EFFECT OF LEAD ACETATE ON SERTOLI CELL LACTATE PRODUCTION AND PROTEIN SYNTHESIS *IN VITRO*

LAYLA I. BATARSEH,* MICHAEL J. WELSH† AND MICHAEL J. BRABEC*

***Department of Environmental and Industrial Health**
School of Public Health
The University of Michigan
Ann Arbor, Michigan

†Department of Anatomy and Cell Biology
The University of Michigan
Ann Arbor, Michigan

The effects of lead acetate on protein synthesis and lactate production by cultures of rat Sertoli cells in vitro *were studied. Sertoli cell cultures prepared from 20 day old Sprague-Dawley rats were exposed to 0.01, 0.05 and 0.10 mM lead acetate. Lactate production was significantly elevated by all concentrations of lead after 3, 6, 9 and 12 hours of exposure. Protein biosynthesis as measured by [^{3}H]-leucine incorporation was significantly depressed by 0.05 and 0.10 mM lead acetate after 2 hours of exposure. These results support the hypothesis that lead acetate may inhibit spermatogenesis by a disturbance of the metabolic activities of the Sertoli cells.*

INTRODUCTION

Lead salts are among the oldest known spermicidal agents, and lead compounds have long been known to be abortifacient (Bell and Thomas, 1980). Lead produces teratospermia, hypospermia, and asthenospermia in man (review: Thomas and Borgan, 1983; Bell and Thomas, 1980).

1. Address correspondence to: Dr. Michael J. Brabec, Department of Chemistry, Eastern Michigan University, Ypsilanti, Michigan 48197.

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3. Abbreviations: MEM, minimal essential medium, TCA, trichloroacetic acid.

Lead is apparently not genotoxic in animals (Forni and Sciame, 1980). Lead acetate is non-teratogenic in rats and mice (Kennedy et al., 1975). Histopathological changes in the testis were shown after administration of 1 g/L of lead acetate in drinking water for 60 days (Chowdhury et al., 1984). Severe atrophic changes, with interstitial cell hyperplasia, Sertoli cell hyperplasia, tubular atrophy and sclerosis were found in testicular biopsies of men occupationally exposed to inorganic lead (Cullen et al., 1984). The histopathological changes correlated with oliogospermia and azospermia, lower sperm motility and a high percentage of abnormal sperm in all subjects.

Lead causes an increase in the activity of testicular oxidizing enzymes in rats (Krasovskii et al., 1979), a supression of FSH receptors, a decrease in cyclic AMP and steroidogenesis (Weibe et al., 1983), a lower ascorbic acid and a higher cholesterol concentration (Chowdhury et al., 1983), as well as a decreased synthesis of testicular RNA (Golubovits et al., 1968) in the rat testes. It is unclear, however, how lead elicits these changes.

The morphological and biochemical complexity of the seminiferous tubule makes the study of spermatogenesis difficult in the intact animal. Thus we examined the mechanism of action of lead in testicular cell cultures. Cell culture allows the use of a smaller number of animals, conditions are easy to manipulate experimentally, and characterized cell populations can be prepared and maintained repeatedly under experimental conditions. This allows the effect of lead on a specific cell population to be examined.

We chose Sertoli cells to study the gonadotoxic mechanism of lead because this cell type provides structural and metabolic support of the spermatogenic cells within the seminiferous tubules (Russell, 1980; Vogl and Soucy, 1985). Therefore, changes in Sertoli cell metabolism might be expected to affect the developing germ cells and consequently alter spermatogenesis.

In the experiments reported here, Sertoli cell cultures were exposed to various concentrations of lead acetate. Three parameters of Sertoli cell function were studied: lactate production, protein synthesis, and morphology. Lactate is an essential substrate for spermatogenesis that is produced by Sertoli cells and utilized by the developing germ cells (Jutte et al., 1983). Protein synthesis may be considered a general indicator of cell function. Moreover, Sertoli cells produce proteins that are believed to be essential for the process of spermatogenesis; some of these proteins have been identified, such as the androgen binding protein (Fritz et al., 1974), plasminogen activator (Lacroix et al., 1977) and testicular transferrin (Skinner and Griswold, 1980). Changes in the morphology of Sertoli cells may also indicate a reaction to the toxic effects of lead. The results suggested that a possible effect of lead acetate on spermatogenesis may be through its effects on Sertoli cell metabolism.

METHODS

Test Material. Lead acetate, approximately 99.8% pure, was purchased as Baker

Analyzed Reagent. Lead acetate solutions were prepared in distilled deionized water that had been boiled for 30 min. The aqueous lead solutions were diluted 10 times each with the culture medium to give the required concentrations. The pH was adjusted to 7.4-7.5. [^{3}H]-leucine, specific activity of 68 Ci/mmole, was purchased from Amersham Corporation, Arlington Heights, IL. All other chemicals were purchased from Sigma Chemical Company, St. Louis, MO.

Animals. Twenty-one day old male Sprague-Dawley rats were purchased from Charles River Breeding Laboratories, Inc., Portage, MI. The animals were killed by cervical dislocation within 3 hr of arrival.

Sertoli Cell Cultures. Sertoli cell cultures were isolated and maintained essentially according to Welsh and Weibe (1975). Testes from 8-10 rats were freed from the tunica albuginea using aseptic techniques; the weighed tissue was cut into pieces of about 1-2 mm. The minced tissue was divided among 50 ml Erlenmeyer flasks with no more than 1 gm per flask. The tissue was washed 3 times with 30 ml ice-cold Ca/Mg-free phosphate isolation solution composed of 97.6 mM NaCl, 25 mM KCl, 3.7 mM Na_2HPO_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 7.4-7.5 (adjusted with KH_2PO_4). After each washing, flasks were placed on ice, the tissue mince was allowed to settle, and the supernatant was decanted. The tissue was treated with 20 ml isolation solution containing 2 mg collagenase (Sigma, type IV, 160μ/mg) per flask for 40-50 min in a shaker bath (37°). The mass of reaggregated tissue, composed mainly of interstitial tissue, was washed twice to harvest more tubule fragments and was discarded. The tubule fragments were allowed to settle, and the supernatant was discarded. The tubules were combined and washed 3 times with 30 ml isolation solution. The tubules were then divided into 50 ml Erlenmeyer flasks with no more than 0.25 g tissue in 20 ml isolation solution per flask. The tubules were treated with 2 mg pancreatin (Sigma, grade VI, porcine activity 4 × NF grade) per flask. After a 10-15 min incubation in a shaking bath (37°), the mass of tissue formed, composed mainly of peritubular cells, was washed twice with isolation solution; the wash was added to the fraction in the flask and the whole suspension was washed by centrifugation in 15 ml isolation solution at 150 × g for 4-5 min. The packed cell volume was estimated, and the cells were suspended in an appropriate volume of serum-free culture medium based on 50:50 Ham's F12 and Minimum Essential Medium (MEM) formula with added insulin, epidermal growth factor and transferrin (Mather, 1980). The Sertoli cells were plated at 250-350 μg protein per plate (Corning, 35 mm) and incubated at 37° under 5% CO_2-95% air. The culture medium was changed once every 24 hr and cultures were used on the fourth day. Cultures were examined by phase contrast microscopy and photographed at 125X with a Leitz Epivert microscope using Panatomic-X film.

Lactate Measurements. On the fourth day of culture, medium was removed from the culture plates and replaced with either fresh medium or solutions prepared in lactate/pyruvate-free medium to give final concentrations of 0.01, 0.05 and 0.10 mM lead acetate. Aliquots of 110 μl were taken from each plate after 3, 6, 9 and 12 hr of

exposure, centrifuged, and lactate in the supernatant was determined according to Hohorst (1965) as modified by Brabec et al. (1984). Lactate concentrations were measured in duplicate by spectrophotometric measurements (340 nm) of the stochiometric conversion of lactate to pyruvate with concomitant reduction of NAD to NADH by lactate dehydrogenase. At the end of 12 hr, the medium was removed from the plates, and cultures were washed with 2.5 ml PBS, then 2 ml 0.5 N NaOH was added to digest the cultures for protein determination (Lowry et al., 1951).

[^{3}H]-Leucine Incorporation. [^{3}H]-leucine incorporation was measured by a modification of the method of Means and Hall (1967). Sertoli cell cultures were prepared and maintained as described previously; on the fourth day, the culture medium was removed and cultures were incubated for 1/2 hr without leucine, followed by incubation with 2.5 ml medium containing 10 μCi/ml [^{3}H]-leucine and lead acetate at 0.01, 0.05, or 0.1 mM. Cycloheximide (50 μg/ml) was used as a positive control. After 2 hr of exposure, the medium was aspirated and the cultures were washed 5 times with regular medium, followed by one rinse with PBS. The cells were dissolved in NaOH (0.5N) for 2 hr at room temperature. An aliquot of 200 μl was taken for protein determination; cold trichloroacetic acid (TCA) was added to the remaining cell suspension (final concentration of 10%), and allowed to stand overnight at 5°. Acid-insoluble radioactivity was collected by vacuum filtration on GF/A 2.4 cm filters. The filters were successively washed once with 5 ml of each of the following: 10% cold TCA, 95% ethanol, ether:ethanol (3:1), and ether. The filters were transferred to scintillation vials containing 10 ml OCS liquid scintillation mixture (Amersham) and radioactivity was determined in a Packard Liquid Scintillation Spectrometer.

Cell Viability. Sertoli cell cultures were treated with lead acetate solutions as described previously. After 12 hr of exposure, trypan blue was added at 2.5% final concentration. Cells were examined microscopically to determine dye exclusion.

Analysis of Data. After establishing homogeneity of variance, one-way analysis of variance was performed. Significance was evaluated by the Bonferonni-Scheffe comparison of means (Neter and Wasserman, 1974), and $P < .05$ was taken as significant. Profile analysis (Morrison, 1967) was also performed on the means of lactate measurements.

RESULTS

Lactate Levels. Lactate levels were measured in the incubation medium after 3, 6, 9 and 12 hr of exposure of Sertoli cell cultures to lead acetate. Profile analysis showed a significant difference in lactate production among the various lead concentrations at each time of measurement (Table 1). The rate of lactate production increased in proportion to the increase in lead acetate concentration in the medium (Table 2).

Protein Synthesis. Protein synthesis as measured by incorporation of [^{3}H]-leucine

TABLE 1
Lactate Levels in Sertoli Cell Culture Medium Following Incubation with Lead Acetate

Incubation Time (hr)	Lactate levels (μmole/μg prot)[a]			
	Control (n = 5)	0.01 mM Pb^{+2} (n = 5)	0.05 mM Pb^{+2} (n = 5)	0.10 mM Pb^{+2} (n = 5)
3	0.16 ± 0.02	0.20 ± 0.05[b]	0.42 ± 0.05[b]	0.53 ± 0.15[b]
6	0.37 ± 0.03	0.50 ± 0.07[b]	0.80 ± 0.14[b]	0.95 ± 0.08[b]
9	0.42 ± 0.05	0.55 ± 0.04[b]	0.99 ± 0.33[c]	1.10 ± 0.10[c]
12	0.47 ± 0.07	0.72 ± 0.23[b]	1.10 ± 0.43[b]	1.21 ± 0.09[b]

[a]Mean ± SD.
[b]$p < 0.001$; significantly different from control values.
[c]$p < 0.01$; significantly different from control values.

into acid insoluble proteins was not significantly different from the control at 0.01 mM lead acetate. However, protein synthesis was significantly lower at 0.05 and 0.1 mM lead acetate (Figure 1). Protein synthesis was reduced to less than 66% and 80% of the control for cultures exposed to 0.1 mM and 0.05 mM, respectively. Inclusion of cycloheximide in the medium reduced [^{3}H]-leucine incorporation to less than 1% of control.

Morphological Appearance. Light micrographs showed changes in Sertoli cell morphology after 4 hr exposure to 0.10 mM lead acetate (Figure 2). The cells appeared to be more stellate and the cell cytoplasm appeared granulated compared to control cells, although cell death was not apparent. Cells exposed to 0.01 mM lead acetate did not show any morphological change, while those exposed to 0.05 mM lead acetate showed the same appearance after 6 hr of exposure as those exposed to 4 hr of 0.10 mM lead acetate. The microscopic examination of the cultures showed very few contaminating germ cells. Sertoli cells in all cultures excluded trypan blue after up to 12 hr of exposure to all tested concentrations of lead acetate.

TABLE 2
Rates of Lactate Production by Sertoli Cells *in Vitro*

Lead Acetate (mM)	μmole/plate/hr[a]
Control	1.13 ± 0.53
0.01	1.63 ± 0.22
0.05	2.04 ± 0.63[b]
0.10	2.48 ± 0.46[b]

[a]Mean ± S.D. of 3 separate experiments.
[b]$p < 0.05$; significantly different from control.

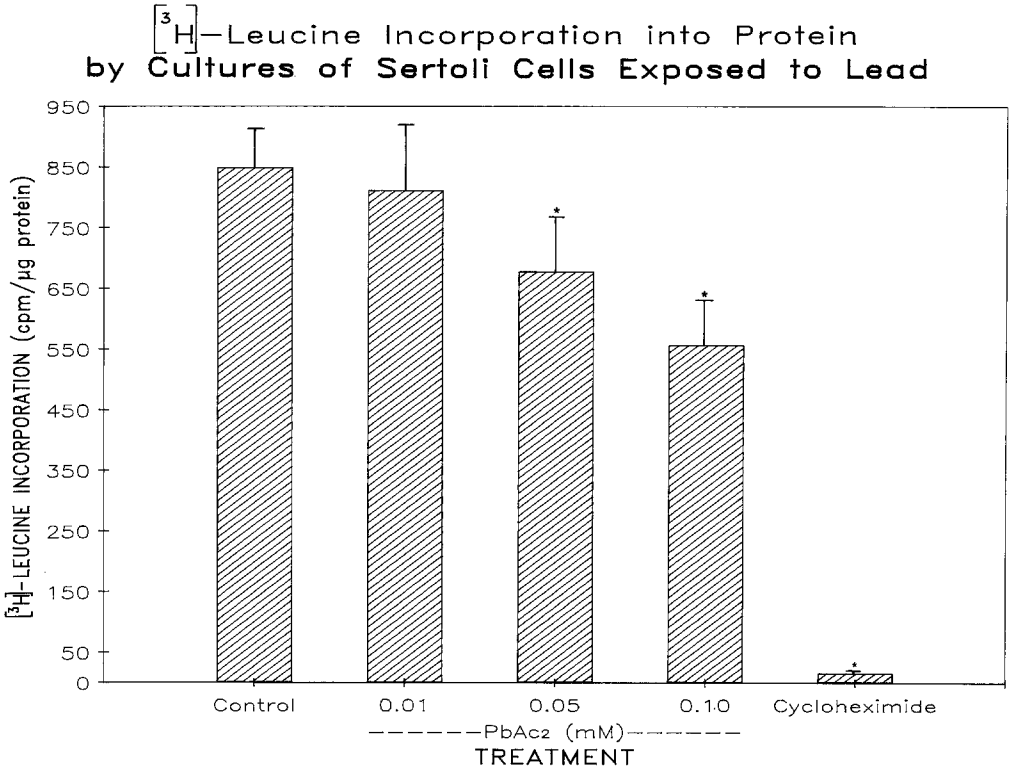

FIGURE 1. Effect of lead acetate on [^{3}H]-leucine incorporation into acid insoluble proteins by four-day-old rat Sertoli cell cultures. (*, $p < .05$, cf. control). Results are representative of 5 experiments; n = 5 plates per group.

DISCUSSION

This work was undertaken to study the mechanism of action of lead on the process of spermatogenesis. Spermatogenesis is a complicated process involving both structural (Russell, 1980) and functional (Jutte et al., 1981; Jutte et al., 1983; Boltani et al., 1983) interactions between Sertoli cells and germ cells within the seminiferous tubules.

We studied the effect of lead acetate on the metabolism of Sertoli cells. It is known that many metabolic products of Sertoli cells are required by the developing spermatogenic cells (Jutte et al., 1981; Jutte et al., 1983), and therefore may be a possible target through which lead might inhibit the process of spermatogenesis.

The viability of Sertoli cells in the cultures as indicated by trypan blue exclusion was not affected by lead acetate under the experimental conditions used in these studies. Lactate production was linear during the course of an experiment (Table 1), and no difference in the total amounts of protein between the control and treated cultures was found after 12 hr of exposure (data not shown). The viability of Sertoli cell cultuers was

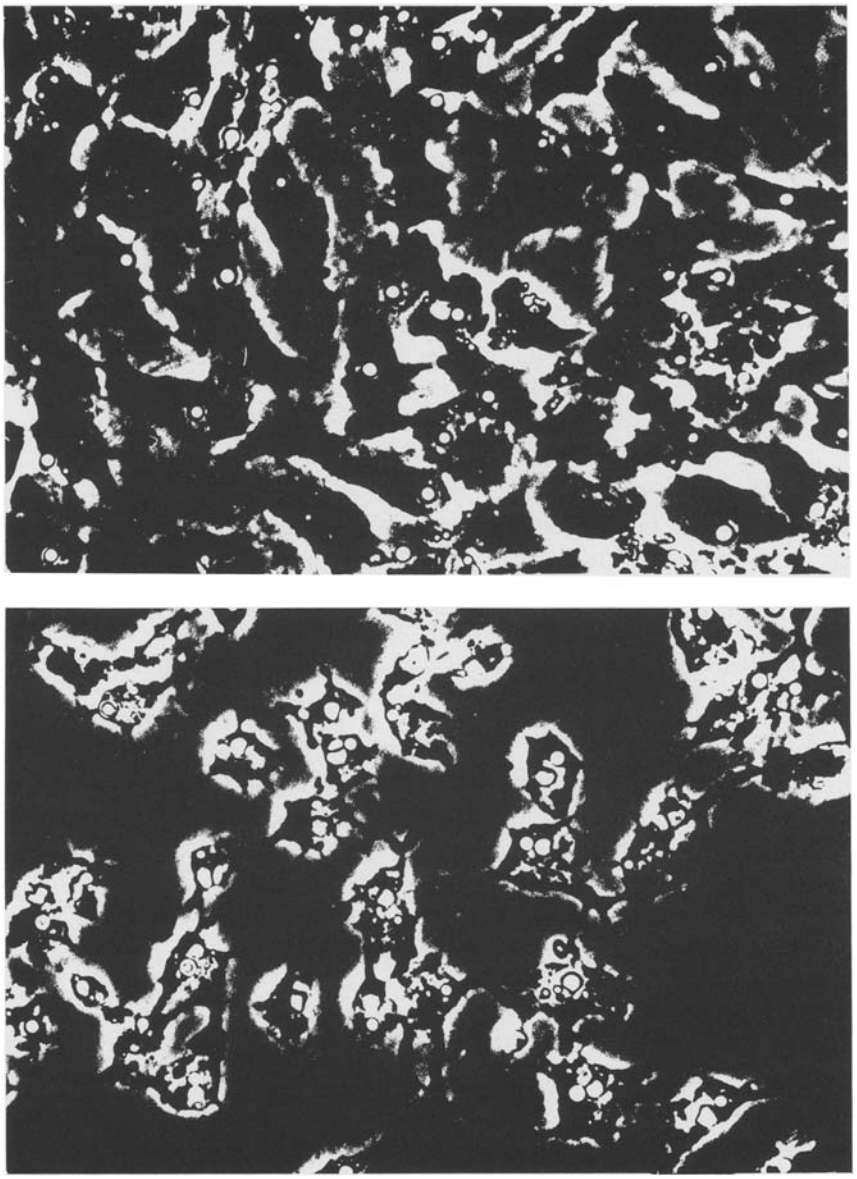

FIGURE 2. Photomicrographs of four-day-old rat Sertoli cell cultures. The control group (a) showed no change in morphology throughout the experiment; the cells treated with 0.10 mM lead acetate (b) for 4 hours became more stellate and the cytoplasm appeared granulated. (125×).

also verified by microscopic examination of the cultures. Although lead-exposed Sertoli cells still adhere to the substrate, light micrographs (Figure 2) showed that a granulated cytoplasm was visible after 4 and 6 hr of exposure to 0.05 and 0.10 mM lead acetate, respectively.

While the viability of Sertoli cells was not affected under the conditions of our experiments, the increase of lactate production with time and dose indicated that energy metabolism of Sertoli cells was disturbed. The increased production of lactate as a response to exposure to lead acetate agrees with another study (Fisher, 1977) with mouse LA fibroblasts. Lactate production increased in a dose-dependent way when fibroblasts were exposed up to 0.16 mM $PbCl_2$ for 24 hr, although cell viability was maintained.

Lactate is an essential metabolite for the survival of germ cells; it is found to support oxygen consumption, [^{3}H]-uridine and [^{14}C]-leucine incorporation by isolated germ cells (Nakamura et al., 1981b), and protein synthesis in round spermatids (Nakamura et al., 1981a). However, the cyclic fashion by which FSH binding and protein and cAMP production varies at different stages of the seminiferous epithelium cycle (Parvinen, 1982) implies that the demand for certain products of the Sertoli cells may vary during the successive stages of spermatogenesis. An excess of lactate during certain stages might be as detrimental to the process of spermatogenesis as its deficiency. Boitani et al. (1983) showed that lactate fails to support spermatid survival when compared to pyruvate, but this was not the case for spermatocytes. Nakamura et al. (1981b) showed that [^{3}H]-leucine incorporation and levels of ATP in round spermatids are directly related, and that both start to drop at higher concentrations of lactate (10 mM).

As mentioned earlier, many of the proteins produced by Sertoli cells may be essential for spermatogenesis. Our results showed a depression of 34% from control levels in protein synthesis in Sertoli cells exposed to 0.10 mM lead acetate. If this inhibition of protein synthesis was preferentially reflected in the output of secreted proteins, this depression of protein synthesis could lead to deficiencies in the proteins required by germ cells in the seminiferous tubules. This might disturb the stages of the cycle where the proteins are most required and consequently might adversely affect spermatogenesis.

Alternatively, lead interference with lactate production and protein synthesis may simply be a reflection of dysfunction at another site in the Sertoli cells. In any case, if spermatogenesis depends upon Sertoli cell function, changes in Sertoli cell metabolism may have substantial effects on spermatogenesis. The mechanism by which lead disturbs the metabolic activities of Sertoli cells remains to be elucidated and is under investigation in our laboratory.

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