., 1988).

hypothalamus-pituitary feedback loop (Goldman *et al.*, 1989; Rehnberg *et al.*, 1989). However, upon further examination of the histopathology, it was found that two sequential events, first in the testis **and then in the** efferent ducts, could answer many of the questions regarding hormonal changes, long-term atrophy of seminiferous tubules and why some males became irreversibly infertile.

#### *Effects on seminiferous epithelium*

Benzimidazole carbamate compounds cause premature sloughing of germ cells along with cleaved processes of Sertoli cell cytoplasm (Hess *et al.*, 1991; Nakai and Hess, 1994), necrosis of mitotic spermatogonia and meiotic spermatocytes (Nakai and Hess, 1997) and seminiferous tubular atrophy (Carter *et al.*, 1987; Hess *et al.*, 1991; Nakai *et al.*, 1992). The proposed mechanism contributing to sloughing is deformation of Sertoli cell cytoplasm due to the disruption of microtubules (Nakai and Hess, 1994; Nakai *et al.*, 1995). Recovery from massive sloughing of germ cells is possible if the efferent ducts remain intact; however, abnormal spermatids are formed several days after a single exposure to carbendazim and may affect fertility long after the initial testicular injury (Nakai *et al.*, 1997). These effects occur in testes with intact efferent ducts. Therefore, carbendazim can have direct effects on the seminiferous epithelium at lower dosages, independent of efferent duct dysfunction. On the basis of the severity of germ cell sloughing, we first hypothesized that occlusions in efferent ducts were caused by cellular debris that clogged the lumen only at ductal junctions. However, other chemicals also induce massive sloughing of germ cells without efferent ductal occlusions; therefore, an alternative explanation was required.

#### *Effects on efferent ducts*

Occlusion of the efferent ducts (Fig. 1) is common after exposure to the fungicide benomyl or its metabolite carbendazim (Hess *et al.*, 1991; Nakai *et al.*, 1992). This response is rapid, as an increase in testis weight is detected as early as 8 h after exposure (Nakai *et al.*, 1992). A single dose appears to be sufficient to induce ductal occlusions, but a comparison of 70 day, single-exposure data (Nakai *et al.*,

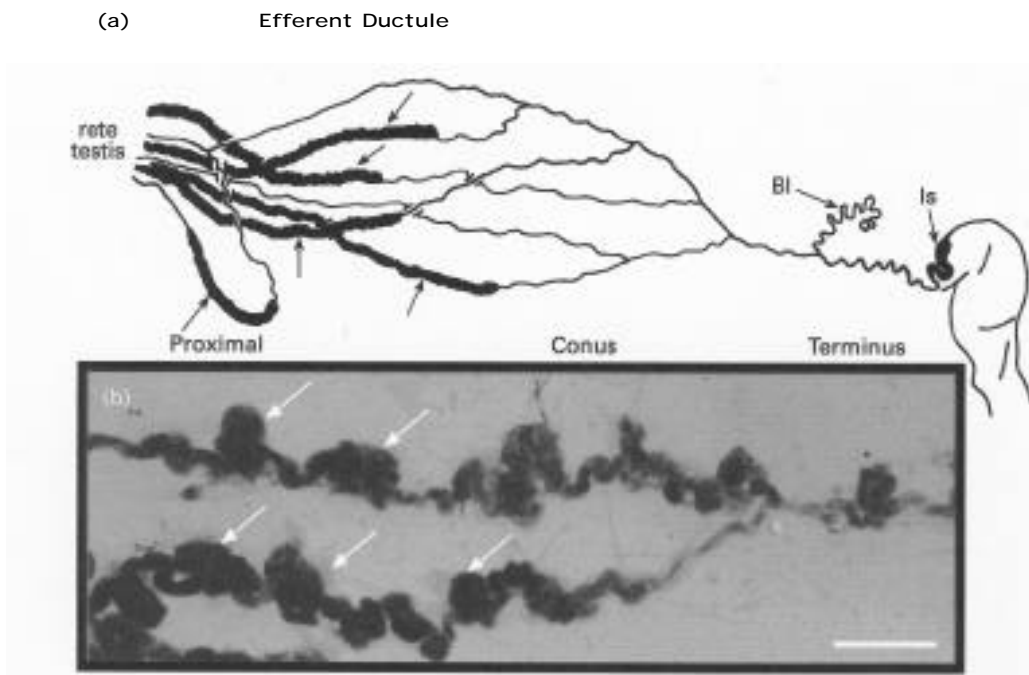


Fig. 1. (a) A drawing of microdissected efferent ducts collected from a rat 48 h after treatment with a single dose of carbendazim (400 mg kg<sup>-1</sup>). This type of data was collected from photographs (similar to (b)) and tabulated in Table 5. Occlusions are noted as thick dark areas (arrows) near the rete testis. Ductal regions are identified as proximal, conus and terminus. The terminus is a single ductule that joins the initial segment epididymidis (Is), and in this case contained a small blind ending tubule (Bl). (b) A photograph of microdissected efferent ducts showing occlusions (arrows). A region of normal size is noted by an asterisk near a junction. Scale bar represents 1 mm.

1992) with 245 days following exposure for 10 days (Carter *et al.*, 1987) is noteworthy because the percentage of testes with 'total seminiferous tubular atrophy' was 21% on day 70 and 50% on day 245. This suggests that either seminiferous tubular regression continues long after treatment has stopped or that the additional doses were effective in spreading damage to a greater number of efferent ducts.

Microdissection of occluded efferent ducts was performed to determine specific locations of the benzimidazole lesions (Table 5). At 12 h after treatment 75.8% of the ductules were occluded and by 24 h nearly 85% were occluded. Overall, 56% of the occlusions were located in the initial zone, 15% at junctions and 44% in the conus vasculosa. No occlusions were observed in the common efferent duct. Further measurements confirmed that the initial zone was the primary site of occlusions, as the ductules in this zone were significantly longer (41.9 mm) in treated animals compared with controls (30.7 mm). The length of the common duct did not change: 19.6 mm and 18.1 mm. in control and treated, respectively.

After occlusions were formed in the efferent ducts, an inflammatory response was initiated, approximately 2-4 h later. The severity of response was dose dependent, but the onset of occlusion and time to first appearance of neutrophils in the connective tissue remained the same regardless of dosage. In controls, occlusions were not present and neutrophilic leukocytes were absent. However, 2 h after treatment, exfoliated spermatids were present in the lumen of the efferent ducts and most ductules were engorged by 4 h. Complete occlusion of the ductules occurred at 6-8 h, before the infiltration of neutrophils, which corresponded to a build-up of fluid in the testis and increased testis weight (Nakai *et al.*, 1992). Neutrophils began to appear in the connective tissue between 8-12 h after

**Table 5.** Carbendazim-induced occlusions of the ductuli efferentes in the adult rat'

Time (h) after treatment	Total		Total Number	%	Location of occlusions'			
	Number of ductules	Number of occluded			Proximal junction	Conus	Terminus	
12	5	33	25	75.8	13	5	12	0
24	5	32	27	84.4	16	3	11	0

1 400 mg kg<sup>-1</sup> by single oral gavage.

2 Overall, 80.1% of the ductules were occluded.

3 Overall, occlusions in the proximal ductule (56%); at junctions (15%); in the conus (44%); in the terminus (0%).

**treatment; therefore, it is concluded that benzimidazole** carbamates have direct effects on efferent ducts rather than an indirect reaction following inflammation.

The neutrophils migrated between the junctions of endothelial cells lining small venules, producing limited haemorrhage into the connective tissue. As the leukocytes migrated between the thin smooth muscle layers of the efferent ducts, some of the muscle cells were destroyed. By 48 h, neutrophils formed between 2-5 solid layers of cells around the base of the ductules (Fig. 2a,b), where they eroded the basal lamina before penetrating between epithelial cells. Neutrophils then phagocytosed luminal debris and caused luminal contents to erupt into the lamina propria. Thus, neutrophils exhibited a specific chemotactic response toward efferent ducts containing stagnant spermatozoa and exfoliated spermatids. The leukocytes attempted to seal off the luminal contents but appeared to damage the epithelium in the process, which may have contributed to subsequent formation of fibrotic lesions and permanent infertility.

The response of efferent ductal epithelium to injury induced by occlusions appears to be dependent upon the degree of inflammation caused by the trauma. An acute inflammatory reaction may be induced by the compacted luminal contents, causing the ductal lumen to dilate (Fig. 2c). It is likely that the ductal epithelium, stretched excessively by a large bolus of testicular debris, releases a chemotactic substance, possibly a cytokine of the interleukin superfamily, which then recruits large numbers of neutrophils. Leakage of sperm antigens may draw the neutrophils toward the lumen and stimulate phagocytic activity. In other organ systems, indirect damage caused by neutrophil emigration into the interstitium and through the epithelium promotes granuloma formation and fibroblast activity (see review by Nakai *et al.*, 1993). Thus, fibrotic lesions may be an indirect result of neutrophil damage rather than direct effects of epithelial injury.

Epithelia with medium inflammatory responses often exhibited irregular epithelial growth along the edge of luminal contents and formed multiple abnormal ductules (Fig. 2d). These abnormal ductules, formed by the migration of the original epithelia and growth at the periphery of the occluded lumen, indicated that recanalization was attempted by 16 days after treatment (Nakai *et al.*, 1993). Epithelial cells of the microcanals were similar in appearance to those of blind ending tubules (Guttruff *et al.*, 1992). No evidence was found to indicate that microcanals formed patent connections between rete

### Conclusion,,

Efferent ducts respond to toxic insult by at least two different means (Fig. 3): an increased rate of fluid reabsorption or decreased secretions (i.e. Cl<sup>-</sup>) ; or a decreased rate of reabsorption or increased secretions. The first response leads to increased viscosity of luminal fluids, sperm stasis, ductal occlusions, granulomas and possibly fibrosis. The second response dilutes the luminal fluid, decreases sperm concentration, and leads to a decrease in sperm transit time through the epididymis. The mechanism by which benzimidazole chemicals disrupt fluid reabsorption is not

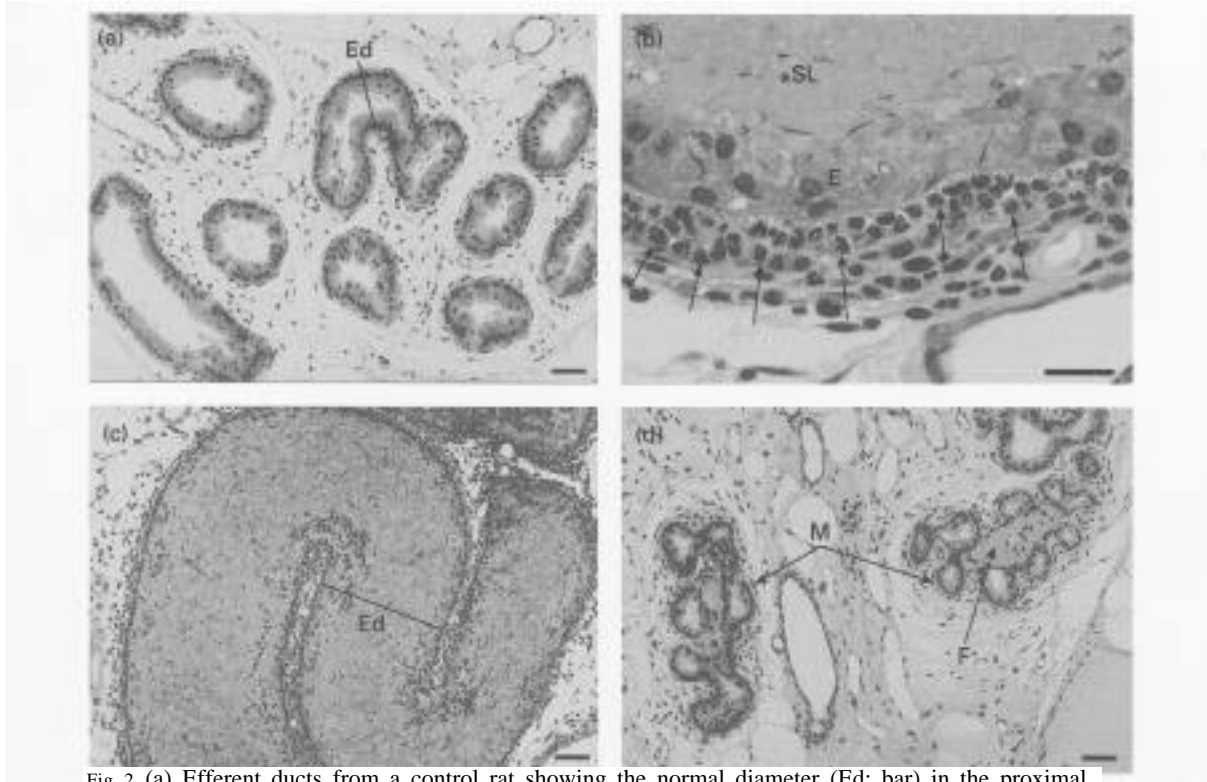


Fig. 2. (a) Efferent ducts from a control rat showing the normal diameter (Ed; bar) in the proximal conus region. (b) Occluded efferent ductule from a rat treated with a single dose of carbendazim (400 mg kg<sup>-1</sup>) and fixed by vascular perfusion 48 h after treatment. The lumen is occluded with sloughed debris (SL) from the testis. The epithelium (E) is disorganized and shows evidence of sperm phagocytosis. Neutrophilic leukocytes (arrows) completely surround the basement membrane. (c) A single occluded efferent ductule 48 h after treatment with carbendazim (400 mg kg<sup>-1</sup>). The lumen is filled with debris and the diameter (Ed; bar) of the ductule has nearly doubled in size. Neutrophils line the basement membrane and are found in the lumen (dark staining nuclei). (d) Occluded efferent ducts 70 days after treatment with carbendazim (400 mg kg<sup>-1</sup>) show both the fibrotic lesions (F) and

known. We **have limited data** showing a dose-dependent increase in the activity of Na<sup>+</sup>,K<sup>+</sup>-ATPase (Table 6), which may result from the disruption of cytoskeletal elements (Jordan *et al.*, 1995). However, effects on other pathways, such as Cl secretion, should also be considered. Regardless of mechanisms or the toxicant involved, once ductules become blocked, long-term results are the same, as testicular atrophy and infertility are produced. Accordingly, long-term testicular atrophy after subchronic and acute multiple exposure to any toxicant could be explained by potential efferent ductal dysfunction, a hypothesis that should be examined routinely by histopathology.

Cessation of fluid reabsorption, as seen in the oestrogen receptor knockout mouse, also leads to fluid build up within the testis and subsequent seminiferous tubular atrophy (Hess *et al.*, 1997). Thus, it appears that oestrogen may be required for normal fertility in the male. At least in the mouse, oestrogen receptor-cc is required. This new role for oestrogens in the male reproductive tract raises renewed interest in the effects of xenoestrogens or environmental oestrogenic chemicals on male fertility and the decline in sperm counts. Are these effects due only to developmental anomalies, as first hypothesized (Sharpe and Skakkebaek, 1993)? Or is it possible that exposure of adults also produces oligospermia or a decline in sperm counts by the dilution of caput spermatozoa due to the inability to reabsorb fluid properly in efferent ducts (Hess *et al.*, 1997)? Increases in

Table 6. The effect of carbendazim on Na,K-ATPase activity in the the ductuli efferentes of adult rats'

Carbendazim (mg kg <sup>-1</sup> )	Number of rats	Na,K-ATPase activity <sup>2</sup> pmol p-nitrophenol mm <sup>-1</sup> tubule <sup>-1</sup> h
0	5	7143 ± 320,
100	5	7767 ± 540ab
400	5	9785 5521

Single exposure by oral gavage. For details of methods, see Ilio and Hess (1992). Significant differences are indicated by different superscripts ( $P < 0.05$ ).

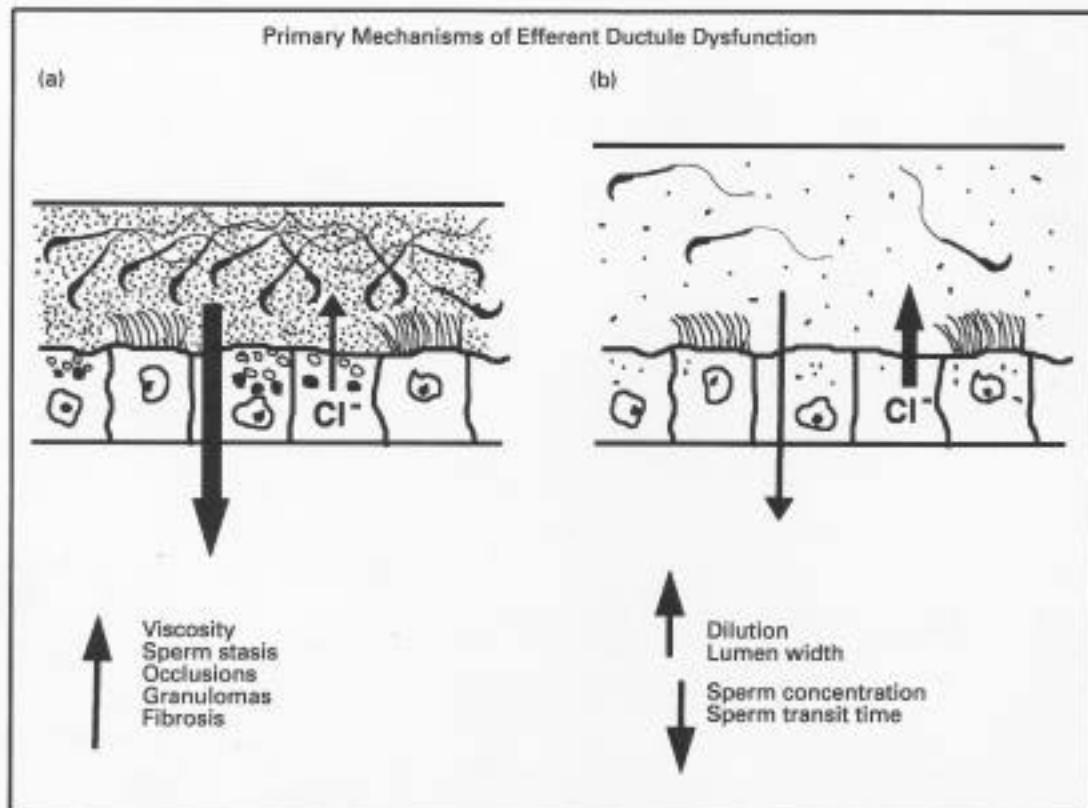


Fig. 3. (a) An illustration of toxicant effects that lead to an excessive amount of fluid reabsorption leaving behind a higher concentration of spermatozoa, protein and cellular debris in the lumen. The consequences of this effect are similar to those seen following exposure to carbendazim and lead to increases in the viscosity of luminal contents, sperm stasis, occlusions, granulomas and fibrosis. (b) An illustration of an effect leading to decreased reabsorption or increased secretions of Cl<sup>-</sup> into the lumen. The consequences of this response are similar to those observed in the oestrogen receptor knockout mouse (Hess et al., 1997), which lead to increased dilution of semen, increased luminal diameter, and decreases in sperm concentration and possibly sperm transit time.

abnormal sperm morphology could also result from abnormal fluid reabsorption, as normal spermatozoa leaving the testis would fail to mature in the epididymis if the surrounding luminal fluid did not contain the necessary concentration of factors required for sperm maturation. Thus, efferent duct dysfunction can interfere with fertility through several mechanisms and environmental chemicals have the potential to cause these effects. Future studies must address not only the pathophysiological mechanisms leading to testicular atrophy, but also the biochemical and molecular mechanisms associated with the regulation of ion and water flux across the ductal epithelium and their relationship to male infertility.

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