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LEAD-INDUCED MALFORMATIONS AND KIDNEY DESTRUCTION OF DEVELOPING CHICK EMBRYOS

By

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التشوهات التي تحدثها مركبات الرصاص وتأثير هذه المركبات على نمو الكلية في أجنة الكتكوت

حمزة الشبكة و جمال أبو سنة و نورة الحنزاب

في هذا البحث استخدمت جرعتان من نترات الرصاص هما ٠,٠٥ ، ٠,١ مليجرام / بيضة حقنت كل منهما بعد اليوم الثالث من التحضين . وعند فحص الأجنة في أعمار مختلفة لوحظ أن نترات الرصاص تحدث زيادة ملحوظة في نسبة الوفيات ونقص واضح في وزن الجسم ونسبة الفقس لهذه الأجنة .

كما لوحظ أن نترات الرصاص أحدثت تشوهات في الأجنة خصوصاً في مناطق الرأس والرقبة والأطراف الخلفية .

ومن الدراسة الهستولوجية لتركيب الكلية وجد أن نترات الرصاص أحدثت تحطيماً للخلايا المبطنة للوحدات البولية خصوصاً الأنيبيبات الملتفة القريبة والقاعدية وكذلك الأنيبيبات الجامعة كما أن الكبة في كلية الأجنة المعالجة بنترات الرصاص غالباً ما تكون صغيرة وكثيفة اذا ما قورنت بمثيلتها في الأجنة غير المعالجة بنترات الرصاص .

Key Words: Kidney destruction,Chick embryos.

ABSTRACT

Two doses of lead nitrate, 0.5 and 0.1 mg/egg, were injected into the eggs after 3 days of indubation. Lead nitrate increased mortality rate and decreased the body weight of developing embryos. The lead nitrate also decreased the hatchability and the weight of hatching youngs. Gross abnormalities over all body especially the head, neck, beak, eyes and hind limbs in addition to retardation of growth were markedly observed in lead-treated embryos. Lead nitrate exhibited a marked destructive effect on the nephric units of kidneys especially the proximal, distal and collecting tubules. Moreover, the glomeruli of such kidneys were abnormally small and compact.

INTRODUCTION

Lead is a serious environmental pollutent. Most of previous studies are confined largely to the effect of this heavy metal on the central nervous system (Karnofsky and Ridgway, 1951; Mc Laughlin *et al.*, 1963; Goyer, 1971; Gilani, 1973; Hirano and Kochen, 1973; Krigman *et al.*, 1974; Roy *et al.*, 1974a and b; De Gennaro, 1978; Narbaitz *et al.*, 1985, Romanoff, 1988). Many investigators have shown that lead compounds produce retardation of growth (Hammett and Wallace, 1928; De Franciscis and Bocalatle, 1962; Gilani, 1973; De Gennaro, 1978), and increase of embryonic mortality (De Franciscis and Bocalatel, 1962; King and Liu, 1974; Carpenter and Ferm, 1977) and external deformities such as hydrocephaly (Butt *et al.*, 1952; Hirano and Kochen, 1973; Gale, 1978), twisted limbs and curled toes (Gilani, 1973; De Gennaro, 1978), eye and neck deformities (Gilani, 1973; King and Liu, 1974) and skeletal defects (Mc Laughlin *et al.*, 1963; Gale, 1978). Surprisingly, little attention has been given to the effect of lead compounds on the internal organs of the developing embryos especially kidneys (Goyer, 1971; Irwin and Karstad, 1972). Hence, the previous results encourage further experimentation to study the effect of this heavy metal on the external features as well as the microscopical structure of the kidney of developing chick embryos.

MATERIAL AND METHODS

Fertilized eggs of domestic fowl; *Gallus domisticus* were incubated at 38° C and about 70% relative humidity. After 3 days of incubation eggs were divided into three groups. The first group served as control and injected with 0.1 ml-egg sterile distilled water. The second and third groups were injected with 0.1 ml/egg sterile distilled water containing 0.05 and 0.1 mg lead nitrate/egg respectively. Eggs were candled daily for checking viability and non viable eggs were discarded. After 9, 12 and 15 days following incubation (stages 35, 38 and 44 according to Hamburger and Hamilton, 1952) eggs were opened and alive as well as dead embryos were scored. Living embryos were examined morphologically to determine types and forms of abnormalities and weighed to obtain an indication of growth.

Living chick embryos were dissected after 9, 12 and 15 days of incubation. Kidneys were fixed in aquous Bouin's fluid for 24 hours, dehydrated, cleared, embedded and sectioned at 7 micron thick. Mounted sections were stained with Harris hematoxylin and eosin.

The data of control and lead-treated embryos were compared using the Student's t-test.

RESULTS

Mortality rate and embryonic weight:

As shown in (Table 1), the mortality percentages were significantly increased in lead-treated embryos during different stages as compared with corresponding controls. Moreover, the mortality percentage was increased as the dose of lead nitrate increased.

In 9-day old embryos treated only with a high dose of lead nitrate as well as all lead-treated embryos obtained during 12th and 18th day of incubation were significantly decreased in weight as compared with that of of corresponding controls (Table 2).

Table 1
Mortality (%) and weight (gm) of control and lead-treated chick embryos at different stages of development.

		9 day			12 day			15 day		
		control	0.05	0.1	control	0.05	0.1	control	0.05	0.1
Mortality(%)		11	47	55	17.9	62	86	20	52	71
Embryonic mean		1.65	1.81	1.91	5.43	4.19	4.1	16.64	14.02	12.58
weight	S.E.	0.06	0.07	0.08	0.15	0.15	0.2	0.63	0.78	0.92
	Sig.	---	#	*	---	**	**	---	*	*

\# not significant
* $P < 0.01$
** $P < 0.001$

Hatchability and hatching weight

The hatching percent of surviving lead-treated embryos was obviously decreased as compared with that of control. The hatchability rate was gradually decreased as the dose of lead nitrate increased (Table 2).

The mean weight of lead-treated hatching youngs was significantly decreased as compared with that of control. This decrease was more pronounced as the dose of lead nitrate was increased (Table 2).

Table 2
Hatching percent and the mean weight of hatching youngs control and lead-treated eggs.

		Control	Lead-treated eggs	
			0.05 mg/egg	0.1mg/egg
Hatchability (%)		80	24.3	15
Hatching	mean	44.9	40.27	32.01
weight	S.E.	1.28	1.7	2
	Sig.	---	P > 0.05	P < 0.001

Gross abnormalities:

The most interesting phenomenon noticed was the high incidence of head and hind limb abnormalities in lead-treated embryos while such abnormalities were not seen among controls (Figs. 1, 2, and 3). Failure of skull roof to develop, brain protrusion, hydrocephaly, hind limb deformities, reduction of digits, eye and neck deformities are the common abnormalities recorded 6 days following injection with the two doses of lead nitrate (Fig. 1 b and c).

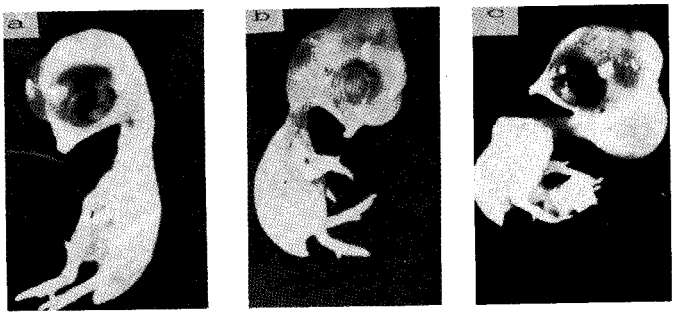

Fig. 1: 9-day old chick embryos.
a-Control.
b-Chick embryo treated with 0.05 mg lead nitrate.
c-Chick embryo treated with 0.1 mg lead nitrate. X 3

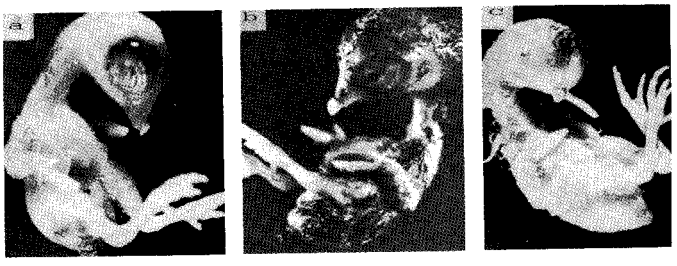

Fig. 2: 12-day old chick embryos.
a-Control.
b-Chick embryo treated with 0.05 mg lead nitrate.
c-Chick embryo treated with 0.1 mg lead nitrate. X 3

After 12 days of lead treatment also serious anomalies such as hydrocephaly, missing of one or both eyes, twisted neck, peak and hind-limb deformities were recorded in the two lead-treated groups (Fig. 2 b and c).

The most common anomalies found after 15 days of lead treatment were the exencephaly, hydrocephaly, shortened dentary, curled and twisted neck (Fig. 3 b and c).

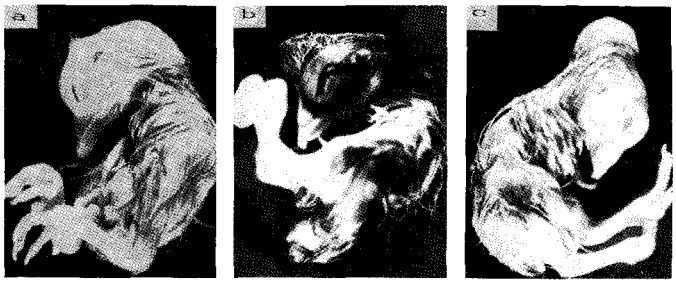

Fig. 3: 15-day old chick embryos.
a-Control.
b-Chick embryo treated with 0.05 mg lead nitrate.
c-Chick embryo treated with 0.1 mg lead nitrate. X 1.5

Effect of lead nitrate on developing kidneys:

9-day old chick embryos:

Each kidney of a control chick embryo is composed of 2 different regions; the outer cortex and the inner medulla. The first part of a nephric unit is a cup shaped Bowman's capsule into which a tuft of blood capillaries is pushed to form the glomerulus. This part is followed by proximal tubule, Henle's loop and distal tubule which discharges into a collecting tubule. The cup-shaped Bowman's capsule is lined by flattened epithelial cells containing oval-shaped nuclei. The proximal tubule is lined by columnar cells containing spherical centrally located nuclei. A conspicuous brush border on the luminal surface is evident. Henle's loop is lined by low cuboidal to squamous cells containing spherical nuclei. The wall in the distal tubules is somewhat thinner than that in the proximal one but the lumen is generally wider and its luminal surface has no brush border. The collecting tubule possesses a wide lumen surrounded by a single layer of cuboidal cells containing spherical nuclei. The spaces between the nephric units are usually occupied by numerous blood capillaries called peritubular capillaries or sinuses (Fig. 4a).

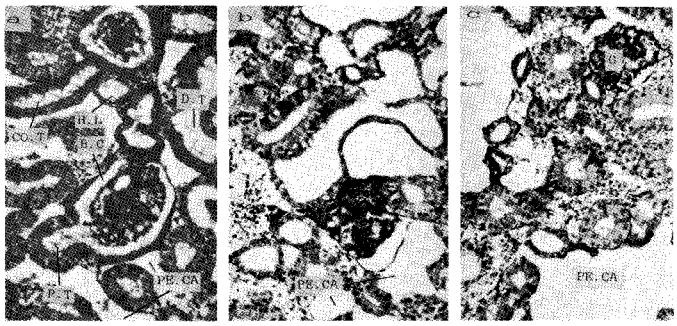

Fig. 4: T.S. of the kidneys of 9-day old chick embryos.
a-Kidney of control embryo showing Bowman's capsule (B.C), collecting tubule (CO.T), distal tubule (D.T.), Henle's loop (H.L.), proximal tubule (P.T.) and the peritubular capillar (PE. CA.).
b-Chick embryo treated with 0.05 mg lead nitrate showing abnormally dilated peritubular capillary (PE. CA).
c-Chick embryo treated with 0.1 mg lead nitrate showing a small and compact glomerulus (G) and dilated peritubular capillary (PE. CA). X 210

Kidneys of chick embryos treated with 0.05 and 0.1 mg lead nitrate/egg indicated some evidence of cell destruction of proximal, distal and collecting tubules. Few cells of such tubules possessed vacuolated cytoplasm and small darkly stained nuclei which represent a sign of degeneration. Moreover, the glomeruli of lead-treated kidneys, especially those received high dose, appeared small and compact. The peritubular capillaries are greatly enlarged and either empty or filled with blood cells (Fig. 4 b and c).

12-day old chick embryos:

Kidneys of control chick embryos, after 12 days of incubation, were obviously increased in size. The nephric units, however, possessed the same basic components described before (Fig. 5 a).

In chick embryos treated with 0.05 and 0.1 mg lead nitrate/egg, the glomeruli were smaller and the glomerular capillaries were somewhat narrow and compact. The epithelial cells of proximal, distal and collecting tubules were cloudy and swollen and numerous residual parts of destroyed cells were noticed in the lumina of such tubules. Pyknosis of the nuclei and vacuolation of cytoplasm were evident. The peritubular capillaries were abnormally dilated and packed with red blood cells (Fig. 5 b and c).

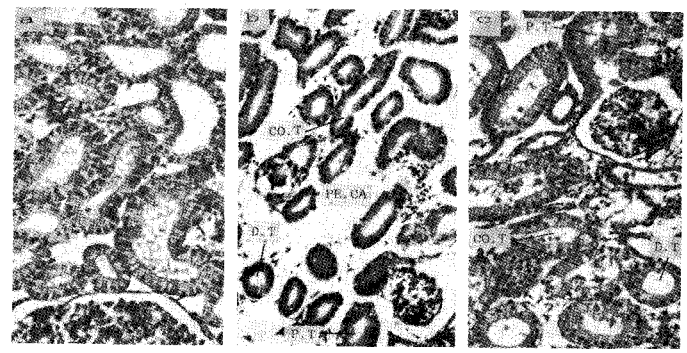

Fig. 5: T.S. of the kidneys of 12-day old chick embryos.
a-Control.
b-Chick embryo treated with 0.01 mg lead nitrate showing the destroyed epithelial cells of collecting tubule (CO. T.), distal tubule (D.T.) and proximal tubule (P.T.). Peritubular capillaries are abnormally enlarged and packed with blood cells.
c-Chick embryo treated with 0.05 mg lead nitrate showing the destroyed epithelial cells of collecting tubule (CO.T), distal tubule (D.T.) and proximal tubule (P. T.) X 210

15-day old chick embryos:

After 15 days of incubation, the kidneys of control embryos were greatly enlarged in size (Fig. 6a).

Cross sections through the kidney of chick embryos treated with 0.05 and 0.1 mg lead nitrate/egg revealed that the glomeruli were abnormally smaller. The tubules of lead-treated kidneys were markedly dilated. Such tubules were lined with thin walls consisting of short columnar to flattened cells. Some of these cells possessed granular cytoplasm and small dense nuclei. The peritubular blood

capillaries were dilated and contained non or few blood cells (Fig. 6b and c).

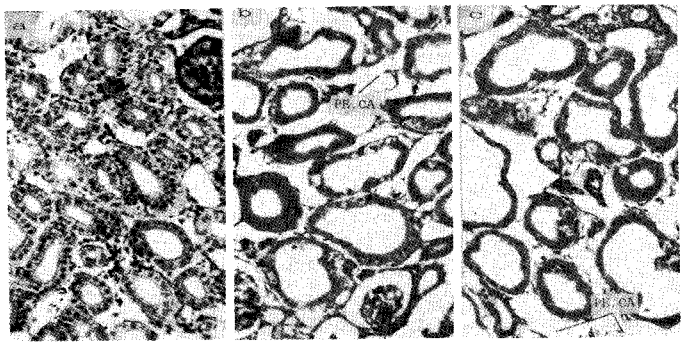

Fig. 6: T.S. of the kidneys of 15-day old chick embryos.
a-Control.
b-Chick embryo treated with 0.05 mg lead nitrate showing the dilated peritubular capillaries (PE. CA).
c-Chick embryo treated with 0.1 mg lead nitrate showing the dilated peritubular capillaries (PE. CA). X 210

DISCUSSION

The results of the present investigation show that the percentage of embryonic mortality was directly proportional to the dose of lead administered. This result agrees with the finding of Catizon and Gray (1941), Butt *et al.,* (1950) and Gilani (1973). Moreover, Karnofsky and Ridgway (1951) reported that a high incidence of embryonic mortality occurs in chick embryos exposed to small amounts of lead compounds during early incubation periods. Also Birge and Roberts (1976) found that lead produces mortality rates of 17-26% when distributed in the egg yolk at much low concentration of 0.001 ppm.

The mean weight of surviving of lead-treated chick embryos decreased compared with corresponding controls. The decrease in body weight of lead-treated embryos may be due to growth retardation since the lead has a general inhibitory action on metabolism (Gilani, 1973). Moreover, the embryonic body weight decreased as the dose of lead nitrate increased. Similar result have been reported in chick embryos injected with lead compounds either before incubation (Hammett and Wallace 1928) or at different stages following incubation (Karnofsky and Ridgway, 1951: Butt *et al.,* 1952; De Franciscis and Bocalatle, 1962; Gilani, 1973: De Franciscis and Bocalatle, 1962; Gilani, 1973; De Gennaro, 1978). The decrease in body weight of newly hatching youngs obtained from eggs injected with lead nitrate is in concert with the decrease embryonic body weight and reflects the inhibitory action of lead on the metabolism during embryonic development.

An inverse relation has been established between hatching percentage and the lead dose administered. Thus the hatching percent of surviving embryos decreased as the dose of lead nitrate increased. In addition, hatching time was also delayed in treated embryos compared with that of controls. The failure of lead-treated embryos to hatch may be due to growth retardation and/or to the malformations, especially beak deformity, which resulted in such embryos. Similarly, Romanoff (1988) recorded that the hatching time was delayed beyond 21 days with increasing amounts of lead and most chicks had to be helped out of the shell. Moreover, McLaughlin *et al.,* (1963) found that lead acetate injected during 10th day of incubation resulted in no hatch at a level of 1 mg/egg. De Gennaro (1978) attributed the failure of lead-treated chick embryos to hatch to injuries of brain and spinal cord which result in loss of behavioural reflexes and coordinated movement that are necessary to move away the egg shell..

Lead nitrate produced also numerous abnormalities which differed from one embryo to another according to the dose used and to the age of the embryo. The common deformities were hydrocephaly, exencephaly, deformities in shape and size of eyes and beak, shortened and twisted neck, shortened and curled toes and paralysis of hind limbs. Similar anomalies have been reported in chick embryos by Hammett and Wallace (1928), Karnofsky and Ridgway (1951), Gilani (1973), Hirano and Kochen (1973), De Gennaro (1978).

In lead-treated chick embryos, the kidneys show dilation of proximal, distal and collecting tubules. The glomeruli appear small and compact. Moreover, most epithelial cells lining the nephric units are swollen, vacuolated and possess pyknotic and irregularly shaped nuclei. The signs of destruction shown in nephric units may be due to accumulation of lead in kidney tissue (Cibulka *et al.,* 1985). Goyer (1971) reported that the renal tubular lining cells in lead poisoning are thick and have a reduced transporting function. Degeneration of kidney was previously reported by Irwin and Karstad (1972) in lead-treated duck. In human kidneys, inflammation, nuclear inclusion bodies and renal tubular degeneration were reported by Dreisbach (1977). The degenerative effect of lead compounds on the kidneys of developing chick embryos may be one of several factors increasing the mortality rate within these embryos.

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