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Male infertility and environmental exposure to lead and cadmium

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Humans are exposed occupationally and environmentally to metal aerosols including lead (Pb2+) and cadmium (Cd2+). These toxicants accumulate in male reproductive organs. Epidemiological studies have been equivocal about effects of Pb2+ and Cd2+ on hormone concentrations, male fertility and sperm parameters. Comparison of Pb2+ and Cd2+ concentrations in fertile and infertile men are problematic. Problem areas include failure to control confounding variables, but genetic polymorphisms as in somatic diseases may modulate Pb2+ and Cd2+ damage. Multiple calcium (Ca2+) and potassium (K^+) channel isoforms have been identified in human testes and spermatozoa. These Ca2+ and K^+ channels are involved in early events of acrosome reactions. Ca2+ channel are susceptible to Cd2+ poisoning and K^+ channels to Pb2+. These channels offer entry paths for metallic toxicants into mature spermatozoa. Ion channel polymorphisms may cause differential sensitivities to Cd2+ and Pb2+, explaining in part prospective blinded studies showing high Cd2+ in varicocele-related human infertility and high Pb2+ in unexplained infertility. In both forms of male infertility the ability to undergo an acrosome reaction decreases. Reverse transcriptase–polymerase chain reaction assays for Ca2+ and K^+ channel isoforms may identify susceptibility subgroups with lower resistance to environmental exposures.

Key words: acrosome reaction/cadmium/ion channels/lead/male infertility

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Introduction

In recent years, there has been an increasing interest in the contribution of occupational and environmental exposures to toxic pollutants to declining sperm concentrations and human male infertility (Carlsen *et al.*, 1992; Auger *et al.*, 1995; Adamopoulos *et al.*, 1996; Becker and Berhane, 1997). We recognize that at least one of these studies (Carlsen *et al.*, 1992) has been criticized because it fails to consider that sperm counts clearly differ by geographical location (Fisch and Goluboff, 1996; Paulsen *et al.*, 1996). Nonetheless, geographic variations in semen quality (Fisch and Goluboff, 1996; Becker and Behane, 1997) may still be influenced by environmental factors (Fisch and Goluboff, 1996) which exhibit considerable variation between climatic seasons (Sram *et al.*, 1996). More importantly, environmental factors differ between areas (Friberg and Vahter, 1983; Svensson *et al.*, 1987; Buchancova *et al.*, 1994; Sram *et al.*, 1996), with higher amounts of pollutants closer to sources of industrialization (Benin *et al.*, 1999).

Unfortunately, relatively few studies have systematically addressed the impact of environmental exposures on human health in general (Buchancova *et al.*, 1994; Staessen *et al.*, 1996) or on human reproductive function (El-Zohairy *et al.*, 1996; Sram *et al.*, 1996). What limited data that does exist suggests that exposure to high values of airborne fine particles is associated with decreased semen quality, i.e. increased aberrant morphology and motility. Heavy and transition metal ions, e.g. cadmium (Cd2+), cobalt (Co2+), copper (Cu2+), iron (Fe2+/3+), lead (Pb2+), mercury (Hg2+), magnesium (Mg2+), manganese (Mn2+), nickel (Ni2+)

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and selenium (Se2+/4+), are common components of such airborne particulates that are within the respirable range (Ragan and Mast, 1990). Pb2+ and Cd2+ preferentially accumulate in male reproductive organs (Danielsson *et al.*, 1984; Oldereid *et al.*, 1993; Jackson *et al.*, 1995). An increase in Pb2+ and Cd2+ often occur simultaneously (Stachel *et al.*, 1989). This review focuses on the effects of Pb2+ and Cd2+. Our studies, which are summarized herein, indicate that Pb2+ and Cd2+ exposures are of particular importance to the production of the infertile state.

Effect of occupational exposure to metal ions

The correlation of trace metal exposures with human sperm production and function has been studied mainly in men occupationally exposed to high concentrations of airborne metal particulates (Danielsson *et al.*, 1984; Assennato *et al.*, 1986; Stachel *et al.*, 1989; Gennart *et al.*, 1992b; Bonde, 1993). The earliest studies were triggered by reports that semen values of another class of environmental toxicants, halogen-containing hydrocarbon pesticides, were associated with decreases in sperm counts and motility, increased percentages of spermatozoa with morphological abnormalities and male infertility (Wong *et al.*, 1979; Dougherty *et al.*, 1986; Ratcliffe *et al.*, 1987; Wagner *et al.*, 1990).

The possible xenobiotic exposures associated with industrial metal aerosols include metals such as aluminum (Al^{3+}), Cd2+, $Fe2+^{/3+}$, chromium (Cr2+), Ni2+, and Pb2+, as well as nitrous gases and ozone (Stern *et al.*, 1986; Mortensen, 1988). Metal workers (e.g. welders, smelters, men engaged in the manufacture of alkaline batteries) comprise 0.5–2.0% of the general working population (Stern *et al.*, 1986). There is epidemiological evidence that exposure to industrial metal aerosols may be detrimental to the male reproductive system; fertility of men employed in the metal industry is apparently reduced when compared with men in other types of work. These statements are based upon studies reporting decreased family size, delay in time to conception and reduced sperm quality in metal workers (Bonde, 1993; Spinelli *et al.*, 1997). These problems have been correlated with both exposure dose and length of exposure (Bonde, 1990a), and are not reversible by short periods of non-exposure (Bonde, 1990b). However, despite these findings, attempts to correlate occupational exposure to Pb2+ and Cd2+ with semen quality and/or infertility have produced conflicting results.

Blood plasma and seminal plasma Pb2+ concentrations are elevated in exposed workers (Stachel *et al.*, 1989; Telisman *et al.*, 1990; Ng *et al.*, 1991; Gennart *et al.*, 1992a; Keck *et al.*, 1995; Aribarg and Sukcharoen, 1996; Robins *et al.*, 1997; see Table I). A positive relationship was detected between blood and seminal plasma Pb2+ concentrations (Aribarg and Sukchareon, 1996). In some studies, evidence is presented suggesting that men with a long duration of Pb2+ exposure in the workplace may have reduced fertility (Lancranjan *et al.*, 1975; Gennart *et al.*, 1992b; Lin *et al.*, 1996). However, in other studies, Pb2+ exposure has not been reported to decrease fertility (Coste *et al.*, 1991) or only exhibits a weak association with infertility (Robins *et al.*, 1997). Dose–response relationships and/or causal mechanisms remain to be defined (Winder, 1993). Seminal plasma Pb2+ concentrations and fertility status have not been correlated (Keck *et al.*, 1995).

Endocrine dysfunction has (Ferioli *et al.*, 1989; MacGregor and Mason, 1990; Ng *et al.*, 1991) and has not (Lancranjan *et al.*, 1975; Assennato *et al.*, 1986) been observed in men occupationally exposed to Pb2+. Where occurring, Pb2+-associated changes in hormone concentrations were thought to be mediated at the hypothalamic–pituitary level (Gustafson *et al.*, 1989; Ng *et al.*, 1991). These data are consistent with findings in animal models, where the primary toxic action of Pb2+ on male reproduction appears to be disruption of the hypothalamus–pituitary–testicular axis (Sokol *et al.*, 1985, 1994; Sokol, 1987; Kempinas *et al.*, 1994).

The majority of reports indicate that workers exposed to Pb2+ exhibit decreased sperm density and a high rate of teratozoospermia (Lancranjan *et al.*, 1975; Hu *et al.*, 1992; Lerda, 1992; Xuezhi *et al.*, 1992; Robins *et al.*, 1997) and may also present with a decreased sperm count (Assenato *et al.*, 1986). An inverse correlation between blood Pb2+ concentrations and decreased semen quality has been observed (Lancranjan *et al.*, 1975; Telisman *et al.*, 1990). The study by Telisman *et al.* (1990) also suggested that reproductive toxicity may occur at blood Pb2+ concentrations below the permissible exposure limit of 40 μg/dl. Thus, current occupational standards may not be sufficiently stringent (Winder, 1993).

In some studies, however, no relationship was detected between seminal plasma Pb2+ concentrations and semen profiles (Aribarg and Sukcharoen, 1996; Hovatta *et al.*, 1998). There are three possible explanations for this failure. Firstly, exposure levels were apparently very low as blood Pb2+ concentrations were generally <40 μg/dl, the recommended limit of exposure for workers in an occupational setting as defined by the World Health Organization (WHO, 1980). Secondly, in some cases the men studied had only short-term exposure to Pb2+ (Aribarg and Sukchareon, 1996). As in animal models (Kempinas *et al.*, 1994), duration of Pb2+ exposure appears to be an important factor when searching for a correlation between Pb2+ and changes in semen parameters (Lerda, 1992). Thirdly, measurements of metal ion concentrations in seminal plasma may be of less significance in the examination of environmental effects than direct measurements of metal ion concentrations in spermatozoa themselves. As an example of the latter, Hovatta *et al.* (1998) reported that sperm motility was inversely correlated with aluminium in spermatozoa but not in seminal plasma.

Whether Cd2+ concentrations in blood and seminal plasma are elevated following occupational exposures is still not clear (Table I). Early studies indicated that, based on autopsy findings, spermatogenesis was reduced in workers exposed to Cd2+ (Smith *et al.*, 1960) and that the family size was smaller than average (Favino *et al.*, 1968). More recent studies did not support these findings. Cd2+ exposures have not been associated with a significant reduction in semen quality (Tielmans *et al.*, 1999) or with a decreased probability of a live birth (Gennart *et al.*, 1992b). In contrast, occupational exposure to Cd2+ has been reported to increase the risk for prostate cancer (Elghany *et al.*, 1990). This is consistent with observations indicating that Cd2+ concentrations are increased in prostate neoplasms, compared with normal tissue (Ogunlewe and Osegbe, 1989; Brys *et al.*, 1997) and studies *in vitro* and in animal models have demonstrated a relationship

Table I. Effects of occupational exposures on the levels of Pb2+ and Cd2+ in blood and seminal plasma

	Unexposed	Exposed	Source
Pb2+ (blood)	13.1 ± 3.5 μg/dl		Plechaty *et al.* (1977)
	23 ± 14 μg/dl	29–100.5 μg/dl*	Lancrajan *et al.* (1975)
	20.8 ± 2.8 μg/dl	50.5 ± 9.6 μg/dl*	Ferioli *et al.* (1989)
	6.7 – 20.8 μg/dl	11.7 – 104.0 μg/dl*	Telisman *et al.* (1990)
	8.3 ± 2.8 μg/dl	35.2 ± 13.2 μg/dl*	Ng *et al.* (1991)
	20.9 ± 11.1 μg/dl	51.0 ± 8.0 μg/dl*	Gennart *et al.* (1992a)
	10.4 ± 3.3 μg/dl	46.3 ± 11.1 μg/dl*	Gennart *et al.* (1992b)
	23.5 ± 1.4 μg/100 g	86.6 ± 0.6 μg/100 g*	Lerda (1992)
	23.6 ± 47.1 μg/dl	47.1 ± 31.0 μg/dl*	Xuezhi *et al.* (1992)
	16.9 ± 2.6 μg/dl	26.7 ± 2.1 μg/dl*	El-Zohairy *et al.* (1996)
		(Fertile)	
		37.0 ± 4.9 μg/dl*	
		(Infertile)	
	27.9 ± 2.5 μg/dl	45.0 ± 9.4 μg/dl*	Aribarg and Sukcharoen (1996)
Pb2+ (semen)	5.9 ± 2.7 μg/dl		Plechaty *et al.* (1977)
	16.5 ± 17.7 μg/dl	66.5 ± 83.6 μg/dl*	Xuezhi *et al.* (1992)
	11.0 ± 1.9 μg/dl	21.9 ± 2.0 μg/dl*	El-Zohairy *et al.* (1996)
		(Fertile)	
		25.8 ± 3.2 μg/dl*	
		(Infertile)	
	18.6 ± 3.3 μg/dl	38.3 ± 10.5 μg/dl*	Aribarg and Sukcharoen (1996)
Cd2+ (blood)	0.22 ± 0.21 μg/dl	0.16 ± 0.14 μg/dl	Xuezhi *et al.* (1992)
Cd2+ (semen)	0.04 ± 0.03 μg/dl	0.05 ± 0.05 μg/dl	Xuezhi *et al.* (1992)
	0.38 ± 0.64 μg/l	3.28 ± 0.40 μg/l*	Keck *et al.* (1995)

*Statistically significant increase, $P < 0.01–0.0001$.

between Cd2+ and malignant transformation of prostate epithelial cells (Voeller *et al.*, 1991; Waalkes and Rehms, 1994).

The controversy concerning whether or not the fertility potential of Pb2+- and/or Cd2+-exposed men is decreased may not be resolved in the near future as the developmental risks, e.g. abortions, stillbirths, postnatal deaths, congenital malformations (Sallmen *et al.*, 1992; Xuezhi *et al.*, 1992), from occupational Pb2+ exposures appears to be of greater concern to employers than male reproductive effects (Paul and Kurtz, 1994). This appear also true of investigators studying the effects of environmental exposures (Sram *et al.*, 1996).

Sources of Pb2+ and Cd2+ found in the environment

Environmental discharges of Pb2+ are a major concern as Pb2+ wastes are tracked as hazardous materials. Pb2+ is probably the most common neurotoxin and suspected reproductive toxicant present in the environment at high concentrations as the result of the use of petroleum products (Hutton and Symon, 1986) and demand for lead-acid batteries, the latter requiring mining, smelting and recycling of Pb2+ (Lave *et al.*, 1995). Combustion of fossil fuels (petroleum, coal) and municipal refuse also contribute to airborne Cd2+ pollution (Hutton and Symon, 1986; Ragan and Mast, 1990). As for Pb2+, regional concentrations of Cd2+ may be higher near industrial operations, such as smelters and battery and paint factories (Benin *et al.*, 1999). In addition, the public may be unwittingly exposed to Pb2+ or Cd2+ via contaminated food or paper (Wu *et al.*, 1995) and cosmetics and herbal folk remedies (Wong and Koh, 1986; Lockitch, 1993). The major risk factors for Pb2+ toxicity include age, nutrition (deficiencies in Ca2+, $Fe2+^{/3+}$ and Zn2+), housing and socioeconomic status and, importantly, smoking (Goyer, 1993).

Cigarette smoking is an important confounding variable when considering the effects of both environmental Pb2+ and Cd2+ exposures on human health (Sram *et al.*, 1996). Cigarette smoke is a major source of airborne environmental Pb2+ and Cd2+ exposures. A single cigarette contains 0.6–2.0 μg Pb2+ (Chiba and Masironi, 1991) and 1–4.5 μg Cd2+ (Chia *et al.*, 1994b;

Saldivar *et al.*, 1991) and at least one tenth of the metal content of a cigarette is inhaled (Elinder *et al.*, 1985).

Examination of the effects of environmental exposure to metal ions

Agents which are reproductive toxicants in the workplace are found in human semen, e.g. Cd2+, dibromochloropropane, lithium, and pesticides (Gagnon, 1988). However, the role of increased seminal plasma trace metal concentrations in regulating reproductive function in the occupationally unexposed male is poorly understood. Geographic differences in the amount of naturally occurring Cd2+ have been correlated with incidence rates of prostate cancer (Garcia Sanchez *et al.*, 1992; Angwafo, 1998). In contrast, it has been difficult to identify an association between environmental metal exposures and male infertility. Since the desire for reproduction occurs only intermittently, environmental exposures that interfere with reproduction may go unnoticed (Mattison *et al.*, 1990).

Tobacco smoke is the best studied environmental exposure. Cigarette smoking impairs both female and male fecundity (Zenses, 1995; Hughes and Brennan, 1996; Curtis *et al.*, 1997). The decrease in fecundity in men who smoke cigarettes may be the result of reductions in semen volume, sperm density, sperm motility, normal sperm morphology and/or sperm viability (Saaranen *et al.*, 1987b; Radcliffe *et al.*, 1992; Chia *et al.*, 1994a,b; Vine *et al.*, 1994; Omu *et al.*, 1995; Sofikitis *et al.*, 1995; Vine, 1996; Merino *et al.*, 1998; Rubes *et al.*, 1998). An increase in sperm aneuploidy, e.g. Y disomy, has recently been noted (Rubes *et al.*, 1998). The latter is consistent with an increase in proportion of female offspring in men exposed to Pb2+ (Dickinson and Parker, 1994). The outcome of sperm function assays was significantly lower in men who smoke, compared with non-smoking controls (Sofikitis *et al.*, 1995). Cigarette smoke may also potentiate the effects of other suspected reproductive toxicants, e.g. caffeine (Bolumar *et al.*, 1997).

Although blood concentrations of Cd2+ and Pb2+ may be increased in smokers (Brockhaus *et al.*, 1983; Friberg and Vahter,

Table II. Comparison of Pb2+ and Cd2+ concentrations in blood and seminal plasma in men who smoke cigarettes and non-smoking control subjects

	Non-smokers	Smokers	Source
Pb2+ (blood)	92.0 μg/l	99.2 μg/l	Brockhaus *et al.* (1983)
		7.09 μg/l	Chia *et al.* (1994b)
Pb2+ (semen)		12.9 μg/l	Chia *et al.* (1994b)
	0.24–0.38 μg/l	0.24–0.34 μg/l	Oldereid *et al.* (1994)
		(<1 pack/day)	
		0.22–0.39 μg/l	
		(>1 pack/day)	
Cd2+ (blood)	0.44 μg/l	1.85 μg/l*	Brockhaus *et al.* (1983)
	0.63 ± 0.44 μg/l	1.66 ± 0.93 μg/l	Moreau *et al.* (1983)
	0.24 ± 0.09 μg/l	0.31 ± 0.06 μg/l*	Saaranen *et al.* (1989)
		(<1 pack/day)	
		0.39 ± 0.05 μg/l*	
		(>1 pack/day)	
	0.84 μg/l	1.55 μg/l*	Chia *et al.* (1994b)
Cd2+ (semen)	0.19 ± 0.21 μg/l	0.23 ± 0.17 μg/l	Saaranen *et al.* (1989)
		(<1 pack/day)	
		0.40 ± 0.40 μg/l	
		(>1 pack/day)	
	0.54 μg/l	0.86 μg/l	Chia *et al.* (1994b)
	0.14–0.32 μg/l	0.19–0.38 μg/l	Oldereid *et al.* (1994)
		(<1 pack/day)	
		0.29–0.49 μg/l*	
		(>1 pack/day)	

*Statistically significant increase, $P < 0.05$–0.001.

Table III. Concentrations of Pb2+ and Cd2+ reported in blood and semen from fertile and infertile men

	Fertile	Infertile	Source
Pb2+ (blood)		6.5 ± 5.4 μg/l	Chia *et al.* (1992)
	16.9 ± 2.6 μg/dl	29.3 ± 3.8 μg/dl*	El-Zohairy *et al.* (1996)
Pb2+ (semen)	7.4 ± 0.7 μg/l		Pleban and Mei (1983)
	255 ± 123 μg/l	243 ± 179 μg/l	Umeyama *et al.* (1986)
	1.7 ± 1.0 μg/l	3.6 ± 3.2 μg/l*	Saaranen *et al.* (1987a)
	8 ± 1.1 μg/l	1.2 ± 1.2 μg/l	Abou-Shakra *et al.* (1989)
		(Oligospermic)	
		6 ± 4 μg/l	
		(Severely oligospermic)	
		1.0 ± 1.3 μg/l	
		(Azoospermic)	
	5.61 ± 0.53 μg/l	11.18 ± 0.62 μg/l*	Jockenhovel *et al.* (1990)
	7.7 ± 5.6 μg/l		Noack-Fuller *et al.* (1993)
	11.0 ± 1.9 μg/dl	19.6 ± 2.4 μg/dl*	El-Zohairy *et al.* (1996)
Cd2+ (blood)		1.35 ± 1.0 μg/l	Chia *et al.* (1992)
Cd2+ (semen)	0.48 ± 0.04 μg/l		Pleban and Mei (1983)
	5 ± 6 μg/l	13 ± 12 μg/l*	Umeyama *et al.* (1986)
	0.34 ± 0.16 μg/l		Noack-Fuller *et al.* (1993)
	0.38 ± 0.64 μg/l	0.43 ± 0.69 μg/l	Keck *et al.* (1995)

*Statistically significant increase, $P < 0.05$–0.005.

1983; Moreau *et al.*, 1983; Xu *et al.*, 1993; see Table II), it is more likely that the negative effects of smoking on semen quality are a result of the observed trend for Cd2+ in tobacco smoke to accumulate in the genital tract of smokers (Saaranen *et al.*, 1989; Pacifici *et al.*, 1993; Oldereid *et al.*, 1994; Keck *et al.*, 1995; Omu *et al.*, 1995; see Table II). In contrast, seminal plasma Pb2+ concentrations appear unaffected by smoking status (see Table II).

Not only are smokers at risk. A positive correlation has been observed between the degree of passive exposure to cigarette smoke and the concentration of components of cigarette smoke in seminal plasma (Pacifici *et al.*, 1995).

Subfertile or infertile men may be more sensitive to the deleterious effects of cigarette smoke than fertile men (Zenses, 1995). Risk factors for unexplained dyspermia in infertile men include smoking (Parazzini *et al.*, 1993). Total sperm count and sperm motility are lower in oligozoospermic men who smoke than in oligozoospermic non-smokers (Rantala and Koskimies, 1987; Vine *et al.*, 1996). An acrosome reaction insufficiency was first suggested from the significant decrease in penetration of zona-free hamster oocytes by spermatozoa from infertile men who smoked cigarettes, compared with infertile men who were non-smokers (Close *et al.*, 1990). A reduced inducibility of the acrosome reaction in subfertile men who smoke has since been confirmed (El Mulla *et al.*, 1995). The latter is consistent with reports of increased 'round-headed' spermatozoa, which lack an acrosome (Zamboni, 1987; Escalier, 1990), in the semen of smokers (Rubes *et al.*, 1998). Hormonal alterations may occur (Gerhard *et al.*, 1992). Anti-sperm antibody production is increased in smokers (Omu *et al.*, 1997). An improvement in semen quality has been noted when subfertile men quit smoking (Vine, 1996). The concept of infertility patients as forming 'susceptibility subgroups' is discussed in greater detail later in this review (see section on genetic determinants of susceptibility below).

Metal ion concentrations in fertile and infertile men

It has long been suggested that at least one half of the cases of human male infertility of unknown aetiology may be attributable to various environmental and occupational exposures (Steeno and Pangkahila, 1984a,b; Gagnon, 1988). Therefore, infertility patients have often been used in attempts to investigate the role of environmental metals. Data from such studies may be skewed as a result of selection bias. Not all couples experiencing infertility seek medical intervention (Olsen *et al.*, 1998).

Semen from men presenting for fertility evaluations generally contain only low concentrations of Pb2+ and Cd2+ (Noack-Fuller *et al.*, 1993). Nevertheless, a considerable body of evidence indicates that decreased concentrations of Zn2+ in seminal plasma and semen occur in some forms of infertility (Marmar *et al.*, 1975; Umeyama *et al.*, 1986; Saaranen *et al.*, 1987a; Hurley *et al.*, 1997;

S.Benoff, A.Hershlag and I.R.Hurley, unpublished observations). In contrast, the majority of reports suggest that environmental exposures to Pb2+ do not significantly contribute to male infertility. In at least half the published studies, seminal plasma Pb2+ values have been reported to be similar in fertile and infertile men (Umeyama *et al.*, 1986; Abou-Shakra *et al.*, 1989; see Table III).

Many studies have failed to find any significant differences in the seminal plasma Cd2+ concentrations of fertile and infertile men (Keck *et al.*, 1995). In contrast, in other studies, seminal plasma Cd2+ concentrations were found to be increased in infertile, compared with fertile, men (Umeyama *et al.*, 1986; Saaranen *et al.*, 1987a; Jockenhovel *et al.*, 1990). In addition, increases in blood plasma Cd2+ concentrations in infertile men have been associated with teratozoospermia (Chia *et al.*, 1992) and an inverse correlation between blood plasma Cd2+/seminal plasma Cd2+ and sperm density and semen volume has been reported (Xu *et al.*, 1993). These conflicting findings are summarized in Table III.

Confounding variables

Confounding variables are likely to be responsible for many of the apparent contradictions between similar studies cited in the preceding section. Groups of environmental toxicologists have been convinced of this, and have proposed guidelines for controlling such variables (Tas *et al.*, 1996; Wyrobek *et al.*, 1997). Central to these strategies is use of multiple study endpoints (Perreault *et al.*, 1989), including biomarkers (Smith and Suk, 1994), to assess exposures to other toxicants which may produce or mask conditions induced by the chemical(s) under study. The extent of knowledge of a particular chemical's toxicology and its underlying molecular biology insofar as it relates to the outcome measure under study will determine the comprehensiveness of the list of confounding variables and endpoints.

Depending upon outcome measure, endpoints may include questionnaires to gather medical and reproductive histories, information on lifestyle factors, e.g. smoking, herbal therapy (Ondrizek *et al.*, 1999) and exposures to unrelated toxicants. Physical examinations of subjects are useful in revealing physical conditions affecting male fertility which may not be evident from questionnaires, including varicocele and small testicular size. A standardized classical semen analysis, computerized sperm morphometry and motility together with blood hormone concentrations are recommended both to characterize the effects of a particular exposure and to identify sub-populations, which presumably differ with respect to some confounding variable. Choice of control groups are also a major issue, e.g. see Favino *et al.* (1968) who used Pb2+-exposed men as controls for the effects of Cd2+ on reproductive hormones.

The state of knowledge of heavy metal confounders is relatively satisfactory. Concentrations of various metal ions have been assayed in the same tissues in animal models. This has revealed that transport and equilibrium concentrations of heavy metals can be altered by concentrations of other metals administered in the course of both acute and chronic exposure studies. For example, similarities of the ionic size and normal ionic charge between Cd2+, Ca2+ and Zn2+ with Pb2+ mean that both Cd2+ and Pb2+ can pass the gut/blood barrier by binding to Ca2+ and Zn2+ transport sites on the surface of intestinal villae (Goyer, 1997). High amounts of Ca2+ in diet are protective against both Cd2+ and Pb2+ poisoning. However, gaps remain. Measurements of effects of Pb2+ upon sperm fertility outcome measures, such as progesterone-induced acrosome loss (see section on ion channels and response to environmental metal exposures below), therefore require assessment of the inter-reacting metal confounders, Cd2+, Zn2+ and Ca2+ in the same samples. These measurements must be both accurate and sensitive, as different methods appear to produce discordant results (e.g. colorimetric versus graphite furnace atomic absorption spectroscopy; Fuentes *et al.*, 1981 and Jeyendran *et al.*, 1989 versus Canale *et al.*, 1986, Xu *et al.*, 1993 and Hurley *et al.*, 1997). Other confounding variables which affect the availability of metals may be addressed using conventional assays. These include transport and storage proteins, and components of diet. For example, high phosphate in the diet potentiates Pb2+ poisoning, by forming insoluble complexes with dietary Ca2+.

There is also a need for reliable molecular biomarkers to assess individual susceptibilities to heavy metal toxicity (Todd *et al.*, 1996). Individual variability could explain why, though blood Pb2+ concentrations can clearly be increased by environmental exposures (Sram *et al.*, 1996), population studies of effects of chronic Pb2+ exposure upon fertility-related outcome measures have not produced consensus. The goal therefore is to develop a group of markers similar to the genetically determined polymorphisms in enzymes susceptible to Pb2+ inhibition, which have been suggested to be useful for predicting renal toxicity (Doss *et al.*, 1982; Todd *et al.*, 1996). We suggest, however, that the most important confounding variable is the choice of the population(s) to be studied.

Genetic determinants of susceptibility

There is an increasing awareness in environmental toxicology for factors that predispose individuals to be sensitive to toxicant exposure (Anderson *et al.*, 1994). One of the primary factors leading to the existence of this susceptibility is genetic polymorphisms.

Somatic diseases

Susceptibility subgroups associated with genetic polymorphisms have been identified in a variety of somatic cell diseases, including sickle cell anaemia (Lu and Steinberg, 1996), tobacco-related lung cancer and gastric cancer (Hirvonen, 1995), breast or ovarian cancer (Suk *et al.*, 1996) and prostate cancer (Kantoff *et al.*, 1998). These polymorphisms may take the form of unstable simple DNA repeats (Panzer *et al.*, 1995) or functional enzyme polymorphisms with marked inter-individual variation (Caraco, 1998) that mediate oxidative metabolism and detoxification reactions, e.g. cytochrome *P*-450 (CYP), monamine oxidase, superoxide dismutase and glutathione *S*-transferase (GST) (Hirvonen, 1995; Raunio *et al.*, 1995; Watanabe, 1998).

Infertility

A large portion of male infertility is thought to have an underlying genetic basis, e.g. gonadotrophin-releasing hormone (GnRH) deficiency, spermatogenic failure and both obstructive (e.g.

congenital absence of the vas deferens) and non-obstructive azoospermia (e.g. associated with Y deletions) (Mak and Jarvi, 1996). An association between expression of certain human leukocyte antigen antigens (e.g. HLA-A28 and Bw40) and azoospermia has also been noted (Bisson *et al.*, 1976; Miura *et al.*, 1998). Studies on genetic factors regulating fertility/fecundity and response to environmental agents have been at best extremely limited. Recent studies suggest that inter-individual differences in expression of isoforms of certain enzymes underlie the response to airborne environmental toxicants (e.g. GST and *N*-acetyltransferase versus effects of polycyclic aromatic hydrocarbons; Sram *et al.*, 1996) and may contribute to the production of endometriosis (Baranova *et al.*, 1997) or an increase in spontaneous abortions (Wang *et al.*, 1998). In contrast, we do not know why occupational exposure to suspected reproductive toxicants appears to have a greater effect on male fecundity than do female occupational exposures (Spinelli *et al.*, 1997), or why men are more sensitive than women to environmental exposure to metals (Staessen *et al.*, 1991; Dickman *et al.*, 1998) or organic toxicants (Welch *et al.*, 1988; Schrader, 1997).

Clearly, there is much to learn about the factors which regulate susceptibility. We believe that analysis of infertility patients offers a unique opportunity to address these factors. Our data suggest that infertility patients may be predisposed to and may respond more strongly to environmental metal exposures than the population in general.

Ion channels and response to environmental metal exposures

Acrosome exocytosis induced by sperm–zona binding and required for sperm penetration through the zona pellucida involves sequential activation of sperm head delayed (outward) rectifier voltage-gated potassium (K^+) channel (VGKC) and L-type voltage-dependent Ca2+ (VDCC) ion channels (L-VDCC) (Benoff, 1999). Distinct binding sites for Pb2+, Cd2+ and other metal ions appear to regulate ion permeation (Leinders *et al.*, 1992). Each type of channel is composed of multiple subunits. When expressed *in vitro*, α subunits possess all characteristics necessary to form channel pores (Catterall, 1995; Benoff, 1998).

The α subunit of the typical VGKC consists of six putative transmembrane segments designated S1-S6 (Catterall, 1995). Transmembrane segment S4 is the voltage sensor. The regions between transmembrane segments S5 and S6 contain the ion conducting pore and binding sites for channel blockers and metals ions (Shieh and Kirsch, 1994; Catterall, 1995; Christie, 1995; Doyle *et al.*, 1998). Different isoforms result from sequence changes in the ion conduction pore (Grissmer *et al.*, 1994). Thus, genetic polymorphisms in these ion channels can regulate resistance or susceptibility to environmental metal exposures.

The α-1 subunits of VDCC are related to those of VGKC, being derived from a common ancester (Catterall, 1995). VDCC α-1 subunits are composed of four repeats (domains I–IV) of the basic structural unit of the VGKC α subunit (S1–S6) (Catterall, 1995; MacKinnon, 1995). Multiple L-VDCC α-1 isoforms (e.g. α-1A, α-1B, α-1C, α-1D, α-1E) are generated by transcription of different genes and by alternate splicing of the primary transcript from each gene (Benoff, 1998). The α-1C subunit forms the pore of the L-VDCC expressed in mammalian testis and spermatozoa (Benoff, 1998,1999; Goodwin *et al.*, 1999a,b). This subunit contains the binding sites for a variety of pharmacological agents, including dihydropyridines which are prescribed for hypertension control and which can produce a reversible infertile state (Benoff *et al.*, 1994; Hershlag *et al.*, 1995). Ion selectivity (e.g. Ca2+ versus Na+) is conferred by single amino acid changes in the extracellular mouth of the pore and/or the pore wall itself (Heinemann *et al.*, 1992). By analogy with Na+ channels, sensitivity or resistance to metals ions will also be conferred by single amino acid substitutions (i.e. Backx *et al.*, 1992; Satin *et al.*, 1992).

Genetic polymorphisms in these ion channels expressed in somatic cells have been associated with the generation of diseases. For example, a study of CCG repeat polymorphisms in human brain cDNA (targeted to develop markers for neuro-psychiatric disorders including autism) amplified a partial clone of BCNG-1 (Kleiderlein *et al.*, 1998). BCNG-1 has been identified as encoding a voltage-gated potassium (K^+) channel (VGKC) with properties indistinguishable from brain pacemaker channels (Santoro *et al.*, 1998). If BCNG-1 shows the sensitivity to Pb2+ of other voltage-gated K^+ channels (Kiss and Osipenko, 1994), its polymorphic expression may provide a mechanism for the variability confounding neuro–psychiatric effects of paediatric Pb2+ exposure (Needleman, 1988).

With regard to VDCC, alternate splicing in the carboxy terminus of the α-1A subunit is linked to a severe form of human ataxia (Zhuchenko *et al.*, 1997). Sequence changes, including one in transmembrane segment IIIS2, of the α-1A result in familial hemiplegic migraine and episodic ataxia type-2 where patients exhibit migraine-like symptoms (Ophoff *et al.*, 1996). These findings are of particular importance given that we have identified multiple splice variants of the human α-1C subunit that are expressed in testis and spermatozoa. The alternatively spliced regions include the amino terminus and transmembrane segments IS6, IIIS2 and IVS3 (see section on varicocele-associated infertility below).

Potential models for metal effects mediated by ion channels

We now present evidence that the interaction between environmental exposure to Cd2+ and L-VDCC results in infertility in the presence of a varicocele. We also report that many cases of 'unexplained' infertility can be attributed to the interaction of VGKC and environmental Pb2+ exposures.

Varicocele-associated infertility

Varicocele-associated infertility is widespread: ~40% of males from infertile couples present with varicocele (Greenberg, 1977; Goldstein *et al.*, 1992; WHO, 1992; Schlesinger *et al.* 1994). The mechanism(s) underlying varicocele-associated infertility, however, are poorly characterized. The most widely accepted explanation for the pathophysiology of varicocele in male infertility is abnormally elevated testicular temperature due to impaired heat transfer by the scrotum and/or changes in testicular blood flow (Comhaire, 1991). Although men with varicocele exhibit higher mean scrotal temperatures, there is a large overlap with the range of scrotal temperatures in fertile men (Mieusset and

Bujan, 1995). More importantly, only 13% of men with varicocele are infertile (Kursh, 1987; Sylora and Pryor, 1994), and, although varicocele repair has been documented to reduce testicular temperature (Agger, 1971; Yamaguchi *et al.*, 1989), only 1/3 of infertile men with varicocele will experience a return of fecundity following varicocele correction (Schlesinger *et al.*, 1994). These findings suggest that varicocele may not be a primary cause of infertility and that it is the interaction of varicocele with other as yet unidentified factors that produces the infertile state (Peng *et al.*, 1990; Gentile and Cockett, 1991; Mieusset and Bujan, 1995).

We suggest that one such factor is the concentration of Cd2+ in the male reproductive tract (Benoff, 1997; Benoff *et al.*, 1997,1998a). In animal varicocele models, exposure to components of cigarette smoke increases impairment of spermatogenesis. Although there is some controversy as to whether smoking reduces semen quality (Goverde *et al.*, 1995; Hughes and Brennan, 1996) or reduces fertility (Vine, 1996) in all men of reproductive age, it is generally agreed that men with marginal semen quality may be made infertile by smoking (see discussion in section on examination of the effects of environmental exposure to metal ions above). Oligozoospermia among men with varicocele who also smoke is: (i) 10-fold more frequent than among other men with varicocele and (ii) five-fold more frequent than among smokers without varicocele (Klaiber *et al.*, 1987).

Our data indicate that the concentration of Cd2+ in seminal plasma from infertile men with varicocele is markedly increased, compared with fertile men with varicocele (Figure 1) or men with other forms of infertility (e.g. see Figure 2). Recent findings indicate that Cd2+ concentrations are similarly increased in the testis of infertile men with varicocele (Figure 1; Benoff *et al.*, 1999b). After varicocele repair, pregnancy by coitus was observed only in those men whose Cd2+ concentrations were at the lower end of the range (Benoff *et al.*, 1998a). These findings suggest that measurements of seminal plasma Cd2+ concentrations may serve as a biomarker for the outcome of varicocele repair.

Men with secondary infertility and varicocele present with seminal plasma Cd2+ concentrations intermediate between those of fertile men and men with primary varicocele-associated infertility (Benoff *et al.*, 1999b), suggesting that the deleterious effects of Cd2+ are time and concentration dependent. Blood plasma Cd2+ concentrations did not, however, differ significantly between infertile and fertile men with varicocele (Fig. 1). These data emphasize that the choice of variable to be analysed influences whether or not an association is detected between an environmental exposure and production of the infertile state.

It is known that infertile men with varicocele are typically oligozoospermic. The sperm populations from these men have increased tapering head forms ('stress pattern'), reduced zona pellucida binding ability and an acrosome reaction insufficiency (Rogers *et al.*, 1985; Naftulin *et al.*, 1991; Vigil *et al.*, 1994; Benoff *et al.*, 1995a, 1997; El Mulla *et al.*, 1995; Benoff, 1997). These defects can clearly result from an interaction between the observed increase in testicular Cd2+ and actin. Cd2+ exposure results in depolymerization of actin filaments and a net decrease in actin monomer protein (Wang *et al.*, 1996). In somatic cells, actin loss increases apoptosis (Russo *et al.*, 1982; Tsukidate *et al.*, 1993) (which could lead to oligozoospermia in infertile men with varicocele) and decreases the ability to undergo exocytosis (Muallem *et al.*, 1995) (which requires both actin polymerization and depolymerization, as in the acrosome reaction; Rogers *et al.*, 1989; Spungin *et al.*, 1995; Benoff *et al.*, 1996; Benoff, 1999).

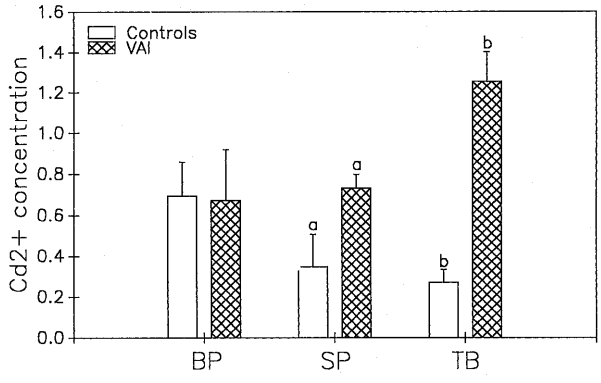

Figure 1. Comparison of the cadmium (Cd2+) content in blood plasma (BP), seminal plasma (SP) and testis biopsies (TB) of infertile men with varicocele (VAI) and men without varicocele (controls). Cd2+ concentrations were determined by graphite furnance atomic absorption spectroscopy (Benoff, 1997; Benoff *et al.*, 1997, 1998a, 1999b; Hurley *et al.*, 1997). The bars in the figure indicate the BP and SP Cd2+ content in μg/l and in testis biopsies (obtained by percutaneous needle aspiration; Marmar, 1998) in ng/mg dry weight. The concentrations of Cd2+ in blood plasma from male partners without varicocele from couples seeking fertility evaluation ($n = 66$) and from VAI males ($n = 14$) were similar. In contrast, SP Cd2+ concentrations were significantly elevated in men with VAI ($n = 14$), compared with fertile men with varicocele ($n = 4$) (a, $P < 0.0001$; *t*-test). Similarly, TB Cd2+ levels were higher in men with VAI [$n = 8$; Johnsen score (Johnsen, 1970) = 8–9] than in TB from men with non-obstructive azoospermia ($n = 4$; Johnsen score = 6) (b, $P < 0.03$; *t*-test).

Preliminary examination of testis biopsies from infertile men with varicocele suggests that this indeed is the case. Actin immunoreactivity was decreased and apoptosis, which normally occurs at low concentrations during spermatogenesis (Furuchi *et al.*, 1996; Tesarik *et al.*, 1998) was increased, compared with biopsies from men without varicocele (Benoff *et al.*, 1999b). These data are consistent with findings in animal models wherein in-vivo administration of Cd2+ induced apoptosis in testicular tissue (Xu *et al.*, 1996; Yan *et al.*, 1997).

In-vitro modelling experiments support our in-vivo findings (Benoff, 1997; Benoff *et al.*, 1997). Cd2+ exposure changed fertile donor sperm protein expression (e.g. loss of sperm head actin, reduced percentages of spermatozoa expressing surface mannose and non-nucler progesterone receptors) and function (e.g. acrosome reaction insufficiency) to mimic varicocele in a dose–response fashion, albeit at Cd2+ concentrations >30-fold higher than in seminal plasma from infertile men with varicocele. After fertile donor spermatozoa were briefly exposed to higher temperatures, lower Cd2+ concentrations produced more drastic sperm function changes, indicating that temperature and Cd2+ act synergistically, as in cryptorchid animal models (Chatterjee and Ray, 1972; Fende and Niewenhuis, 1977).

Increased testicular Cd2+ concentrations are probably derived from the increased transvascular fluid exchange which occurs with varicocele (Shafik and Bedeir, 1980; Sweeney *et al.*, 1995).

This means that there is the potential for more of the Cd2+ in serum to enter the testis. As there is no active pump to remove this Cd2+ (Gunn *et al.*, 1961), testicular Cd2+ concentrations would be elevated over time. Results from autometallography (Benoff, 1998,1999; Benoff *et al.*, 1999b) indicate that this Cd2+ can enter spermatogenic cells via L-VDCC (Kiss and Osipenko, 1994; Atar *et al.*, 1995), which are expressed in all cells within the germinal epithelium (Goodwin *et al.*, 1998). The multiple isoforms of the testis-specific L-VDCC which have been identified result from alternate splicing of a primary transcript from a single gene (Benoff, 1998). Preliminary findings suggest that each man expresses only one L-VDCC splice variant in his ejaculated spermatozoa (Goodwin *et al.*, 1999a,b). The Cd2+ sensitivity or resistance of L-VDCC is conferred by the amino acid sequence within a short segment of the protein (domain I, transmembrane segment S6; e.g. Backx *et al.*, 1992). This region is alternatively spliced in the testis-specific L-VDCC and a series of amino acid substitutions within this region have also been observed (Benoff, 1999). Thus, our working hypothesis is that infertility in these men results from the interaction of three factors: (i) the presence of a varicocele; (ii) an increase in testicular Cd2+ concentrations; and (iii) the expression of particular L-VDCC isoforms.

Lead and unexplained infertility

We have been prospectively evaluating men from infertile couples undergoing their first IVF cycle in order to identify biomarkers which predict fertilization outcome and which are not related to sperm count, motility, viability or morphology (Benoff *et al.*, 1999a). An unexpected finding was that >40% of these males, who were not exposed to Pb2+ in their places of work and who did not smoke cigarettes, exhibited blood and seminal plasma Pb2+ concentrations that were above the permissible limit in men occupationally exposed to Pb2+ (Benoff *et al.*, 1998b). Both blood and seminal plasma Pb2+ concentrations in these men were inversely correlated with the rate of fertilization of metaphase II oocytes in IVF (Benoff *et al.*, 1998b; Benoff, 1999; see Figure 2). The negative effect of increased Pb2+ on IVF outcome was attributable to altered sperm function, i.e. increased spontaneous acrosome loss and a decreased ability to undergo an acrosome reaction induced by exposure of capacitated spermatozoa to progesterone or model zona ligands containing mannose. The progesterone-stimulated acrosome reaction was the parameter most severely affected by Pb2+. Mannose receptors and non-nuclear progesterone receptors are co-expressed on a subpopulation of motile human spermatozoa (Benoff *et al.*, 1995c) and the response to progesterone determines whether or not human spermatozoa undergo an acrosome reaction after zona binding and fertilize human oocytes (Jacob *et al.*, 1998c).

Results from cDNA cloning and in-vitro modelling studies employing K^+ channel inhibitors provided evidence that the effects of progesterone on the human sperm acrosome reaction were mediated by a delayed rectifier VGKC (Jacob *et al.*, 1997a,b, 1998a,b) and that this VGKC is upstream of the L-VDCC which regulates the sustained Ca2+ influx required for the AR (Benoff, 1999). K^+ channels in somatic tissues can be inhibited by Pb2+ (Kiss and Osipenko, 1994) and environmental Pb2+ exposures have been reported to alter K^+ transport by red blood cells (Hajem *et al.*, 1990).

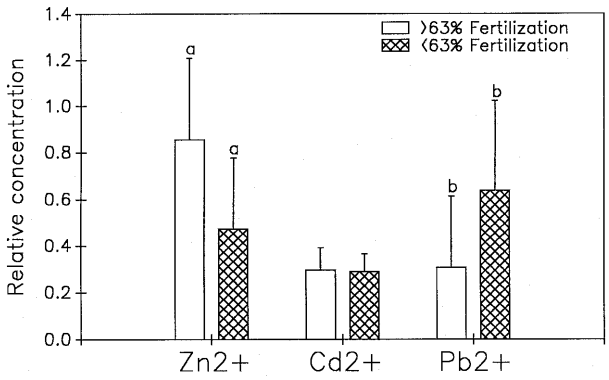

Figure 2. Comparison of the concentrations of zinc (Zn2+), cadmium (Cd2+) and lead (Pb2+) in seminal plasma from men exhibiting high rates of fertilization following conventional IVF and men exhibiting reduced or failed fertilization. In a prospective analysis, we evaluated the concentrations of metals in seminal plasma (SP) from men from consecutive couples undergoing their first cycle of conventional IVF (n = 96). The number of spermatozoa used in each inseminate was normalized on the basis of acrosomal morphology, so that at least 25 000 spermatozoa with an oval head with a well-defined acrosome covering >50% of the head were inseminated per oocyte cultured in 1 ml of medium (Benoff *et al.*, 1999a). The mean fertilization for this study population was 85.2 + 11.1% (Jacob *et al.*, 1998c; Benoff *et al.*, 1999a). Cases of 'reduced' fertilization were defined as exhibiting fertilization rates >2 SD below the mean, e.g. <63%. Cases of 'high' fertilization were defined as exhibiting fertilization rates >63%. SP metal concentrations were determined by graphite furnance atomic absorption spectroscopy (Benoff, 1997; Benoff *et al.*, 1997, 1998b; Hurley *et al.*, 1997; S.Benoff and I.R.Hurley, unpublished). SP Zn2+ concentrations are presented as millimolar concentrations while SP Cd2+ and SP Pb2+ concentrations are reported as μg/l. Although SP Cd2+ did not differ between the two groups, SP Zn2+ was markedly decreased (a, $P < 0.0001$; t-test) and SP Pb2+ was significantly increased (b, $P < 0.0002$; t-test) in cases of reduced fertilization, compared with cases with high fertilization rates.

Sperm surface VGKC were mapped using a biotinylated charybdotoxin (CBTx) probe (Figure 3a,b). Of spermatozoa from fertile donors ~38% exhibited head-directed CBTx binding. Sites of exogenous Pb2+ entry into the human sperm head were similarly localized by autometallography (Figure 3c,d). The same percentages of spermatozoa that bound CBTx also bound Pb2+, providing strong evidence that a VGKC capable of transporting Pb2+ is expressed on the human sperm head. Preliminary results from in-vitro modelling studies confirmed these findings (Figure 4). CBTx strongly inhibited the increase in spontaneous acrosome loss which normally results from Pb2+ exposure. These data are consistent with findings in animal models that in-vivo Pb2+ exposure causes premature acrosome reactions (Johansson, 1989) and provide strong support for our hypothesis that Pb2+ action to reduce human sperm fertilizing potential is mediated by a sperm head VGKC.

Analysis of the effects of the K^+ channel inhibitors CBTx, tetraethylammonium ion (TEA), and 4-aminopyridine (4AP) on progesterone-stimulated acrosome loss by fertile donor spermatozoa provided evidence for inter- male variability in VGKC isoform expression (Benoff, 1999). The existence of multiple VGKC isoforms would help to explain why two out of 53 men who exhibited high rates of fertilization had seminal plasma Pb2+ concentrations that were >10 μg/dl (the upper limit of the range of blood Pb2+ concentrations in unexposed men) and three

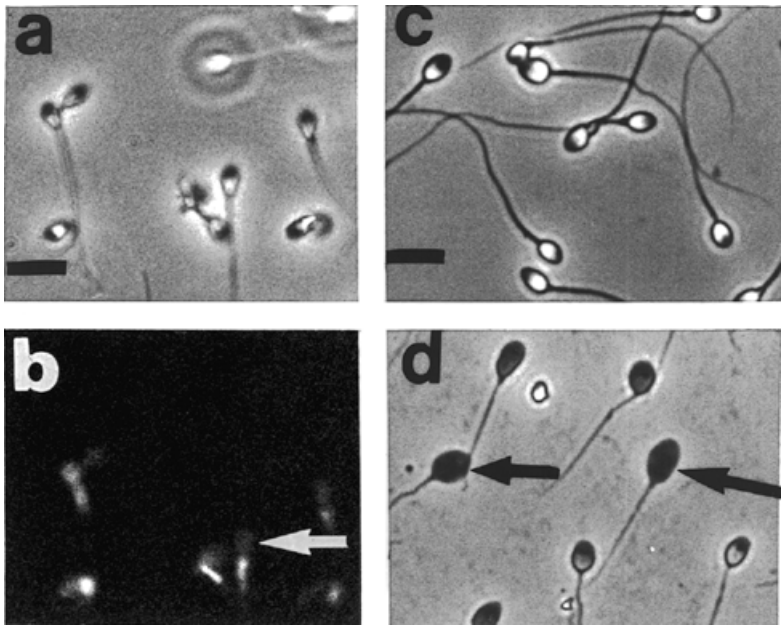

Figure 3. Voltage-gated potassium channels and sites of exogenous lead entry co-localize on the human sperm head surface. Specimens were viewed at ×400 original magnification with an Olympus microscope (Olympus Corp., Lake Success, NY, USA) and photographed at ×600 on 35 mm/400 ASA black and white film (Eastman Kodak Co, Rochester, NY, USA) with automatic exposure for phase-contrast images (**a**, **c**, **d**) and 50 s exposure for the epifluorescence image (**b**). (**a**, **b**) A minimally biotinylated charybdotoxin probe was prepared by modifying the protocols supplied with commercial reagents (Pierce, Rockford, IL; Bio Rad Laboratories, Hercules, CA, USA) (A.Jacob, I.R. Hurley and S.Benoff, unpublished observations). Motile spermatozoa from fertile donors were surface labelled with the biotinylated charybdotoxin and sperm-bound charybdotoxin was visualized by indirect immunocytochemistry using polyclonal antibodies to biotin prepared in rabbit (Enzo Diagnostics, New York, NY, USA). (**a**) Paired phase-contrast and (**b**) epifluorescence images are shown. The arrow in the figure points to charybodtoxin binding over the entire sperm head surface. Scale bar = 12.5 μm. (**c**, **d**) Motile spermatozoa from fertile donors were incubated overnight in capacitation media supplemented with 2.5 μmol/l lead (Pb2+). Sperm-bound Pb2+ as localized by autometallography (Stoltenberg *et al.*, 1997a,b). Pb2+ bound over the entire sperm head is visualized as black silver deposits (**d**, arrow) after conversion of Pb2+ to Pb sulphide and physical development with silver lactate. Black silver deposits are not detected in control aliquots (**c**) exposed to Pb2+ but not treated with sodium sulphide prior to physical development. Scale bar = 12.5 μm.

out of 21 men who exhibited reduced or failed fertilization had Pb2+ concentrations <10 μg/dl. Further studies are now in progress to identify the specific structural variations in the human sperm VGKC α subunit which regulate sensitivity to Pb2+.

Conclusions

We are left with a puzzle. The clinical data presented above show that two groups of infertile men have abnormally high metal concentrations in seminal plasma, without any obvious recent high exposure to these toxicants. The in-vitro studies and the molecular genetics provide, in outline, a plausible mechanism showing how these toxicants could produce the characteristic clinical symptoms of each type of infertility. However, this does not explain the aetiology of high metal concentrations. Both Cd2+ (characteristic of the varicocele-related infertile group) and Pb2+ (high spontaneous acrosome reaction group) are metals with a low clearance rate. It is possible that members of both groups had some unrecorded high exposure to these metals in the past, and their blood and seminal plasma concentrations are equilibrium concentrations reflective of normal clearance. This is hard to credit, as an exposure large enough to give the concentrations we observed after an uncertain number of years of the initial exposure is likely to have exhibited clinically significant symptoms at the time of that exposure. Another possibility is that the individuals with high metals concentrations have some other condition which predisposes them to accumulate high amounts of toxicant from exposures no higher than those most of us encounter. This

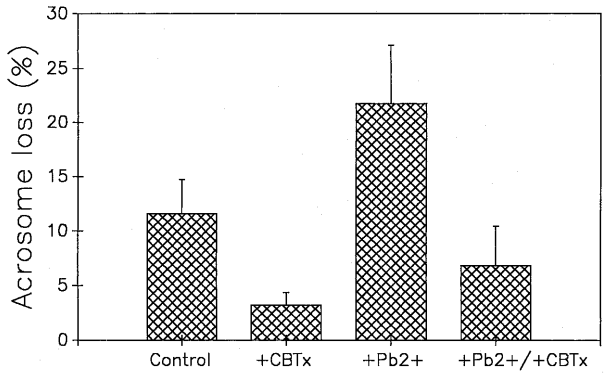

Figure 4. Opposing effects of lead (Pb2+) and charybdotoxin (CBTx) on the spontaneous acrosome reaction. Motile sperm populations from fertile donors ($n = 5$) were divided and exposed to 50 nmol/l CBTx or 2.5 μmol/l Pb2+, alone or in combination, and compared with aliquots not exposed to either agent (control). Spontaneous acrosome loss was assessed following reaction with rhodamine-labelled *Pisum sativum* agglutinin, which binds to acrosome content (Benoff *et al.*, 1995b). Pb2+ exposure significantly increased acrosome loss ($P < 0.007$). CBTx decreased both the level of control acrosome loss ($P < 0.005$) and that observed following Pb2+ exposure ($P < 0.006$). The percentages of spermatozoa undergoing an acrosome reaction in the presence of both Pb2+ and CBTx were similar to that in the presence of CBTx alone (not significant).

condition may be dietary, related to intake of some other metals or minerals which alter the bio-availability of toxicants. Alternatively, these conditions may be genetic, involving isoforms of proteins involved in transport, membrane passage or storage of metals. If genetic predisposition does indeed create susceptibility subgroups with significantly lower tolerance of toxicant exposures, it may be necessary to reconsider the standards embodied in current legislation.

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