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STUDIES ON THE EFFECT OF CADMIUM CHLORIDE ON THE GONADS OF THE DEVELOPING CHICK EMBRYOS

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Key words : Chick embryo – gonad – cadmium.

ABSTRACT

Cadmium chloride was injected in doses of 5, 10, 15 and 20 μg/egg into the yolk sac of 4–day–old chick embryos. Mortality due to cadmium intaxication increased with dose increase. The mortality rate of chick embryos injected with 10 μg/egg cadium chloride increased gradually for 6 days post injection and then decreased significantly. The gonads of surviving embryos were smaller and possessed fewer primordial germ cells than those of control embryos. Testicular destruction, especially the primordial germ cells, was associated with abundant inter–tubular connective tissue. Extensive cortical damage of the ovary included primordial germ cells and oogonia. Recovery of most destructed parts of the ovary took place within 10 days following cadium injection.

INTRODUCTION

Cadmium, the industrial pullutent (Flick, *et al.,* 11971) may occur in high concentrations in oysters (Fox, 1979; Siewicki *et al.,* 1983) and crabs (Newton *et al.,* 1984). Heavy smokers may take in from cigarettes as 20 μg of cadmium per day (Kuhnert *et al.,* 1982; Sangster *et al.,* 1984). The risk of cadmium contamination is high because of its long biological half life resulting in a cumulative effect (Friberg *et al.,*1974).

Cadmium salts cause sterility in adult rats, mice and hamsters by interfering with spermatogenesis (Parizek, 1960; Paufler and Foote, 1969; Saksena *et al.,* 1977; Aoki and Hoffer, 1978; Wong and Klaasseen, 1980), ovulation (Saksena and Salmonsen, 1983) and reducing genital ridge size and germ cell population of mouse embryos (Tam and Liu, 1985).

In birds, cadmium salts induced lesions in adult domestic pigeon testes (Sarsak and Mondal, 1973) and testicular hypoplasia and growth retardation in 4–week–old quail (Richardson *et al.,* 1974). In chick embryos, cadmium salts were highly toxic when administered on the 4th or 8th day of incubation (Ridgway and Karnofsky, 1952) and induced highly mortality and skeletal and nervous malformations when injected before incubation (Birge and Roberts, 1976). However, the effects of cadmium chloride on the gonads and their germ cells in avian embryos are not clearly understood. The aim of the present study was to investigate the toxicity and teretogenic effects of cadmium chloride on the gonads of the developing chicks embryos.

MATERIALS AND METHODS

Fertile eggs of *Gallus domesticus* were incubated at 38°C and 70% relative humidity. A sterile cadmium chloride solution in 0.9% saline solution was used for injecting embryos at early stage of development. Injection were made horizontally through the air space and into the yolk sac using a 1ml tuberculin syringe and a 1 inch 26–gauge needle. The injection hole was sealed with melted paraffin.

Embryotoxicity

In the first experiment, five groups of eggs, each of 15–38 eggs, were used. The first four groups were injected with 5, 10, 15 and 20 μg cadmium chloride / 0.05 ml solution after 4 days of incubation. The fifth control group was injected with 0.05 ml saline solution. Two days post–injection, the eggs were opened and the mortality percentage was recorded for each group.

In a second experiment, two groups of eggs were injected with 10 μg/egg cadmium chloride solution (90 eggs) and saline solution (61 eggs), respectively, after 4 days of incubation. These eggs were opened at different incubation periods to determine the post–injection period of maximum mortality. Data were compared using the student t–test and test of proportions.

Histological effects

Control and cadmium injected (10 μg/egg) eggs were examined 2, 6 and 10 days post–injection. The embryos were staged according to Hamburger and Hamilton (1951), and then fixed in 10% neutral formalin or Bouin's fluid. After dehydration in an ascending series of ethyl alchol and clearing in terpeniol, whole embryos or only the gonads wee embedded in paraffin, sectioned at 7 microns and stained with Delafield haematoxy and eosin.

RESULTS

Embryotoxicity

(a) Dose effect

The mortality rates observed in the cadmium-treated and control embryos are presented in Table (1). The mortality percentage increased gradually with dose icrease becoming significant ($P < 0.05$) in embryos injected with a dose of 10 μg/egg and highly significant ($P < 0.001$) in those injected with higher doses.

(b) Toxicity of 10 μg cadmium chloride dose at different post-injections periods

Following cadmium chloride injection, significant mortality rate ($P < 0.02$) occured after 2 days, increased ($P < 0.01$) after 6 days, and decreased significantly differed ($P > 0.4$) from that of the control after 10 days.

Table 1

Mortality rate of chick embryos post-injection with various doses of cadmium chloride.

Injection	No.	Mortality %	P value[o]
Saline Solution (Control)	15	6.67%	—
5 μg Cadmium Chloride	33	21.21%	> 0.2
10 μg Cadmium Chloride	27	33.33%	< 0.05
15 μg Cadmium Chloride	38	71.05%	< 0.001
20 μg Cadmium Chloride	29	93.10%	< 0.001

[o]P Values when the mortality percentage was compared with that of the control group.

Table 2

Mortality rate of chick embryos at different periods post-injection with 10μg cadmium chloride.

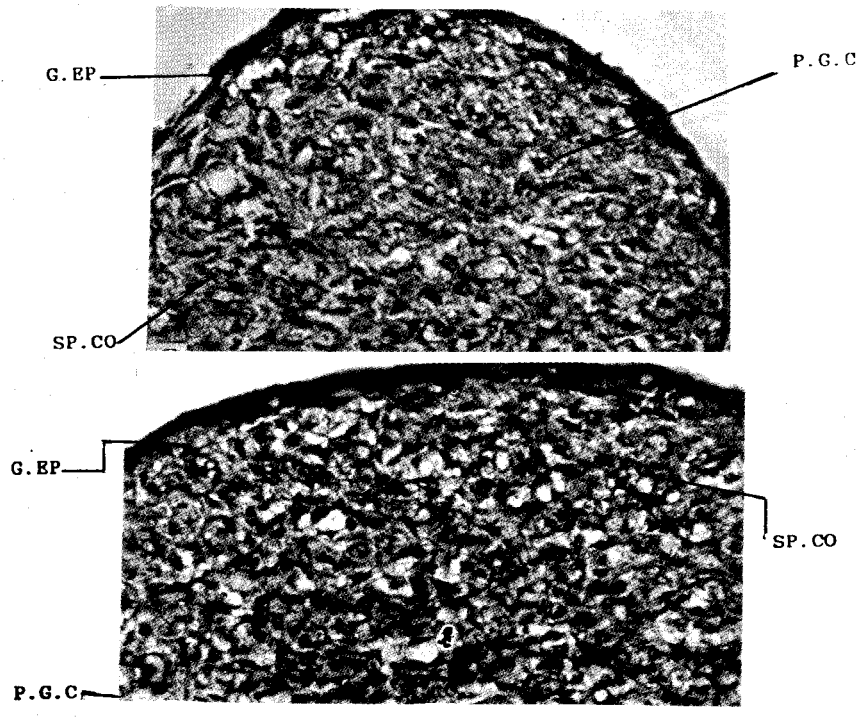
Post-injection period (days)	Saline-injected eggs		Cadmium chloride injected eggs		
	No.	Mortality %	No.	Mortality %	P value
2	18	5.56	26	34.62	0.02
6	22	9.09	30	46.67	0.01
10	21	9.52	34	17.65	0. 4

Histological Effect of Cadmium Chloride on the Gonads

The Undifferentiated Gonads

In 6–day old control embryos, the gonads are undifferentiated and all embryos possess the same basic structure. The left gonad is surrounded by a germinal epethelium consisting of cuboidal to columnar cells possessing deeply stained nuclei and a homogenous cytoplasm. From the germinal epithelium, the sexual cords protrude (Fig. 1). The premordial germ cells usually occur among the germinal epithelium and sexual cord cells. Such cells posssess eccentric spherical nuclei, each containing one or two distinct nucleoli (Fig. 1).

In treated embryos, the gonads are smaller in size than those of the control embryos. In the left gonads, the germinal epithelium is thinner, the sexual cords are less developed and the primordial germ cell number is smaller than those in the control embryos (Fig. 2).

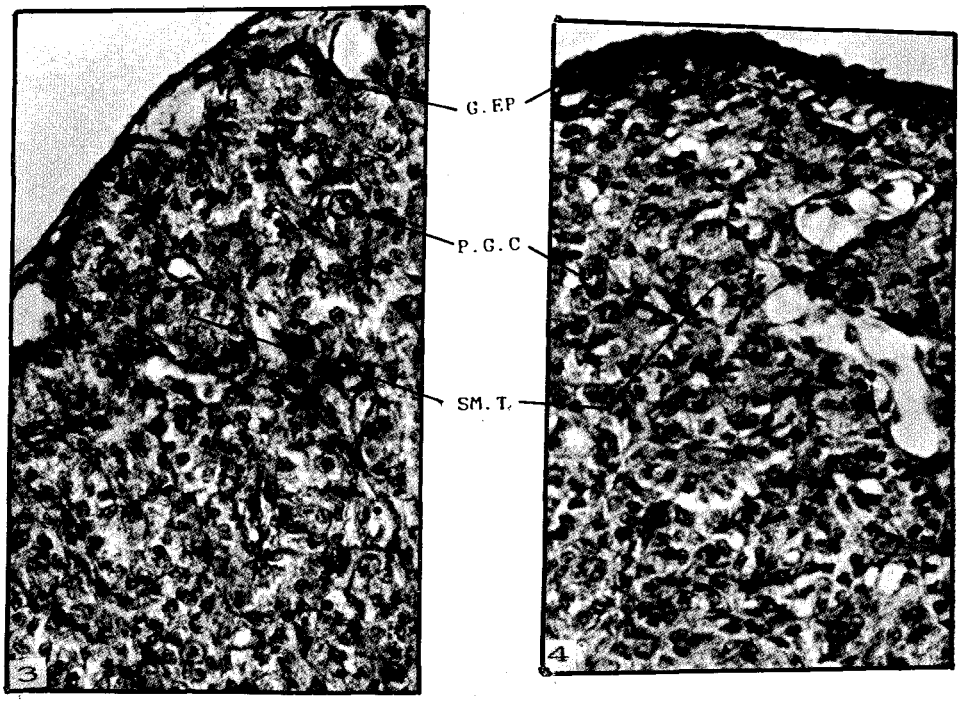


Figures 1 & 2 : T.S. of the undifferentiated left gonads of 6–day–old chick enbryos injected with (1) saline (control) and (2) cadmium chloride solution showing the germinal epithelium (G.EP), primordial germ cells (P.G.C.) and spermatic cords (SP.CO).

The Testis

In 10-day-old control embryos, the right and left testes are nearly equal in size. Each testis is surrounded by a thin layer of squamous or cuboidal cells. The sexual cords are hollowed out to form seminiferous tubules which they contain primordial germ cells and numerous mesenchyme cells. The seminiferous tubules are separated from each other by a richly vascular connective tissue containing numerous mesenchyme cells (Fig.3).

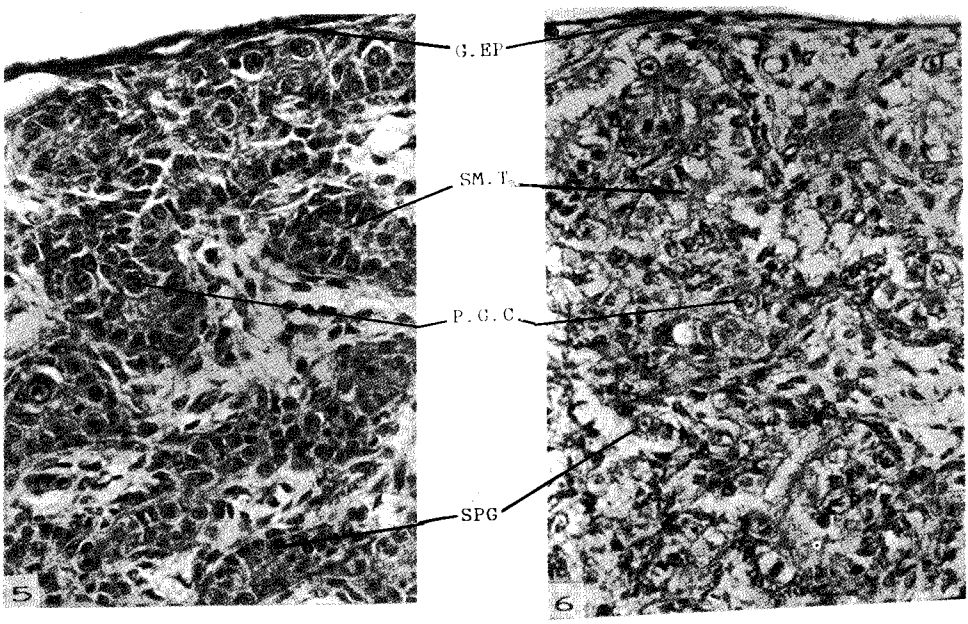
In treated embryos (6 days posr-injection) the testes are smaller and tubular formation is delayed than in the control embryos. The primordial germ cell number is smaller and the extra-tubular tissue is markedly more abundant than in the testes of the control embryos (Fig. 4).



Figures 3 & 4 : T.S. of the left testis of 10-day-old chick embryos injected with (3) saline (control) and (4) cadmium chloride solution showing the germinal epithelium (G.EP), primordial germ cells (P.G.C.) and seminiferous tubules (SM.T).

In 14–day–old control embryos, the testes contain large, ramified seminiferous tubules. Some primordial germ cells are transformed into spermatogonia and some Sertoli cells appear among them. The interstital cells usually apprear as small groups of polygonal cells scattered between the seminiferous tubules (Fig. 5).

In treated embryos (10 days post–injection) the testes are smaller than in control ones. The seminiferous tubules are less ramified, the primordial germ cells and the spermatogonia are fewer in number and the inter–tubular spaces contain abundant connective tissue than in the control embryos (Fig. 6). Also, many germ cells possess pyknotic nuclei.

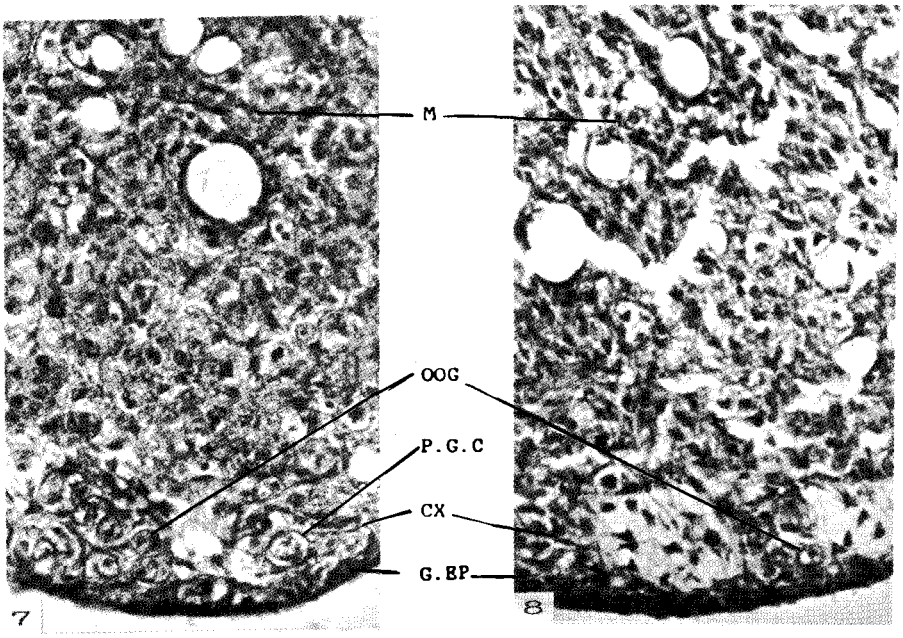


Figures 5 & 6 : T.S. of the left testis of 14–day–old chick embryos injeted with (5) saline (control) and (6) cadmium chloride solution showing the germinal epithelium (G.EP.), primordial germ cells (P.G.C.) seminiferous tubules (SM.T.) and spermatogonia (SPG).

The Ovary

In 10-day-old control embryos, the left ovary is differentiated into two regions; a peripheral cortex and a central medulla. The cortex is surounded by a thick germinal epithelium consisting of cuboidal cells with spherical nuclei. The cortical layer contains numerous primordial germ cells and a few number of oogonia. The primordial germ cells occur singly and possess large, eccentric spherical nuclei. The oogonia are usually smaller than the primordial germ cells and their nuclei are more or less centrally located. Numerous small round or oval cells scattered among the oogonia and primordial germ cells forming the sexual cords. The medulla consists of cords of epithelial cells and contains cavities especially where the medulla is attached to the mesonephros (Fig. 7).

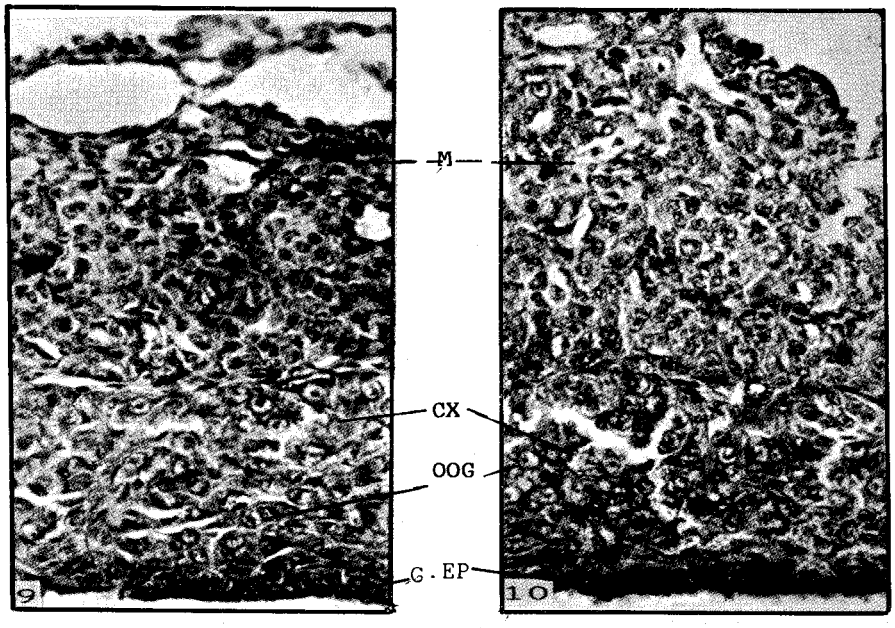
In the left ovary of treated embryos (6 days post-injection), sever destruction of the cortical and medullary cells are observed. Most primordial germ cells and oogonia are destructed and the stromal connective tissue replace most of the cortical elements. The medullary region contains numerous cavities and occupy most of the ovary (Fig. 8).



Figures 7 & 8 : T.S. of the left ovary of 10-day-old chick embryos injected with (7) saline (control) and (8) cadmium cdhloride solution showing the cortex (CX), germinal epithelium (G.EP), medulla (M), oogonia (OOG) and primordial germ cells (P.G.C.).

In 14–day–old control embryos, the cortical cords occupy a thick region below the germinal epithelium in the left ovary. These cords are separated into lobules by trabeculae of mesenchyme cells extending from the stromal connective tissue. These cords consist mainly of oogonia and follicular cells scattered at random throughout the cortex. The medulla is differentiated into the outer compact zone of solid cords and an inner zone containing large cavities (Fig. 9).

In 14–day–old treated embryos, the histological structure of the left ovary is normal except for the cortex which contains a fewer number of oogonia than that of the control embryos (Fig. 10).



Figures 9 and 10: T.S. of the left ovary of 14–day–old chick embryos injected with (9) saline (control) and (10) cadmium chloride solution showing the cortex (CX), germinal epithelium (G. EP), medulla (M), oogonia (OOG) and Primordial germ Celis (P.G.C).

DISCUSSION

In the present investigation, cadmium chloride administrated to 4-day-old chick embryos caused high mortality rate. These results are in accordance with those reported by Ridgway and Karnofsky (1952) and Birge and Roberts (1976). Mortality occurs during the few days following cadmium injection and embryos surviving the first week post-injectin continue to develop. These results are similar to those observed by Narbaitz *et al.,* (1983). Based on prior investigation, three possible mechanisms of action have been postulated: (1) cadmium salts may induce lesions in various organs and tissues in birds (Snarkar and Mondal, 1973; Richardson *et al.,* 1974; Richardson and Fox, 1975) and mammals (Parizek, 1975; Itokawa *et al.,* 1974 Singhal et al., 1976). (2) cadmium may displace zinc from metalloenzymes such as carboxypeptidase (Folk *et al.,* 1962) or carbonic anhydrase (Holgen *et al.,* 1969). The carbonic anhydrase activity in chorionic epithelium of chick embryos was reported to be important for cadmium absorbtion (Tuan and Zrike, 1978; Narbaitz *et al.,* 1981). (3) cadmium may effect the yolk sac and hence the nutrition of the developing embryo (Record, *et al.,* 1982). The high mortality may also due to nervous intoxication (Birge and Roberts, 1876) or/and cadmium intoxication of heart, kidney and liver (Meek, 1959; Richardson *et al.,* 1974).

In 6-day-old chick embryos, the gonads are described as indifferent since they possess the same structure without sexual differentiation (Ghorab and Shahin, 1975). In the present study, the injurious effects of cadmium chloride occured in undifferentiated gonads. The fewer primordial germ cells in the undifferentiated gonads of treated embryos may be a result of retarded cell migration (Tam and Liu, 1985). In a survey of teratogenicity of the heavy metals, arsenic, molybdenum, lead, mercury and selenium were found to affect the fertility of the offspring of treated pregnant hamster and rats (Earl and Vish, 1979). Cadmium chloride also produced a marked reduction of germ cells in gonads of mouse embryos treated prenatally during the early organogenesis stage (Tam and Liu, 1985).

Sexual differentiation takes place in the chick embryos on the 7th day of incubation (Amer, *et al.,* 1977). The testes of cadmium-injected embryos showed scarcity of destruction of primordial germ cells, increase in the amount of inter-tubular tissues and the seminiferous tubules are less extensively

ramified than those of the controls. In the present study, even after 10 days post–injection no regeneration of the testis was noted. Cadmium chloride may have interfered with development of the germ cells during early organogenesis of the chick embryos. Such interference has been reported to occur in the testes of mouse embryos Tam and Liu, (1985). It is quite possible that these changes may be associated with enzyme inhibition by cadmium salts. These enzymes may be general ones such as succinic dehydrogenase or characteristic for testis such as hyaluronidase (Parizek, 1960). Testicular hypoplasia was one of the most obvious lesions produced by dietary cadmium in quail during the first 4 week of life, (Richardson, *et al.*, 1974) and in adult pigeons (Sarkar and Mondal, 1973). Teratogenic effects of cadmium chloride have been noted in the testes of adult rats (Parizek, 1960; Saksena *et al.*, 1977; Aoki and Hoffer, 1978; Wong and Klaassen, 1980) and Rabbits (Paufler and Foote, 1969).

Cadmium chloride also caused marked destruction of a large number of germ cells in the cortical region and increase the ovarian stromal tissues. Such cortical degenerative changes are repaired after 10 days post–injection when most ovarian elements and transformation of primordial germ cells into oogonia occured. These observations support those of Tam and Liu (1985) who stated that cadmium chloride administration prenatally to the mouse embryos caused degenertion of most germ cells and regeneration took place at 14th day of pregnancy.

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دراسة تأثير كلوريد الكادميوم
على مناسل جنين الكتكوت

حمزة أحمـد الشبكة

تم في هذا البحث دراسة تأثير أربعة جرعات هي ٥ ، ١٠ ، ١٥ ، ٢٠ ميكروجرام من كلوريد الكادميوم حُقِنَت في اليوم الرابع من التحضين . وبعد يومين من الحقن وُجد أن معدل الوفيات إزداد بزيادة الجرعة . كما حُقنت جرعة واحدة (١٠ ميكروجرام) في اليوم الرابع من التحضين وَوُجد أن معدل الوفيات يزداد تدريجياً بزيادة فترة التحضين ثم يقل بدرجة ملحوظة بعد عشرة أيام من الحقن .

وأظهـرت الـدراسـة أن الخـلايا التنـاسلية تتأثر كثيراً بمركبات الكادميوم خصـوصـاً الخـلايا الجـرثـومية الأولية كما أن الخصية تتأثر تأثراً بالغاً بكلوريد الكادميوم ، بينما يختفي تأثير هذا المركب على المبيض بعد عشرة أيام من الحقن .