PRIMARY RAT SERTOLI AND INTERSTITIAL CELLS EXHIBIT A DIFFERENTIAL RESPONSE TO CADMIUM[1]

STEPHEN R. CLOUGH,[a] MICHAEL J. WELSH,[c] ANITA H. PAYNE,[de] CARL D. BROWN,[b] AND MICHAEL J. BRABEC[b]

Eastern Michigan University, Department of Chemistry,[b] The University of Michigan Department of Environmental and Industrial Health, Toxicology Program[a] Department of Anatomy and Cell Biology,[c] and Department of Obstetrics and Gynecology[d] and Biological Chemistry[e]

Two cell types central to the support of spermatogenesis, the Sertoli cell and the interstitial (Leydig) cell, were isolated from the same cohort of young male rats and challenged with cadmium chloride to compare their susceptibility to the metal. Both cell types were cultured under similar conditions, and similar biochemical endpoints were chosen to minimize experimental variability. These endpoints include the uptake of ^{109}Cd, reduction of the vital tetrazolium dye MTT, incorporation of ^{3}H-leucine, change in heat-stable cadmium binding capacity, and production of lactate. Using these parameters, it was observed that the Sertoli cell cultures were adversely affected in a dose-and time-dependent manner, while the interstitial cell cultures, treated with identical concentrations of $CdCl_2$, were less affected. The 72-hr LC_{50}'s for Sertoli cells and interstitial cells were 4.1 and 19.6 μM $CdCl_2$, respectively. Thus, different cell populations within the same tissue may differ markedly in susceptibility to a toxicant. These in vitro data suggest that the Sertoli cell, in relation to the interstitium, is particularly sensitive to cadmium. Because the Sertoli cell provides functional support for the seminiferous epithelium, the differential sensitivity of this cell type may, in part, explain cadmium-induced testicular dysfunction, particularly at doses that leave the vascular epithelium intact.

.1. All correspondence should be sent to: Michael J. Brabec, Department of Chemistry, Mark-Jefferson Building, Eastern Michigan University, Ypsilanti, MI 48197; 313-487-2039.

2. Key words: cadmium, Sertoli cell, interstitial cell, testis, rat.

3. Abbreviations: CBF, cadmium-binding factor; DMEM, Dulbecco's modified Eagle's medium; FSH, follicle stimulating hormone; ICC, interstitial cell culture; IGF-I, insulin-like growth factor-I; LC(50), 50% lethal concentration; MTT, 3-(4,5-dimethylthiazol-2-yl); SCC, Sertoli cell culture.

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INTRODUCTION

Experimentally, cadmium is the most potent male reproductive toxicant known. A single injection of the metal salt (>10 μmol/kg) will cause complete "necrosis" of the testis in most laboratory mammals, despite the fact that the testis accrues, in relation to other organs, the smallest percentage of the dose (Parizek and Zahor, 1956; Gunn et al., 1968a). It has been suggested that the histopathological changes seen (and subsequent infertility) are secondary to the destruction of the testicular vasculature by cadmium (for Review: see Lee, 1983; Parizek, 1983; Singhal et al., 1985).

However, acute cadmium administration to rats below 4 μmol/kg will also produce significant reproductive effects (Meek, 1959; Kar and Das, 1960; Lee and Dixon, 1973) in the absence of any adverse effects to the testicular vasculature (Gunn et al., 1966). Recent studies have shown that changes in serum FSH or testosterone concentration may represent the most sensitive endpoints after acute cadmium administration in rats (Laskey et al., 1984; Laskey et al., 1986). Thus, either the Sertoli or the Leydig cell may be the initial testicular target of cadmium.

Although many studies have been performed with regard to the effect of cadmium on cells isolated from other tissues, little information exists on the effect of cadmium on testicular cells *in vitro*. Espevik et al. (1982) investigated the effects, as well as the prevention, of cadmium cytotoxicity in primary cultures of rat (20 day) Sertoli cells. They reported a 72 hr LC_{50} of 3.6 μM $CdCl_2$ and found that pretreatment with selenium, zinc, or cadmium had a prophylactic effect toward subsequent cadmium exposure, whereas FSH, estradiol, or testosterone did not. Waalkes and Poirer (1985) observed that the uptake of cadmium by freshly isolated adult rat interstitial cell preparations is multiphasic, with the secondary phase being saturable, energy dependent, and inhibited by zinc. Cell viability was not reduced by cadmium concentrations up to 100 μM and the efflux of metal ion from these cells at a fairly significant rate.

This study examined differences in susceptibility between Sertoli and interstitial cells to low cadmium concentrations *in vitro*. These two cell types were chosen because they play central roles in the support and control of spermatogenesis, are potential targets for cadmium *in vivo*, and are amendable to culture *in vitro*. A consanguinous preparation of Sertoli cells and purified interstitial cells was utilized. This design allowed the comparison of common metabolic endpoints and minimized experimental variability. Morphology, respiratory activity, total protein synthesis, appearance of a heat-stable cadmium binding factor, and lactate synthesis were compared between the two cultures. These observations suggest that the Sertoli cell is much more sensitive to the effects of cadmium than the interstitial cell *in vitro*. The results, along with those of others (Boscolo et al., 1985; Chung and

Maines, 1987), support the premise that the reduction of male fertility could be the result of an early, direct toxic effect of cadmium on the Sertoli cell.

MATERIALS AND METHODS

Media and Reagents. All reagents and medium components were obtained from Sigma Chemical Co., St. Louis, MO. Cadmium chloride (Baker Chemical Company, Phillipsburg, N.J.) was recrystallized 2X, filtered, and desiccated. Stock $CdCl_2$ solutions were prepared in 0.9% saline and sterile filtered; these solutions were analyzed against a commercial cadmium standard using a Perkin-Elmer 403 atomic absorption spectrophotometer. ^{109}Cd and ^{3}H-leucine were obtained from New England Nuclear, Boston, MA. The activity per ml of medium was 0.2 and 1.0 μCi, respectively. Metrizamide was purchased from Accurate Chem. & Scientific Corp., Hicksville, N.Y. Nitex mesh was purchased from Wilflife Biological Supply, Saginaw, MI. Anti-desmin monoclonal antibody and mouse anti-IgG-FITC were obtained from Boehringer-Mannheim, Indianapolis, IN.

Animals: Male Sprague-Dawley CD-1 rats were purchased from Charles River Breeding Laboratories, Inc., Wilmington, MA. The age at arrival was 19–21 days. The rats were weaned, caged with bedding, and held in an environmentally controlled room until 27–30 days of age. At this time they were killed by cervical dislocation.

Cell Preparation. Culture medium (DME/F12) used throughout this study was a serum-free medium based on a 50:50 mixture of Ham's DMEM/F12 formula with insulin (10 mg/l), epidermal growth factor (25 μg/l), and transferrin (5 mg/l) (Mather, 1980). Lactate was omitted from the formulation. Testes from 6–10 rats were aseptically removed and placed in ice cold calcium, magnesium-free, phosphate buffered saline containing 97.6 mM NaCl, 25 mM KCl, 3.7 mM Na_2PO_4, 8.3 mM glucose, 50 U/ml penicillin, 50 μg/ml streptomycin sulfate, and 0.008 mM phenol red in deionized, distilled water, pH 7.4. Testes were then decapsulated and weighed. The method of Chase and Payne (1983) was then followed for the isolation of partially purified Leydig cells, except for the following modifications: 1) all medium used was DME/F12; 2) a linear 11-23% gradient was prepared by layering, from bottom to top, three 5 ml volumes of media containing 23, 17, and 11% Metrizamide into a 38 ml roundbottom polycarbonate centrifuge tube and stored for 48 hr at 4°. Following isolation, the partially purified interstitial cells were counted in a hemocytometer and plated at a density of $0.8—1.2 \times 10^6$ cells/35 mm plate into a total volume of 2.5 ml medium. The cells were allowed to attach (2 hr at 32°) and the medium was then replaced with 2 ml of fresh medium and the plates incubated at 32° in a 5% CO_2-95% air atmosphere. The average yield of this preparation was 1.7×10^6 cells/testis. Staining for 3-beta-hydroxysteroid dehydrogenase activity with nitro blue tetrazolium (NBT) revealed an average Leydig cell purity of 42%. Indirect immunofluorescence using anti-desmin monoclonal antibody conjugated with mouse anti-IgG-FITC (supplier

procedure) revealed an average peritubular cell composition of 11%. The tubules remaining after enzymatic digestion (above), were transferred to 20 micron Nitex mesh and blotted from below the gauze. The procedure for isolation of Sertoli cells was then followed according to the method of Welsh and Wiebe (1975) as modified by Beattie et al. (1984), with the following modifications: 1) the incubation temperature during the first digestion was 34°; 2) the incubation time was 35–40 min; 3) the pancreatin digestion period was 15 min. After isolation, 10–12 μl (0.8 - 1.2 $\times$ 10^6 Sertoli cells) of packed cell pellet was plated per 35 mm petri dish. Sertoli cells were plated into a total volume of 2 ml medium, and allowed to attach to culture dishes at 37° for 24 hr under the same gas composition as above.

Culture Conditions. The day of isolation was designated Day 0. On day 1 (24 hours post-isolation), the medium was changed and both cell types were placed into the same incubation unit (32°). On day 3, the Sertoli cells were treated for 2 min with 20 mM Tris/HCl, pH 7.4 to eliminate germ cells as described by Galdieri et al. (1981). The medium was then changed for both cell populations. Media containing cadmium was prepared fresh before each experiment. All experiments were initiated on day 4, and each figure shown, unless noted otherwise, represents the mean (+/− range) of two experiments. Cultures remained viable over a four day incubation period, as indicated by no significant change in their ability to reduce the vital dye MTT (data not shown).

Reduction of MTT. Cell preparations on Day 0 were routinely examined and counted in 0.04% trypan blue and consistently exceeded 95% viability as measured by dye exclusion. Respiratory activity of cadmium exposed cells (Day 4–7) was assessed by the ability of live cells to reduce the tetrazolium dye MTT as described by Mosmann (1983). Cells were plated into 24 well plates at the same density as above. After treatment, 100 μl of dye (4 mg/ml in Dulbecco's phosphate buffered saline) was added to each well and incubated for 2 hr at 32°. Following incubation,

each plate was rotated at 100 cycles/min for 5 min to dislodge any unattached cells. The medium was aspirated, and 1 ml of a 50:50 Dulbecco's PBS:acid isopropanol solution added to each well. The optical density of each well was read at 560 nm, and the amount of formazan produced calculated by using a molar extinction coefficient of 51,000 (Pearse, 1960).

Lactate Determinations. Lactate concentrations in media (frozen aliquots) were determined (in duplicate) enzymatically by the method of Hohurst (1965) as modified

by Brabec et al. (1984). PROTEIN SYNTHESIS. Protein synthesis was measured by the incorporation of ^{3}H-leucine into TCA insoluble material by a modification of the method of Tash et al. (1981). Briefly, after cadmium exposure, cells were incubated in medium containing 2 μCi ^{3}H-leucine. After a 3 hour incubation, the plates were washed with complete medium, Dulbecco's PBS and finally solubilized

in 1.1 ml 0.5 N NaOH/1% sodium deoxycholate. Following transfer to disposable test tubes, a 100 μl aliquot was taken for protein determination and the remainder acidified with 3 ml of ice cold 40% TCA. After 24 hours at 4°, the suspensions were rinsed onto a glass fiber filter (Whatman GF/A, 2.4 cm) in a BioRad vacuum manifold, washed with 3 ml 10% TCA with 40× cold leucine, 95% EtOH, 50:50 95% EtOH:ethyl ether, and finally 100% ethyl ether. The filter paper was placed in a plastic minivial with 6 ml ACS scintillation cocktail (Amersham), and the radioactivity in each sample determined in a Delta 300 liquid scintillation spectrometer.

Induction of Heat-Stable Cadmium Binding Factor (CBF). Following cadmium exposure, each plate was rinsed once with fresh medium and Dulbecco's PBS. One ml of 20 mM Tris/HCl, pH 7.4 was then added and the plates were frozen and thawed twice. The contents of each plate was subsequently sonicated for 1 min, transferred to glass test tubes, and assayed directly for 'cadmium binding capacity' by the method of Onsaka et al. (1978) as modified by Eaton and Toal (1982).

Cadmium Uptake and Efflux. To each culture dish, 1 ml of medium containing 1 μM $CdCl_2$ (0.2 μCi ^{109}Cd/ml) was added and the cultures were incubated as above. Uptake and efflux of the metal was then measured according to the procedure of Failla and Cousins (1978).

Protein Determination. Protein determinations were made by the method of Lowry (1951) as modified by Peterson (1977). Sertoli cell and interstitial cell cultures were plated at an equal cell density (1×10^5 cells/cm^2). On the average ($n = 30$ plates per experiment), Sertoli cell cultures had 1.6 times the protein content of the interstitial cell cultures.

Data Analyses. Means were compared by two-way analysis of variance (ANOVA using SPSS) and, for pair-wise comparisons, Duncan's multiple range (Gad and Weil, 1982).

RESULTS

Morphology. Microscopic observations of control and cadmium-treated Sertoli cell cultures (SCC) showed dose-related responses, primarily granular cytoplasm, pyknosis, cell rounding and detachment. Observations of interstitial cell cultures (ICC) treated at identical concentrations (0 - 4.5 μM $CdCl_2$) revealed no apparent morphological changes. Similar responses in ICC could only be seen at concentrations exceeding 8 μM $CdCl_2$.

Cadmium Uptake. At a cadmium concentration of 1 μM, the rate of transport of the metal were similar for both cell types (Figure 1). The accumulation of cadmium,

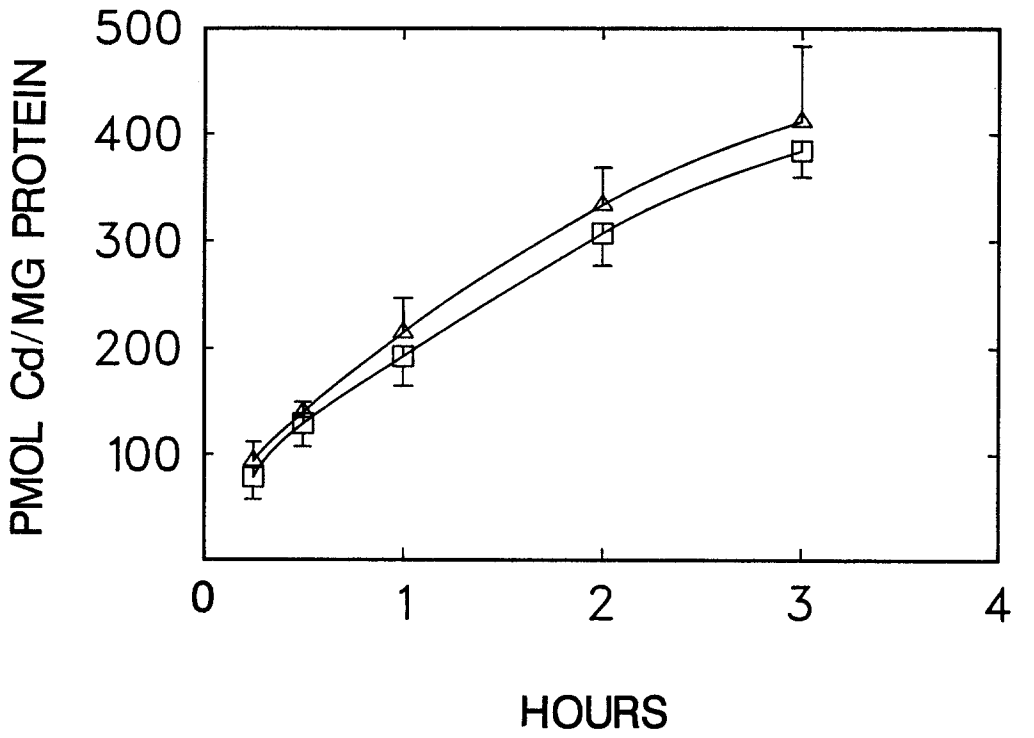

FIGURE 1. Uptake of cadmium by Sertoli (□) and interstitial cell (Δ) cultures. Cultures (35 mm plates) were incubated in media containing 1 μM cadmium chloride and 0.2 μCi Cd-109 at 32° C. At each time point, culture dishes (N = 3) were removed from incubation and washed 2× with ice-cold chelation buffer (10 mM EDTA, 10 mM HEPES, 0.9% NaCl, pH 7.4), solubilized, and assayed for activity by liquid scintillation spectrometry. Each point represents the mean (+/− range) of two experiments.

normalized per mg protein, also showed no significant differences between the two types of cells.

Reduction of MTT. Only viable cells with intact mitochondria can reduce the tetrazolium dye MTT, and the amount reduced is directly proportional to the number of viable cells present (Mosmann, 1983), an observation that this laboratory has subsequently confirmed using either protein content or cell number as an endpoint (data not shown).

Figure 2 shows the reduction of MTT as a function of cadmium exposure for both cell types at 12, 24, 48 and 72 hour intervals. Cadmium stimulated formazan production well above that of control for both cell types. However, the maximal response was at a higher cadmium concentration (at all time points) for the ICC than for the SCC. Since both cell types are non-dividing, the increase in formazan production seen most probably reflects an increase in respiratory activity.

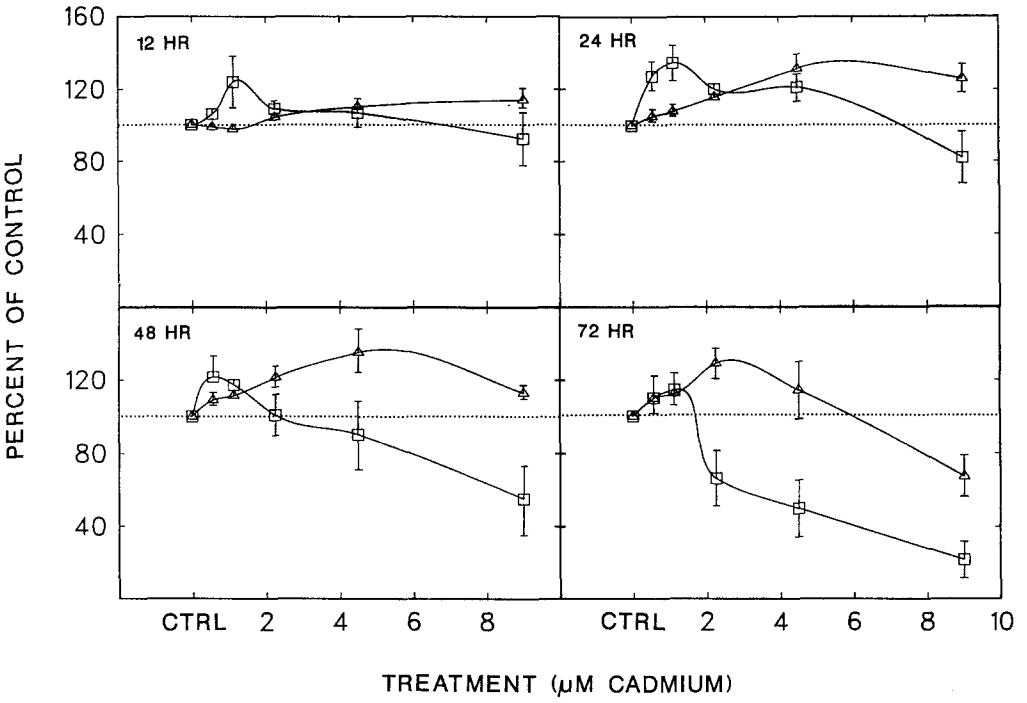

FIGURE 2. Reduction of tetrazolium dye MTT in Sertoli (□) and interstitital (Δ) cell cultures, measured over a 72 hr period (32° C). The horizontal stippled line indicates the control value, normalized as a percentage, for both cell populatios at each time point. Mean (+/− SEM) control values for SCC and ICC were 3.8 (0.30) and 2.38 (0.10) μg formazan/well, respectively. Cultures are non-dividing, and an increase above control values represents an increase in mitochondrial activity. Each point represents the mean +/− SEM for three experiments (4 wells/treatment).

At 48 and 72 hours, concentrations of cadmium >2.25 μM inhibited the reduction of MTT in the SCC (Figure 2). These exposures were accompanied by detachment of dead or dying cells in the SCC. In contrast, the reduction of MTT by ICC was stimulated at all exposures of cadmium tested except at the highest concentration at 72 hours.

When the concentration range for the ICC was extended (9–64 μM) and the 72-hr data for both dose response curves (not shown) analyzed by a log-probit method (Litchfield and Wilcoxon, 1949), both plots were linear and parallel. The estimates of the 72 hr LC_{50} for SCC and ICC were 4.1 and 19.6 μM $CdCl_2$, respectively.

Production of Lactate. Cadmium causes a marked increase in lactate production at both 24 and 48 hours in SCC (Figure 3); ICC show a slight but insignificant decrease at the lower dose levels at both time points. The ICC show a significant increase above control ($p < 0.01$) only at 48 hours and at the highest treatment level.

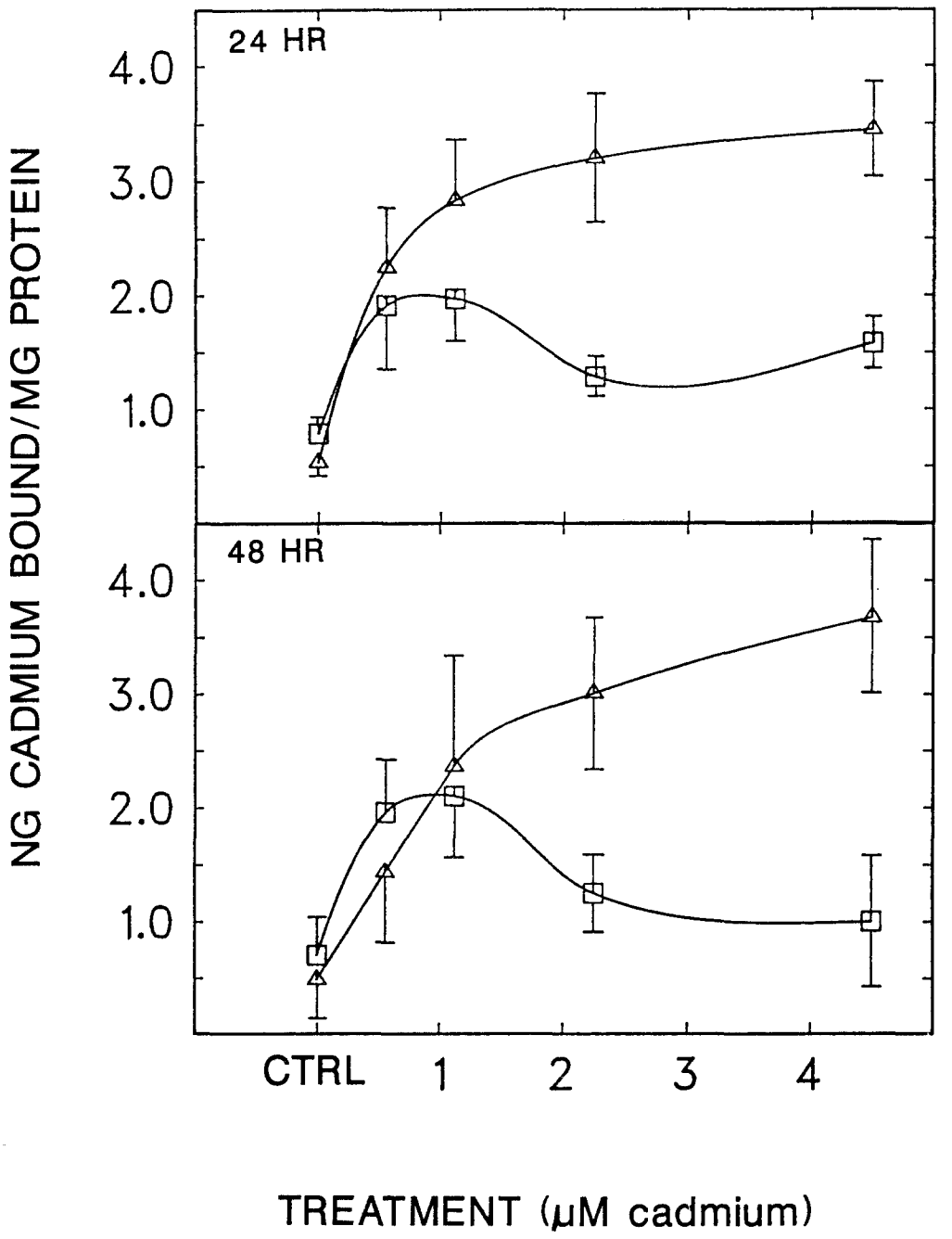

FIGURE 3. Lactate production by Sertoli (□) and interstitial (Δ) cell cultures after incubation at 32° C for 24 and 48 hr with increasing concentrations (0.56-4.5 μM) of cadmium chloride. Media was removed and lactate concentrations determined enzymatically. Each point represents the mean +/− range of two experiments (3 dishes/treatment).

Protein Synthesis and Induction of Cadmium Binding Capacity. Cadmium effected a dose-dependent decrease in the incorporation of ^{3}H-leucine into TCA insoluble protein of the SCC, whereas the ICC were largely unaffected (Figure 4). As can be observed in Figure 5, the production of a heat-stable cadmium binding factor (CBF) was dose-dependent in ICC, whereas SCC exhibited a non-linear dose-response, most probably due to overt cytotoxicity at the higher cadmium concentrations. SCC are apparently able to maintain production of CBF at concentrations that appreciably inhibit protein synthesis (see Figure 4).

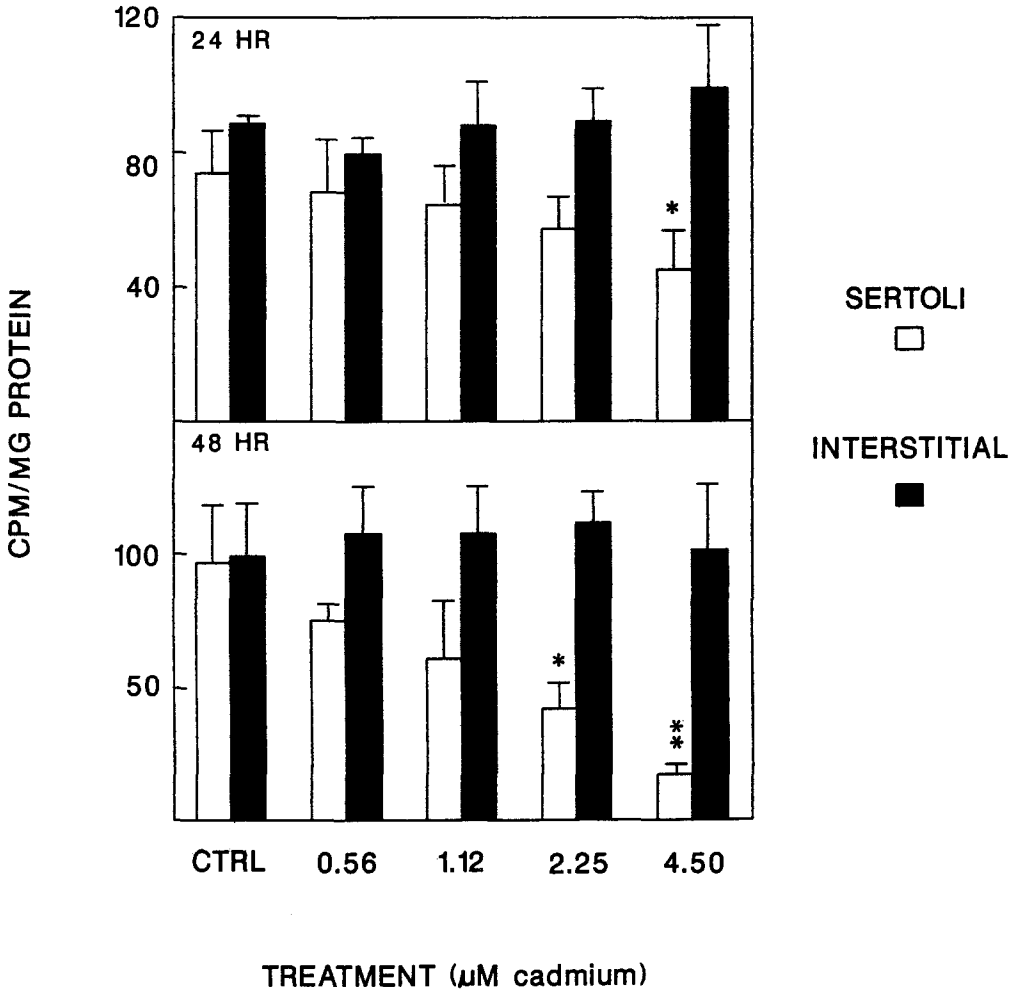

FIGURE 4. Protein synthesis, measured by incorporation of ^{3}H-leucine, in Sertoli (open) and interstitial (filled) cell cultures after incubation at 32° C for 24 and 48 hr with increasing concentrations (0.56-4.5 μM) of cadmium chloride. Cycloheximide treated cultures were less than 10% of control values (data not shown). Each point represents the mean +/− range of two experiments (3 dishes/treatment).

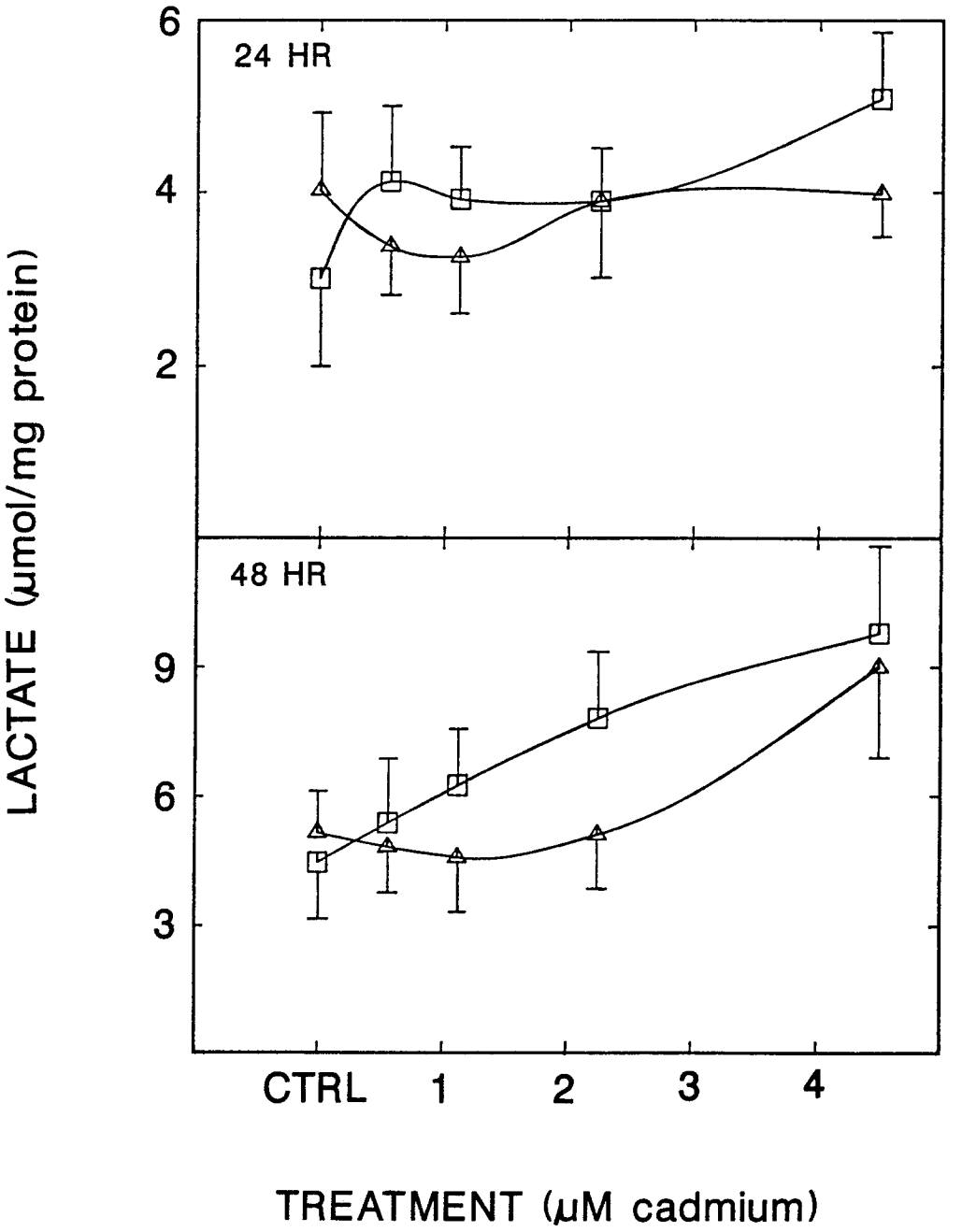

FIGURE 5. Production of heat-stable cadmium binding factor in Sertoli (□) and interstitial (Δ) cell cultures after incubation at 32° C for 24 and 48 hr with increasing concentrations (0.56-4.5 µM) of cadmium chloride. After incubation, the cells were rinsed, frozen and thawed twice in 1 ml PBS, and sonicated for 1 min. The homogenate was then assayed for protein and 'cadmium binding capacity.' Each point represents the mean +/− range for two experiments (3 dishes/treatment).

DISCUSSION

The response of two testicular cell types after exposure to cadmium was compared. The design centered around a dual cell culture system that allowed the concomitant isolation, culture, and treatment of primary rat Sertoli and interstitial cells (from a consanguineous source) under identical environmental conditions. Common morphological and biochemical endpoints were selected to compare the response of the two cell cultures to low concentrations of cadmium. With the exception of the high cadmium concentrations for determination of the LC_{50}, all of the endpoints were measured at non-lethal concentrations. The results indicated that the Sertoli cell was markedly more susceptible to the toxic effects of cadmium than the interstitial cell *in vitro*.

The lowest cadmium concentration required to produce observable changes in Sertoli cell morphology (12–24 hour observation) was approximately 1.2 μM $CdCl_2$ (data not shown). The interstitial cells appeared unaffected at concentrations up to 8 μM. Once adverse changes had begun, both cell types exhibited similar responses that included cytoplasmic granulation, increased vacuolization, cell contraction and detachment.

The most sensitive endpoint was the reduction of MTT. Because this reagent is reduced by the mitochondrial electron transport chain, an increase in cellular respiration will increase the production of formazan above that of control. This stimulation is apparent in both cell types after exposure to cadmium, as can be clearly seen in Figure 2. This observation is consistent with that of others who have shown that mitochondria will concentrate significant amounts of cadmium in relation to other organelles, and that both the glycolytic and oxidative phosphorylation pathways are affected (Jarvisalo et al., 1980; Toury et al., 1985; Rao, 1983).

The most notable observation to be made with regard to the reduction of MTT by both SCC and ICC is the concentration of $CdCl_2$ required to produce the maximum respiratory stimulation for each cell type. Both maxima differ, by at least a factor of 5, for the first 48 hours of exposure. Additionally, cell detachment (indicated by formazan concentrations dropping below that of the control group) for the ICC was only observed at the highest concentration (9 μM $CdCl_2$) for the longest exposure period (72 hr).

Cadmium also inhibited protein synthesis (as measured by ^{3}H-leucine incorporation), stimulated lactate production (an indicator of mitochondrial inhibition), and induced cadmium binding capacity in Sertoli cells at concentrations that did not affect the interstitial cells. Cadmium will elicit similar responses in other cell types, albeit at higher concentrations (Muller and Ohnesorge, 1984; Waalkes et al., 1984; DeRuiter et al., 1985; Fischer, 1985; Wade et al., 1986). The uptake of radioactive cadmium revealed that there was no significant difference in the rate of metal transport

between the two cell types. Thus, the different responses seen in this study cannot be explained by one cell type accumulating more of the metal than the other. Waalkes and Perantoni (1986) reported that the major cadmium binding protein of the testes was distinct from that of liver and kidney in rats and mice. However, the cell population in the testes that is responsible for synthesizing the cadmium binding protein has not been identified. Our data would suggest that the predominant site of synthesis would be the interstitial cell, since this population possessed a higher cadmium binding capacity than the Sertoli cell.

How might these *in vitro* effects seen in this study be related to the effects seen after administration of low levels of cadmium to the rat *in vivo*? The Sertoli cells provide structural support for the differentiating germ cells, as well as nourishment (Jutte et al., 1983; Grootegoed, 1988). If cadmium selectively alters Sertoli cell function, as might be suggested by these data, then the seminiferous epithelium would be compromised. The interstitium, however, is anatomically situated to receive the "first pass" of an acute dose and, being less sensitive to the metal, may afford the spermatogenic components some degree of protection. This premise is supported by past studies which have demonstrated that low, non-necrotizing doses (<4 μmol/kg) of cadmium will not pass the blood-testis barrier (Gunn et al. 1968b) and that most of the metal absorbed by the testis can be found in the interstitial compartment (Berlin and Ullberg 1963; Gunn et al., 1968b; Danielsson et al., 1984).

As in any *in vitro* study, extrapolation of the results to the *in vivo* situation must be made cautiously. The range of cadmium concentrations used in this study were selected by calculating testicular concentrations in the rat after a sub-necrotic exposure *in vivo*. Assuming 1) and average weight of 1 gram/testis 2) that the testis absorbs 0.1 - 0.2% of the cadmium dose (Gunn et al., 1968a) and 3) the density of the testis is one, the calculated range would fall between 1-10 μM. However if there is a differential uptake between the interstitium and the seminiferous tubule *in vivo*, then the exposures in this study may be unrealistically low for ICC and high for SCC.

The resistance to cadmium seen in the interstitial cells might be attributed to using a heterogeneous population of cells. In the present culture system the peritubular and non-specific interstitial cells, as seen *in vivo*, are closely associated with the Leydig cells, providing a possible means of support, as well as an additional experimental variable not seen in the Sertoli cell cultures. We observed, however, that high concentrations of cadmium selectively destroyed the peritubular and non-specific interstitial cells, whilst the Leydig cells survived. Further, although both cell types were plated at the same cell density, the Sertoli cell has a relatively larger cell volume than the interstitial cell, and yields slightly more protein per dish, thus providing a confounding variable between the two cell cultures. However, this should afford the Sertoli cells an advantage over that of the interstitial cells,

since they possess a greater amount of metal buffering capacity (i.e. more protein per cell and per dish) than the latter, especially at the low cadmium concentrations used in this study.

Because the rats used in this study are not fully mature (27–30 day), the relevance to the effects of cadmium on spermatogenesis in the mature animal may be in question. Although not yet fertile, 28 day rats show the typical cellular associations (Perey et al., 1961) up to Stage IX of the seminiferous cycle. Sertoli cells isolated from three week old animals bind FSH, insulin, and IGF-I, and respond with increased rates of lactate synthesis to hormone exposure (Oonk and Grootegoed, 1988). Sertoli cells from three week old rats secrete androgen binding protein, a Sertoli cell marker (Kühn-Velten et al., 1987) and inhibin (Toebosch et al., 1988). It would appear that Sertoli cells from 4 week old rats perform at least qualitatively as well as do cells isolated from older rats. In addition, Wong and Klaassen (1980) have shown that significant effects can be seen in the seminiferous epithelium of cadmium-treated rats as early as 3 weeks of age.

When comparing the response of two cell populations to a toxicant, care must be taken in defining one as "sensitive" or "resistant," since these are relative terms. From the data presented here, it appears that ability of the interstitial cells to tolerate cadmium may be comparable to that seen in studies with primary cells isolated from non-reproductive tissue, e.g. hepatocytes (Muller and Ohnesorge, 1984), kidney cells (Jin and Nordberg, 1986) and white blood cells (Stacey, 1986; Coin and Stevens, 1986). Sertoli cells, on the other hand, appeared to be "sensitive" to the metal, exhibiting even less of a tolerance seen in the more fastidious established cell lines (Fisher, 1985; Borenfreund and Puerner, 1986). Additionally, the log-probit dose-response plots for both cell populations were different by a factor of approximately 5, although the plots were parallel. Thus, one could argue that the mechanism of toxicity may be similar for both cell types, but that the ICC are better able to sequester cadmium in a manner that favors less interference with vital cellular functions. Since the interstitium lies outside the blood-testis barrier and is situated to receive the initial dose of a potentially toxic metal, it is logical that this population be inherently more "resistant" to toxic insult.

In summary, we have shown, using common biochemical endpoints, that Sertoli cells are significantly more sensitive to the effects of cadmium than partially purified interstitial cells. The dichotomous response seen in both cell populations may cast a different perspective on the toxicity of heavy metals in the testis. Specifically, the most susceptible cell population in an organ may account for the susceptibility of the organ (to a toxicant) as a whole. In addition, the *in vitro* dual cell model we have developed and employed in this study reduced animal use and served as a cost-effective tool in the investigation of the reproductive toxicity of cadmium. It shows potential for elucidating some of the early biochemical alterations that

this metal may induce in the cell. The model may also be helpful in the investigation of the mechanism of other xenobiotics that may be potentially harmful to the testis.

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