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GONADAL DEVELOPMENT OF LEAD INTOXICATED RAT EMBRYOS

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ABSTRACT

Pregnant rats injected with 60 μg lead nitrate/gm body weight on day 8 of gestation showed a significant fetal resorption and reduced size of gonads on day 13.5 gestation. Incidence of fetal death and fetal resorption decreased and embryo gonads attained a normal size on day 18 of gestation. The developing gonads of lead treated embryos possessed a smaller number of primordial germ cells, spermtogonia and oogania compared with those of controls.

INTRODUCTION

Human intoxication with lead, the serious environmental pollutent, may be acquired mainly from three sources. Firstly, the painters using lead paint are always subject to lead intoxication if proper precautions are not taken (Lambie, 1967). Secondly, children may chronically ingest small amounts of lead from walls covered with lead-containing paint (Griggs *et al.*, 1964; Barltrop and Killaka, 1969). Thirdly, the greatest source of environmental lead is gasolin produced from automobiles (Goyer, 1971). Data from the National Health and Nutrition Examination Survey (Mahaffey *et al.*, 1982) reported that in children, from 6 months through 5 years of age, the prevalance of elevated blood lead levels (above 30ug/dl) was 4%.

It has been also know that lead can pass through the mother placenta to the fetus (Buchet *et al.*, 1977; Karp and Robertson, 1977; Hubermont *et al.*, 1978; Lauwerys *et al.*, 1978; Roels *et al.*, 1978).

Exposure of human, rats, mice and hamsters to lead may be associated with decreased fertility (Rom, 1976; Earl and Vanish, 1979; Gerber *et al.*, 1980; Wide, 1985). but the mechanism of this effect is not fully understood.

At certain life stages, lead exposure is practically hazardous to reproduction. In the mouse, those stages are either around the stage of blastocyst implantation (Jacquet, 1977; Odenbro and Kihlstrom, 1977; Wide and Nilson, 1977) during which lead causes disturbance in the endocrine interaction between the ovaries and uterus (Petruz *et al.*, 1979; Wide and Wide, 1980) or at the early organogenesis stage on day 8 of gestation (Wide, 1985). However, the effects of inorganic lead compounds on the developing gonads and their germ cells are not clearly known. The present study was undertaken to investigate the development of gonads of rat embryos exposed to lead nitrate on day 8 of their intrauterine life.

MATERIALS AND METHODS

Virgin white rats of about 15 weeks old and weighing 250–270 gm were caged with males overnight. Females were considered 1 day pregnant on demonstrating sperms in the vaginal smear. The females were kept in wire cages at 22±1°C. On day 8 of gestation, pregnant rats were divided into two groups. In the first group (12 rats), each pregnant rat was injected in a tail vein with 0.1 ml sterile destilled water containing 60 μg lead nitrate (Merck)/gm body weight. In the second group (10 rats), each pregnant rat was injected with 0.1 ml saline solution and served as control. Half the number of experimental and control animals were decapitated on days 13.5 and 18 of gestation respectively. The uteri of the animals were removed, carefully examined and live and resorption sites as well as dead embryos were recorded.

Embryos were fixed in Boiun's fluid, dehydrated in an ascending series of ethyl alchol and cleared in terpeniol. Whole embryos (on day 13.5) or only the genital ridges and the adjacent tissues (on day 8) were embedded in paraffin, serially sectioned at 7 microns and stained with Delafield's hematoxylin and eosin.

From each pregnant rat, four embryos, 2 males and 2 females, were collected at random for scoring the germ cell number. In four sections of each embryo, all germ cells in the gonads were counted. In all cases, only germ cells whose nuclei were seen in the section were counted. Average gonad diameter and the mean number of germ cells/section were calculated for the control and treated embryos. Student's t–test was used for the statistical comparison between the control and lead treated groups.

RESULTS

Embryos surviving lead nitrate treatment were smaller in number ($P < 0.01$) than in control ones (Table 1). The lead treated embryos had a higher frequency of fetal resorption on day 13.5 of gestation ($P < 0.05$) than the control embryos

of the same age. On the other hand, fetal death and fetal resorption in treated embryos was not sigificantly different from those of control ones ($P > 0.4$) on day 18 of gestation. (Table 1).

Table 1

The number of living and dead or resorbed embryos of control and lead-treated rats.

Embryo Age (Days)	Mean number of living embryos ± SE		Mean number of dead and resorbed embryos ± SE	
	control	treated	control	treated
13.5	10.0 ± 0.55	6.0 ± 0.56 ($P < 0.01$)	0.6 ± 0.46	2.2 ± 0.37 ($P < 0.05$)
18	9.6 ± 0.25	6.4 ± 0.51 ($P < 0.01$)	0.4 ± 0.4	1.2 ± 0.37 ($P > 0.4$)

The testes

In 13.5 day control embryos, the average diameter of the testis is 174 ± 5.1 micron (Table II). The seminiferous tubules consist of regularly arranged solid strands of cells containing numerous primordial germ cells (Fig. 1). Near the testicular surface, condensed cells form the germinal epithelium.

Table 2

Gonad size and germ cell number in embryo of control and lead-treated rats.

Embryo Age (Days)	Mean diameter of Gonads ± SE (Micron)		Mean germ cell number/section ± SE	
	control	treated	control	treated
13.5				
Testis	174.0 ± 5.10	118.0 ± 3.74 ($P < 0.01$)	103.3 ± 10.72	60.6 ± 6.99 ($P < 0.01$)
Ovary	223.6 ± 8.89	170.0 ± 7.07 ($P < 0.01$)	82.8 ± 2.51	42.3 ± 3.50 ($P < 0.001$)
18				
Testis	368.0 ± 14.97	366.0 ± 8.12 ($P < 0.9$)	1411.3 ± 58.41	798.0 ± 60.85 ($P > 0.001$)
Ovary	263.0 ± 5.39	253.0 ± 7.00 ($P > \times = \mu$)	832.0 ± 41.89	594.0 ± 49.75 ($P < 0.01$)

In 13.5 day treated embryos, the testis are smaller ($P < 0.01$) than those of the controls (Table II). The testis possesses less ramified solid seminiferous tubules (Fig. 2) containing a fewer number of primordial germ cells ($P < 0.01$) than those of the control testis (Table II & Fig. 2).

Figures 1 & 2 : T.S. of the testis of 13.5 day rat embryo treated on day 8 of intrauterine life with (1) saline solution and (2) lead nitrate solution showing the Germinal epithelium (GR.EP), Primodial germ cells (P.G.C.) and Seminiferous tubules (SF.T.).

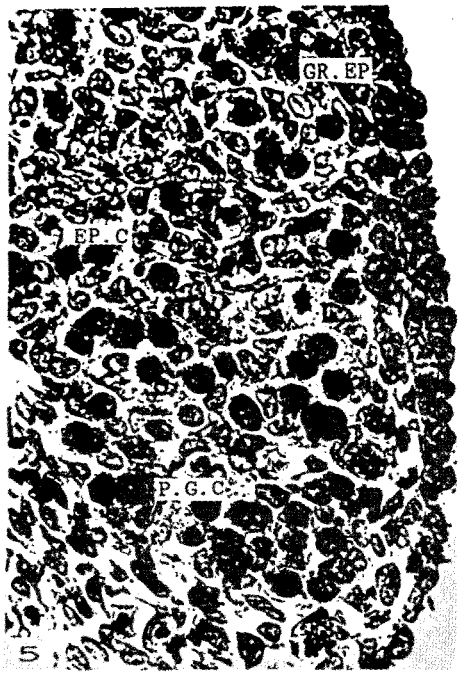
The testis of 18 day control embryos are greatly enlarged in size and are covered externally with a thick connective tissue layer; the tunica albuginea. The solid seminiferous tubules are well differentiated and highly ramified. These tubules contain numerous large spermatogonia and many Sertoli cells. The seminiferous tubules are separated by intertubular connective tissue containing groups of polygonal interstitial cells (Table II & Fig. 3).

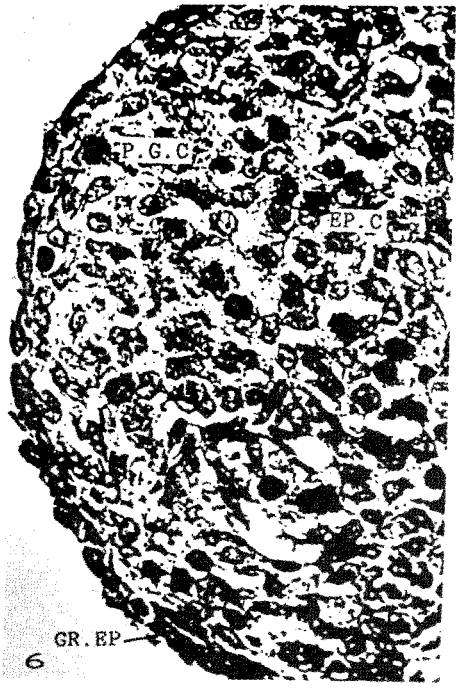
In 18 day treated embryos, the testis appear to be normal and to be similar in size ($P > 0.9$) to those of the control embryos. However, the seminiferous tubules are narrower and contain a fewer number of spermatogonia ($P < 0.001$) than those of the control embryos (Table II). The intertubular spaces are large and are occupied by large amount of intertubular connective tissue (Fig. 4).

Figures 3 & 4 : T.S. of the testis of 18 day rat embryo treated on day 8 of intrauterine life with (3) saline solution and (4) lead nitrate solution showing the Interstitial cells (IS.C), Sertoli cells (SE.C), Seminiferous tubules (SF.T), Spermatogonia (SPG) and Tunica albuginea (TU.AL).

The Ovaries

The ovaries of 13.5 day control embryos are closely attached to the lower poles of the kidneys. Each ovary is covered with a short columnar cell layer; the germinal epithelium. The ovaries contain many primordial germ cells and numerous epithelial cells (Table II and Fig. 5).



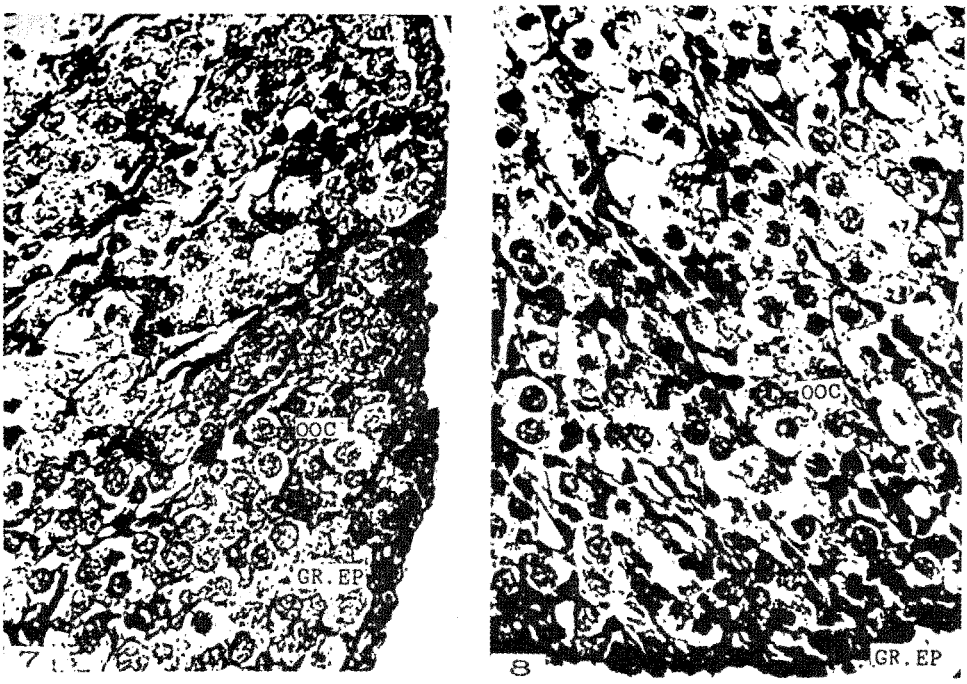


Figures 5 & 6 : T.S. of the ovary of 13.5 day rat embryo treated on day 8 of intrauterine life with (5) saline solution and (6) lead nitrate solution showing the Germinal epithelium (GR.EP), Epithelial cells (EP.C.) and Primordial germ cells (P.G.C.).

In 13.5 day treated embryos, the ovaries are smaller in diameter ($p < 0.01$) and contain a fewer number of primordial germ cells ($P < 0.001$) than those of the control ovaries (Table II and Fig. 6).

The ovaries of 18 day control embryos are much larger than those of 13.5 day control embryos and are covered with a thick germinal epithelium. Each ovary contains numerous dividing oocytes which are often grouped in clusters. Some oocytes, near the central region of the ovary, are encircled by flat epithelial cells which form the primary follicles (Fig. 7).

In 18 day treated embryos, the ovaries are nearly similar in size ($P > 0.4$) to the control embryos (Table II). The ovaries of treated embryos contain a smaller number ($P < 0.01$) of less condensed oocytes (Table II and Fig. 8) than those of the control embryos. Groups of necrotic germ cells are frequently found in some ovarian sections of lead treated embryos.



Figures 7 & 8 : T.S. of the ovary of 18 day rat embryo treated on day 8 of intrauterine life with (7) saline solution and (8) lead nitrate solution showing the Germinal epithelium (GR.EP) and Oocytes (OOC).

DISCUSSION

In the present study, a single dose of 60 μg/gm body weight lead nitrate injected on day 8 of intrauterine life has been found lethal to a significant number of the embryos. Lead nitrate may be toxic to the fetus (Wide, 1985) and/or cause reduction of blastocyst implantations; lead is suspected to cause disturbance in the central neuroendocrine reaction between the ovaries and uteri (Petruz *et al.*, 1979; Wide, 1985).

The gonads of 13.5 day lead treated embryos are smaller than those of the control embryos. Similar results have been reported in mice treated prenatally with cadmium chloride (Tam and Liu, 1985). The lead treated gonads resume their growth so that the diameter assumes a normal size on day 18.

In the present study, the reduced number of germ cells in the testes and ovaries of lead treated embryos may explained by the interference of lead with primordial germ cell migration and/or delay in the growth of the developing gonads. Such interference has been reported in mice embryos injected with mitomycine C (Tam and Snow, 1981) and cadmium chloride (Tam and Liu, 1985). Lead nitrate, on the other hand, may have interfered with mitosis by inhibiting the formation of microtubules making up the mitotic spindle (Zimmermann *et al.*, 1984).

Reduction in the number of germ cells in lead treated embryos may result in reduction of F_1 rat fertility as has been reported in prenatally lead treated F_1 mice by Wide (1985). However, fertility may be conserved by restorative proliferation of primordial germ cells in the early developing embryos, but restoration is highly restricted if a toxic agent is given at the time of meiotic division of the germ cells (McLaren *et al.*, 1972; Ballve, 1979).

In the present study, lead nitrate induced subtle histological changes, specially reduction of germ cell number in the developing gonads. Therefore, the lead nitrate interference with the development of the gonad may effect the fertility of the offspring. This presumption requires further investigation.

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نمو المناسل في أجنة الفئران المعالجة بنترات الرصاص

حمزه أحمد الشبكة

في هذا البحث تم حقن إناث الفئران البيضاء في اليوم الثامن من الحمل بجرعه واحدة من نترات الرصاص مقدارها ٦٠ ميكروجرام لكل جرام من وزن الجسم ، وفي اليوم الثالث عشر والنصف من الحمل أثبتت الدراسة أن نسبة الوفيات في الأجنة التي تعرضت لمركب الرصاص زادت زيادة ملحوظة ونقص حجم المناسل في هذه الأجنة نقصاً واضحاً .

وفي اليوم الثامن عشر من الحمل قلتّ نسبة الوفيات كثيراً في الأجنة التي تعرضت لمركب الرصاص ، كما أن حجم المناسل أصبح عادياً .

وقد أثبتت الدراسة أيضاً أن مناسل الأجنة التي تعرضت لنترات الرصاص تحتوى على عدد قليل من الخلايا التناسلية مما يشير إلى أن مركبات الرصاص لها تأثير مباشر على خصوبة هذه الأجنة .