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# Cadmium Concentrations in the Testes, Sperm, and Spermatids of Mice Subjected to Long-Term Cadmium Chloride Exposure

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**Background:** Exposures to cadmium have been reported to reduce male fertility and there are several hypotheses that suggest how reduced male fertility may result from incorporation of cadmium into sperm chromatin The purpose of this study was to determine whether mice subjected to long-term intraperitoneal cadmium exposure incorporated cadmium into their sperm chromatin.
**Methods:** Male mice were exposed to 0.1 mg/kg body weight cadmium in the form of $CdCl_2$ via intraperitoneal injection once per week for 4, 10, 26, and 52 weeks and then sacrificed. The cadmium contents of the liver, testes, pooled sperm, and pooled spermatids from dosed and control animals were determined by atomic absorption spectroscopy. Cadmium and zinc contents in individual sperm and spermatid heads were determined by particle-induced x-ray emission.
**Results:** Atomic absorption spectroscopy revealed that although cadmium accumulated in the liver and testes, cadmium was not detected in pooled sperm or spermatid samples down to minimum detectable limits of 0.02 μg/g dry weight. Particle-induced x-ray emission analyses did not show the presence of cadmium in any sperm or spermatid head down to minimum detectable limits of 15 μg/g dry weight. Particle-induced x-ray emission analyses also demonstrated that phosphorus, sulfur, and zinc concentrations in individual sperm and spermatid heads were not altered by exposure to $CdCl_2$.
**Conclusions:** Because cadmium was not incorporated into sperm chromatin at levels above 0.02 μg/g dry weight, the data cast doubt on hypotheses that suggest that reduced male fertility may result from incorporation of cadmium into sperm chromatin. Cytometry 35:30–36, 1999. **Published 1999 Wiley-Liss, Inc.†**

**Key terms: cadmium; sperm; testes; fertility; reproduction**

The roles that heavy metals play in the etiology of reproductive pathology have been debated for several decades. Exposures to lead and cadmium have been reported to reduce male fertility in both humans and rodents (1–6) and there are several hypotheses that suggest how reduced male fertility may result from incorporation of heavy metals into sperm chromatin (7,8). One hypothesis suggests that these metals, which bind tightly to free thiols, replace or compete with the zinc that is normally bound to the cysteine residues in protamine, forming more stable metal - SH bonds that ultimately prevent proper decondensation of sperm chromatin following fertilization (8). An alternate hypothesis is that the presence of tightly bound cadmium may prevent normal disulfide bond formation within and among protamines during the final stages of sperm maturation. The disruption of this process, or the sequestration of free thiols that may be required for protamine removal from DNA after fertilization, could lead to dominant lethal mutations, similar to those observed in mice exposed to thiol alkylating agents (9). Heavy metals carried into the egg by sperm may also pose a significant risk to the developing embryo via their toxicity. However, some of the information implicating the involvement of heavy metals in the etiology of reduced male fertility has been anecdotal (10) and there are few studies that demonstrate the capacity of semen and sperm to accumulate these metals.

The purpose of the present study was to measure the concentrations of cadmium and zinc in the testes, sperm, and spermatids from mice that have been exposed to



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cadmium via intraperitoneal injection in order to determine whether cadmium is incorporated into sperm chromatin following long-term cadmium exposure.

## MATERIALS AND METHODS

Sexually mature 12-week-old male mice (*Mus musculus*, Swiss Webster strain) were acquired from Simonsen Laboratories (Gilroy, CA). Mice were divided into five groups of at least three animals. In four of the groups each mouse was dosed via intraperitoneal injection once a week with 0.1 mg/kg body weight cadmium in the form of $CdCl_2$ (Sigma Chemical Company, St. Louis, MO) dissolved in saline (0.008 mg/ml $CdCl_2$ in 0.9% NaCl). An equivalent volume of saline (0.9% NaCl) was administered to a fifth set of mice as a vehicle control. The LD50 for a single intraperitoneal dose of cadmium (in the form of $CdCl_2$) in mice is 6.75 mg/kg body weight (11). The dose of 0.1 mg/kg body weight cadmium per week was derived so that over a 52-week exposure the sum of the individual exposures would not exceed a single LD50 dose. (For comparison, cigarette smoke contains up to 6.67 µg cadmium/cigarette (12). For a 70-kg human, this data indicates that up to 13 µg/kg cadmium per week could be inhaled from smoking 20 cigarettes per day. Such a dose is consistent with cadmium contents of 0.4 µg/kg found in blood serum from human males who smoke approximately 20 cigarettes per day (13).)

Four groups of mice were exposed to $CdCl_2$. One group of mice were sacrificed 4, 10, 26, and 52 weeks after initiation of exposure. At least three mice from the control group were sacrificed at each of the 4-, 10-, 26-, and 52-week time points as well as at the beginning of the intraperitoneal exposure (0 weeks). Exposed and control animals were sacrificed by cervical dislocation following anesthesia in $CO_2$. Testes, liver, and caudal portions of the epididymides were removed. The external physical appearance of the testes and epididymes were recorded.

### Isolation of Sperm

Sperm were isolated from the epididymides by teasing apart each epididymis in 2 ml, 0.15 M ammonium acetate (Ameresco, Cleveland, OH), pH 7.4, aspirating the suspension to release the sperm and filtering it through a fine silk filter (No. 11 Standard Swiss Silk Doufour, Abbe Engineering Co., Brooklyn, NY). The suspension was centrifuged at 5000 *g* for 3 min. The sperm pellets were washed twice in 2 ml ammonium acetate, pH 7.4, and once in 2 ml water. Each wash was performed by resuspending the pellet with sonication using a W-220 Sonicator (Heat Systems - Ultrasonics, Farmingdale, NY) equipped with a Teflon-encased tip. Following sonication, each suspension was centrifuged at 5000 *g* to re-pellet the sperm. The final sperm pellet was resuspended in water. Sonication breaks off the majority of sperm tails but leaves the vast majority of sperm cell heads intact. Optical microscopy studies of small aliquots from the final pellets revealed that the pellets were comprised of intact sperm heads and tails.

### Isolation of Spermatids

Spermatids were released from one testis in water by removing the outer membrane, teasing apart the tissue into small pieces, and aspirating the tubules with a Pasteur pipette. The suspension was filtered as described above. The filtering process effectively separates the spermatids from the supporting testicular tissue, which was discarded. The filtered spermatids were centrifuged at 5000 *g* for 3 min and the spermatid pellets were subsequently washed three times in water with sonication as described above. Sonication destroys early-stage spermatids and any other cells that may remain following the filtration and initial pelleting processes, as well as breaking off tails from the majority of spermatids. However, the vast majority of late-stage spermatid heads (stages 12 to 16) are left intact. Optical microscopy studies of small aliquots from the final pellets revealed that the pellets were comprised of intact late-stage spermatid heads and tails.

### Validity of Sperm and Spermatid Extraction Procedures

The sperm and spermatid samples were isolated using a method that ensured they are representative of the entire population of sperm or late-step spermatid cells in the epididymis or testis. The tissues were teased apart and aspirated, releasing the sperm or spermatids into 0.15 M ammonium acetate or water. The resulting suspensions were then filtered to separate the sperm/spermatids from the supporting epididymal/testicular tissue. The method is so efficient at releasing sperm and spermatids from these tissues that it is used to measure sperm numbers produced by the animal (14), and also used to evaluate sperm head shape morphologies (15,16). Although the sonication step is employed to selectively disrupt and remove immature spermatids and somatic cells by lysing their nuclei, mouse stage 12–16 late-stage spermatid and mature sperm (those cells in which DNA is packaged by protamine) nuclei have been shown to be resistant to sonication (17) and this method is used routinely to prepare samples of these cells.

### Rationale for Analytical Techniques

Atomic absorption spectroscopy (AAS) has been routinely used to detect and quantify cadmium contents in human seminal plasma (13,18,19). Consequently, AAS was selected as the analytical technique to determine cadmium contents in pooled sperm, pooled spermatids, liver, and a testis from each sacrificed animal. Procedures involving $^{109}Cd$ uptake (20) can be more sensitive than AAS but they are more cumbersome to use. Furthermore, procedures studying $^{109}Cd$ uptake also involve higher costs because animals and waste generated are subject to radioactive materials regulations and management.

Particle-induced x-ray emission (PIXE) has been utilized in prior studies to accurately and precisely determine protamine and zinc contents in individual sperm (21–23). Consequently, PIXE was utilized in this study to determine, at the individual sperm level, whether exposure to

cadmium altered the zinc content in sperm/spermatid chromatin. In addition, although it is at least 100 times less sensitive than AAS for the detection of cadmium, PIXE was utilized to determine whether cadmium was compartmentalized in a small subpopulation of sperm.

### Preparation of Sperm and Spermatids for Quantitative Elemental Analysis

Portions (1 to 2 μl) of the final aliquots containing sperm or spermatids heads were mounted on nylon foils and freeze-dried for PIXE analysis and stored as previously described (21–23). The remainder of the sonicated sperm and spermatid samples were frozen to −40°C in small plastic vials and lyophilized to remove unbound water prior to analysis by AAS. The other testis and liver were sealed in plastic vials, immediately frozen, and stored in a −40°C freezer.

### Exposure to a 6.75-mg/kg Dose of $CdCl_2$

In addition to the long-term exposure study, another study was performed in which five mice (*Mus musculus*, Swiss Webster strain) were subjected to a single intraperitoneal dose of 6.75 mg/kg body weight cadmium (LD50 equivalent dose) in the form of $CdCl_2$ dissolved in 0.9% sodium chloride. Three of these mice were sacrificed 11 days later by cervical dislocation following anesthesia by $CO_2$, and the liver, testes, and caudal portions of the epididymides were removed. Sperm and spermatid samples were prepared for both AAS and PIXE analyses as described above.

### Analysis of Individual Sperm and Spermatid Heads

The phosphorus, sulfur, zinc and cadmium contents within individual sperm and spermatid heads were measured with the nuclear microprobe located at the Lawrence Livermore National Laboratory (24). PIXE data were obtained using incident 3 MeV proton beams. For the measurements reported here, beam currents of up to 1.5 nA focused down to spot sizes of 3 μm were scanned over areas of $\sim 20 \times 19$ μm$^2$ as previously described (21–23). Each area was irradiated with an exposure of up to 5.0 μC and contained the head from a single sperm or spermatid. Data were reduced off-line and analyzed with x-ray spectrum fitting codes as previously described (21–23). Phosphorus and sulfur counting statistics from each sperm/spermatid head were better than 1.5%, whereas zinc x-ray counting statistics were better than 4%. Background levels of phosphorus, sulfur, cadmium, and zinc on the nylon foils were below minimum detectable limits of $\sim 1$ ng/cm$^2$ for each sample. A series of thin film standards containing phosphorus, sulfur, zinc, and cadmium were used to calibrate the x-ray detection system and elemental masses within sperm/spermatid heads were calculated with the thin film approximation as previously described (21–23). The measurement of elemental masses is accurate to within 7% (4% detector efficiency/charge collection and 5% scan area calibration/thin film approximation added in quadrature).

The DNA and protamine content inside the nucleus of a mature sperm can be derived from the phosphorus and sulfur content of the sperm nucleus (21–23). The DNA and protamine mass calculations assume that the phosphorus signal arises from DNA and the sulfur signal arises from protamine. Although we have analyzed sperm and spermatid heads in this study rather than amembraneous nuclei, the phosphorus and sulfur contents in the sperm and spermatid heads are similar to those found previously in amembraneous nuclei (21,23). Consequently, the phosphorus and sulfur masses can be used as a rough guide to the DNA and protein content within individual sperm and spermatid heads. To obtain the approximate DNA mass (assuming the phosphorus signal derives entirely from DNA) the phosphorus mass is multiplied by 10.66, whereas the approximate protein mass in sperm and spermatids (assuming the sulfur signal derives entirely from protamine) can be obtained by multiplying the sulfur mass by 33.5 (21,23) .

For each of the 0-, 4-, 10-, 26-, and 52-week time points, sperm and spermatid heads from three control animals were analyzed. Sperm and spermatids from three cadmium-dosed animals were analyzed at each of the 4-, 10-, 26-, and 52-week time points. Nine sperm heads and nine spermatid heads were analyzed per animal. For each animal mean phosphorus, sulfur, and zinc contents within the analyzed sperm and spermatids were calculated and subsequently used for statistical analysis. Statistical analysis was performed separately on the phosphorus, sulfur, and zinc data, by one-way analysis of variance (ANOVA) with a significance level of 0.01. (For the ANOVA studies, nine different exposure conditions were considered corresponding to the five control time points and four dosed time points. Each exposure condition had three data points with each data point corresponding to the mean elemental concentration measured in the sperm/spermatids from one animal).

### Measurement of Cadmium Levels in Sperm/Spermatid Wash Media

Washing the sperm cells could result in the possibility of a loss of cellular cadmium due to redistribution of cadmium with the wash media. Consequently, several samples of used sperm and spermatid wash media were selected and analyzed for cadmium content via PIXE. A 5-μl aliquot of each selected sample was deposited on a nylon foil as described above and freeze-dried. The mass of freeze-dried material on the nylon foils was determined by weighing the sample frame before deposition of the media and after lyophilization and was found to be $<50$ μg in all instances. PIXE data from these samples were obtained using incident 3 MeV proton beams. Beam currents of up to 2 nA focused down to spot sizes of 10 μm were scanned over areas of $\sim 100 \times 100$ μm$^2$ on each freeze-dried media deposit for an exposure of up to 5.0 μC.

Table 1
*Concentrations (μg/g) of Cadmium in Liver, Testes, Pooled Sperm and Pooled Spermatids Following 4, 10, 26, and 52 Weeks of $CdCl_2$ Exposure and Associated Controls*

| Exposure | Liver[a] | Testis[a] | Sperm[b] | Spermatid[b] |
|---|---|---|---|---|
| 0 weeks | 0.05 ± 0.03 | not detected[c] | not detected[c] | not detected[c] |
| 4 weeks dosed | 2 ± 1 | 0.2 ± 0.1 | not detected[c] | not detected[c] |
| 4 weeks control | 0.07 ± 0.02 | not detected[c] | not detected[c] | not detected[c] |
| 10 weeks dosed | 7.6 ± 0.8 | 0.4 ± 0.1 | not detected[c] | not detected[c] |
| 10 weeks control | 0.08 ± 0.03 | not detected[c] | not detected[c] | not detected[c] |
| 26 weeks dosed | 4 ± 2 | 1.6 ± 0.2 | not detected[c] | not detected[c] |
| 26 weeks control | 0.02 ± 0.01 | not detected[c] | not detected[c] | not detected[c] |
| 52 weeks dosed | 16 ± 2 | 3 ± 1 | not detected[c] | not detected[c] |
| 52 weeks control | 0.21 ± 0.02 | not detected[c] | not detected[c] | not detected[c] |

[a]Concentrations are wet weight with each result expressed as the mean ± standard deviation of measurements from three animals.
[b]Concentrations are dry weight with each result expressed as the mean ± standard deviation of measurements from three animals.
[c]Not observed at levels at or above the minimum detection limit of 0.02 μg/g.

### Analysis of Cadmium Contents in Testes, Livers, and Pools of Lyophilized Sperm and Spermatids

The cadmium content of the lyophilized pooled sperm and pooled spermatid samples, a testes and the liver from each sacrificed animal was determined using AAS. Frozen liver and testes samples were allowed to thaw immediately prior to digestion. Sample masses were determined by weighing the sample container before and after sample transfer into analytically clean 50 mL beakers containing 5 mL of Aqua Regia (50:50 HCl:$HNO_3$). Lyophilized sperm and spermatid samples typically had a mass of 0.7 mg, liver samples had a mass of 1.5 g, and testes had a mass of 0.15 g. The digestion was carried out at 80°C on a hot plate and the mixture was allowed to retort for 6 hours. Testis samples typically had small amounts of lipids present (<10 μL), which were dealt with by adding <1 mL of 3% hydrogen peroxide in 300 μL aliquots. Larger amounts of lipids (< 100 μL) were present in liver tissue. These lipids were extracted with hexane. Cadmium was not found at or above the limits of detection in the extraction media.

Once digestion was complete, sample volumes were reduced by allowing water to evaporate until the sample digest volume was approximately 4 mL. The samples were then removed from the hot plate and allowed to cool to room temperature. Samples were transferred to analytically clean 50-mL plastic graduate conical vials. The beaker was rinsed with several 750 μL aliquots of analytical grade water, which were decanted into the 50 mL conical vial. The process was repeated until the sample final volume was 10 mL. It was at this stage that the liver lipids were extracted with hexane.

Digest controls consisted of positive and negative controls. Negative controls were employed to ensure the reagents and glassware used were clean of cadmium. Two negative control beakers were employed during the digest. Two hexane extract negative controls were also employed. Positive controls were employed to ensure that cadmium was not lost during the digestion. Two positive controls were used. The positive control consisted of a beaker containing all reagents added to the samples, as well as a 3 mg/kg cadmium spike. The positive controls were put through the same digest and analysis procedure as the samples. Two hexane positive controls were also employed.

Cadmium analysis was performed using a Perkin Elmer 5100 series Atomic Absorption Spectrophotometer (Norwalk, CT). Both flame (parts per million range) and graphite furnace (parts per billion range) modes were used. Perkin Elmer hollow cathode lamps, appropriate for cadmium were employed. The spectrophotometer, regardless of flame or furnace mode, was calibrated using six to eight calibrants. Calibrants, samples, matrix spikes, calibration checks, and blanks were analyzed in triplicate. Calibrants, samples, matrix spikes, and calibration check data were deemed acceptable if the percent relative standard deviation was <10. Calibrants, matrix spikes, and calibration check data were deemed acceptable if cadmium recovery was 90–110% of the calculated value.

The sperm and late-stage spermatid samples were analyzed for cadmium using graphite furnace AAS. Liver and testis samples were analyzed for cadmium using flame AAS and, for samples with low cadmium content, graphite furnace AAS. The flame AAS minimum detection limit for cadmium was 0.10 μg/g sample. The graphite furnace AAS minimum detection limit for cadmium was 0.020 μg/g sample. These limits of detection take the instrument limit of detection, sample mass, and sample dilutions into consideration.

## RESULTS

Table 1 shows the results from the AAS analyses of the liver, testes, pools of sperm, and pools of spermatids from the sacrificed animals. Using linear regression analysis the concentration of cadmium in the liver was found to be positively correlated with its concentration in the testes ($r^2$ = 0.87). No cadmium was detected in any pooled sperm or pooled spermatid sample from the twelve

Table 2
*Measured Masses (Picogram) of Phosphorus, Sulfur, and Zinc Within Individual Freeze-Dried Sperm and Spermatid Heads Following 4, 10, 26, and 52 Weeks of $CdCl_2$ Exposure and Associated Controls*

| Exposure[a] | Sample type | Phosphorus | Sulfur | Zinc |
|---|---|---|---|---|
| 0 week | sperm | 0.31 ± 0.02 | 0.087 ± 0.007 | 0.015 ± 0.005 |
| 4-week Cd dosed | sperm | 0.31 ± 0.02 | 0.087 ± 0.008 | 0.013 ± 0.006 |
| 4-week control | sperm | 0.30 ± 0.03 | 0.084 ± 0.009 | 0.013 ± 0.007 |
| 10-week Cd dosed | sperm | 0.32 ± 0.02 | 0.088 ± 0.006 | 0.017 ± 0.005 |
| 10-week Control | sperm | 0.29 ± 0.04 | 0.083 ± 0.008 | 0.016 ± 0.007 |
| 26-week Cd dosed | sperm | 0.32 ± 0.02 | 0.085 ± 0.006 | 0.016 ± 0.005 |
| 26-week Control | sperm | 0.30 ± 0.03 | 0.088 ± 0.008 | 0.015 ± 0.006 |
| 52-week Cd dosed | sperm | 0.31 ± 0.02 | 0.088 ± 0.006 | 0.014 ± 0.005 |
| 52-week Control | sperm | 0.31 ± 0.02 | 0.085 ± 0.008 | 0.015 ± 0.006 |
| 0 week | spermatid | 0.31 ± 0.02 | 0.092 ± 0.007 | 0.014 ± 0.004 |
| 4-week Cd dosed | spermatid | 0.32 ± 0.02 | 0.090 ± 0.008 | 0.013 ± 0.005 |
| 4-week control | spermatid | 0.33 ± 0.04 | 0.094 ± 0.008 | 0.015 ± 0.005 |
| 10-week Cd dosed | spermatid | 0.32 ± 0.02 | 0.092 ± 0.007 | 0.016 ± 0.004 |
| 10-week Control | spermatid | 0.30 ± 0.04 | 0.090 ± 0.009 | 0.014 ± 0.005 |
| 26-week Cd dosed | spermatid | 0.31 ± 0.02 | 0.090 ± 0.007 | 0.015 ± 0.004 |
| 26-week Control | spermatid | 0.32 ± 0.03 | 0.094 ± 0.007 | 0.016 ± 0.006 |
| 52-week Cd dosed | spermatid | 0.32 ± 0.02 | 0.093 ± 0.006 | 0.015 ± 0.004 |
| 52-week Control | spermatid | 0.30 ± 0.04 | 0.095 ± 0.009 | 0.016 ± 0.006 |

[a]Data expressed as the mean ± standard deviation of measurements from 27 heads from 3 animals with nine heads measured per animal.

cadmium dosed and fifteen control animals down to limits of detection that were 0.02 μg/g sample.

ANOVA revealed no differences in the phosphorus, sulfur, and zinc contents of the sperm heads from the nine exposure groups (4-, 10-, 26-, and 52-week cadmium-dosed animals and 0-, 4-, 10-, 26-, and 52-week control animals) at significance levels of 0.01. Likewise, ANOVA indicated there were no differences in the phosphorus, sulfur, and zinc contents within the spermatid heads from the nine exposure groups at significance levels of 0.01. Consequently, Table 2 summarizes the average measured masses of phosphorus, sulfur, and zinc present in the analyzed sperm and spermatid heads obtained from the nine different exposure conditions.

No sperm or spermatid head analyzed with PIXE contained detectable levels of cadmium. The cadmium minimum detection limit in the sperm and spermatid heads was determined by integrating the energy window corresponding to the background signal under the cadmium Lα peak. Following the definition of Currie (25) the minimum detection limit was taken to be 3.29 × (background. Using this formalism, the minimum detection limit for cadmium in a freeze dried sperm or spermatid head was determined to be 15 μg/g sample.

PIXE analyses also did not reveal the presence of cadmium in any of the freeze-dried samples of media used to wash the sperm and spermatids from cadmium-dosed and control animals. The minimum detection limit for cadmium in these freeze-dried samples of media was determined using the formalism described above and determined to be approximately 5 μg/g sample. (The minimum detection limit of cadmium is lower for the freeze-dried samples of wash media than for the individual sperm cell analyses as the sperm cells contained significantly higher chlorine concentrations than the freeze-dried samples of wash media. Owing to the small energy separation between chlorine x-rays and cadmium L x-rays a higher chlorine content degrades the detection sensitivity for cadmium.) Because the mass of the freeze-dried samples of media was ≤1/100 that of the 5-μl aliquots of media deposited on the nylon foils this detection limit translates to a minimum detection limit for cadmium in the washing media of approximately 0.05 μg/g sample.

There were no significant differences in the external appearance of testes and epididymes between dosed and control subjects apart from one animal dosed with cadmium for 52 weeks. This animal had testes and epididymes that were approximately half the normal size of those found in other subjects. However, despite their small size these organs did not appear to be atrophied or damaged. For this subject the mass of the testis analyzed via AAS was 49 mg whereas all other testes (both control and dosed at all time points) analyzed via AAS had a mass between 113 and 160 mg with a mean value of 137 mg and a standard deviation of 13 mg. Testicular masses from the other subjects showed no correlation with exposure to cadmium.

We observed that three mice subjected to a single intraperitoneal dose of 6.75 mg/kg body weight cadmium (LD50 equivalent dose) in the form of $CdCl_2$ and sacrificed 11 days later had severely atrophied testes. However, AAS and nuclear microprobe analyses did not detect the presence of cadmium in sperm or spermatids from the 6.75 mg/kg body weight cadmium-dosed animals. Furthermore, phosphorus, sulfur, and zinc contents in sperm from these animals were not signifi-

cantly different than those found in the sperm of control animals.

## DISCUSSION

Studies in rats and mice have provided evidence to suggest that exposures to cadmium disrupt spermatogenesis, damage supporting testicular tissue, and reduce male fertility, but the mechanisms that lead to these effects remain unknown. While it has been hypothesized that elevated cadmium concentrations might disrupt sperm function by competing for zinc binding sites in sperm chromatin, this has never been confirmed experimentally. The present exposure study was designed to specifically address this question and to determine to what extent cadmium might be taken up by sperm and pose a threat to the embryo after fertilization.

The results of this study show that cadmium accumulates within the liver and testes of the animals exposed to $CdCl_2$. Corresponding changes in cadmium content are not observed in the tissues of control animals. In spite of the observed increase in testis cadmium levels during the course of the exposure (up to 52 weeks) cadmium is not incorporated into sperm at levels above 0.02 µg/g dry weight (the minimum detection limit for AAS in this study) for any of the 12 cadmium-dosed animals. Furthermore, the results demonstrate that exposure to 0.1 mg/kg body weight cadmium once per week for up to 52 weeks does not alter the zinc content of individual sperm and spermatid heads.

Very little data has been published on the concentrations of heavy metals present in sperm cells. Concentrations of cadmium and other metals were reported for intact spermatozoa obtained from a population of patients undergoing tests for suspected infertility (26), but similar data were not obtained for spermatozoa produced by normal, fertile males. Instead, most studies involving cadmium exposures have focused on measuring cadmium concentrations in blood or seminal fluid. Increased levels of cadmium have been reported in the seminal plasma of men with varicole (27) and high levels of cadmium have been detected in the semen of smokers who had asthenozoospermia (19). Cadmium in seminal plasma has also been associated with low semen volume (28) and sperm motility. Changes in the linear and curvilinear velocity of sperm have also been correlated with semen cadmium levels (18). On the other hand no significant increase in seminal cadmium was observed in smokers whose consumption is less than 20 cigarettes per day (29).

We have found only one other study (30) that has examined the metal content of individual sperm exposed to cadmium, and our data are not consistent with the results obtained in this earlier study. In the experiments performed by Battersby et al. (30), human semen samples were exposed to a high concentration of cadmium in vitro by incubating sperm in 2 mM $CdCl_2$ for 24 hours. Electron microprobe analysis of portions of air dried sperm from semen incubated in the heavy metal solution revealed a cadmium content of 0.025% (250 µg/g) dry weight and a 50% drop in sperm zinc content, whereas cadmium was not detected in controls. Because the concentration of cadmium added to the sample was so high, the retention of cadmium by the treated sperm in the Battersby study might be partially explained by insufficient removal of the culture medium from the sperm prior to analysis. This would not, however, explain the attendant decrease in zinc.

With the possible exception of one animal with small testes that did not appear to result from the exposure, prolonged exposure of mice to a low dose of cadmium (0.1 mg/kg Cd in the form of $CdCl_2$) via intraperitoneal injection once a week did not significantly alter the physical appearance of the testes. Although, there are reports of cadmium-induced testicular damage (31–33) and failure of spermiation in rodents subjected to cadmium exposure (31), the doses used typically have been a single dose of approximately 1 mg/kg body weight or more (31,33). Our observations of atrophied testes resulting from a single 6.75 mg/kg body weight cadmium dose are consistent with findings from these higher dose regimes.

The data obtained from the 6.75 mg/kg body weight exposure also suggest that the effect of cadmium on fertility is not the result of the metal damaging or inactivating the spermatid or sperm cell. Testicular damage is observed at these higher doses, but cadmium is not found at detectable levels in either spermatids or sperm. This observation suggests instead that the metal must be toxic to the earlier stages of spermatogenesis or to the supporting testicular tissue, either of which could reduce sperm production. This is consistent with the results reported in a study of $^{109}Cd$ uptake by rat testes (20), which indicated that cadmium uptake by the testis was limited to nonspermatogenic cells.

In summary, the data presented here show that cadmium does not enter the maturing spermatid or sperm at levels above 0.02 µg/g, nor does it replace zinc in sperm chromatin. Although the involvement of cadmium in the etiology of reproductive biology is still controversial, the results of this study cast doubt on hypotheses that suggest that reduced male fertility may result from incorporation of cadmium into sperm chromatin. However, it could be hypothesized that exposure to cadmium may have a detrimental effect on testicular function (the metal could be toxic to the supporting testicular tissue or to the earlier stages of spermatogenesis) that could result in reduced sperm production leading to reduced male fertility.

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