

EFFECT OF LEAD NITRATE ON THE LIVER OF THE DEVELOPING CHICK EMBRYOS

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ABSTRACT

The liver of chick embryos treated with 0.03, 0.05 and 0.1 mg lead nitrate/egg showed disorganization of hepatic cords, abnormally enlarged blood sinusoids and extensive cell necrosis. The hepatocytes of lead-treated embryos possessed vacuolated cytoplasm beside small irregular and darkly stained nuclei.

Liver proteins of lead-treated chick embryos show insignificant variations during almost all developmental stages. However, a significant increase in liver proteins was noticed in 9-day embryos treated with 0.1 mg lead nitrate/egg. The percentages of all liver protein bands of lead-treated embryos during different stages varied markedly from those of corresponding controls.

Different doses of lead nitrate caused an increase of the activity of all investigated enzymes, with a variable values, at almost all developmental stages. Liver alkaline phosphatase and GOT of 9-day chick embryos appeared to be sensitive even to the low doses of lead nitrate while those of late stages can tolerate the same doses.

INTRODUCTION

It is well known that accumulation of lead may reach a toxic level that produces serious effects to living systems. Data from National Health and Nutrition Examination Survey (Mahaffy, *et al.*, 1982) reported that in children from six months through 5 years of age, the prevalence of elevated blood lead levels was 4%. The best organ for chemical analysis to confirm a diagnosis of lead-poisoning is the liver (Salisbury, *et al.*, 1958). Metallic lead causes liver atrophy and gall bladder distention (Irwin and Karstad, 1972). A marked increase of activity of liver acid phosphatase of lead-treated chick embryos was reported by King and Liu (1974, a). They reported also that, liver alkaline phosphatase activity of lead-treated chick embryos showed insignificant differences. Kucharz (1986) found that rats intoxicated with lead acetate showed an increase of both total and insoluble collagen

content of the liver. However, there is a scarcity of studies dealing with the biological effects of lead on the liver of developing embryos. Hence, the aim of the present investigation is to study the effects of lead nitrate on the structure, proteins and some enzymatic activities of liver of developing chick embryos.

MATERIALS AND METHODS

Fertilized eggs of *Gallus domesticus* were collected, cleaned and incubated at 38°C and about 70% relative humidity. After three days of incubation, eggs containing living embryos were divided into four groups. The first, second and third groups were injected with 0.1 ml/egg sterilized distilled water containing 0.03, 0.05 and 0.1 mg lead nitrate respectively. The fourth group was injected with 0.1 ml/egg sterilized distilled water and served as a control. After 9, 12 and 18 days following incubation (stages 35, 38 and 44 according to Hamburger and Hamilton, 1952), eggs were opened and the living embryos were separated from underlying yolk, then washed in a warm saline solution.

Histological studies

Control and lead-treated chick embryos were dissected after 9, 12 and 18 days of incubation and the liver was excised immediately and fixed in aqueous Bouin's fluid. Fixed tissues were dehydrated, cleared, embedded and sectioned at 7 microns thick. Mounted sections were stained with Harris hematoxylin and eosin.

Physiological studies

The liver of control and lead-treated chick embryos were immediately excised, homogenized in 0.85% saline at a ratio of 50 mg/ml (w/v) using an all glass homogenizer immersed in an ice water bath. The liver extracts were centrifuged in a refrigerated centrifuge at 15,000 rpm for 25 minutes at 4°C. The clear supernatants were stored at -30°C.

A. Liver proteins

The total proteins were estimated according to Biuret method of Boehringer Mannheim Automated Analysis for BM/Hitachi system 705 according to the method of Witt and Trendelenburg (1982).

For electrophoresis, the liver extract of control and lead-treated chick embryos were concentrated 50 times using Minicon A 25 concentrator. Four applications (5 ul) for each sample were loaded on wetted titan III cellulose acetate membrane using super Z applicator. The current used was 180 volts for 15 minutes. Protein bands were stained in Panceau S solution and excess dye was removed in 5% acetic acid. The plate was dehydrated, cleared and dried in an oven at 50°C. Scanning was carried out using Econoscan densitometer at 525 nm.

B. Liver enzymes

Glutamate oxaloacetate transaminase (GOT) and alkaline phosphatase activities of liver extract of control and lead-treated chick embryos were estimated according to the method of Bergmeyer *et al.* (1976 and 1978) using Boehringer Mannheim Automated Analysis for Hitachi 705.

Lactate dehydrogenase, glutamate pyrovate transferase (GPT) and acid phosphatase activities were estimated according to the methods described by Bio Merieux kits using spectronic 20 apparatus.

RESULTS

Histological studies

a. 9-day chick embryos

The liver of 9-day old chick embryo consisted of hepatocytes and blood sinusoids. The hepatocytes are arranged in a series of anastomosing hepatic cords each being composed of a double layer of polygonal cells. These cells contain round nuclei each possesses a small deeply stained and centrally located nucleolus. The hepatic cells are attached apically with the bile canaliculi and basely with blood sinusoids. The latter have relatively wide lumina anastomosing irregularly and the wall of each consisted of a discontinuous layer of flattened endothelial cells containing oval shaped and darkly stained nuclei (Fig. 1, a).

Sections through the liver of chick embryos treated with 0.03, 0.05 and 0.1 mg lead nitrate/egg showed disorganized hepatic cords and blood sinusoids. The hepatic cords became thinner and their cells contained numerous vacuoles. Moreover, extensive cell necrosis was reported in many hepatocytes and endothelial cells lining the blood sinusoids (Fig. 1, b, c and d). In chick embryos treated with 0.03 mg lead nitrate/egg blood corpuscles, mostly hepatocytes, were abnormally accumulated inside destructed blood sinusoids (Fig. 1, b). In addition, the liver of chick embryos treated with 0.1 mg lead nitrate/egg possessed abnormally enlarged sinusoids and the cytoplasm of hepatocytes appeared swelled and contained large vacuoles (Fig. 1, d).

b. 12-day chick embryos

The liver of 12-day old control chick embryo was similar in structure to that of previous stage. It is composed of two-cell thick hepatic cords. The hepatic cells appeared irregular in shape and arranged around a fine bile canalicule. The hepatic cords were separated from each other with narrow branching blood sinusoids lined by endothelial cells containing flattened darkly stained nuclei (Fig. 2, a).

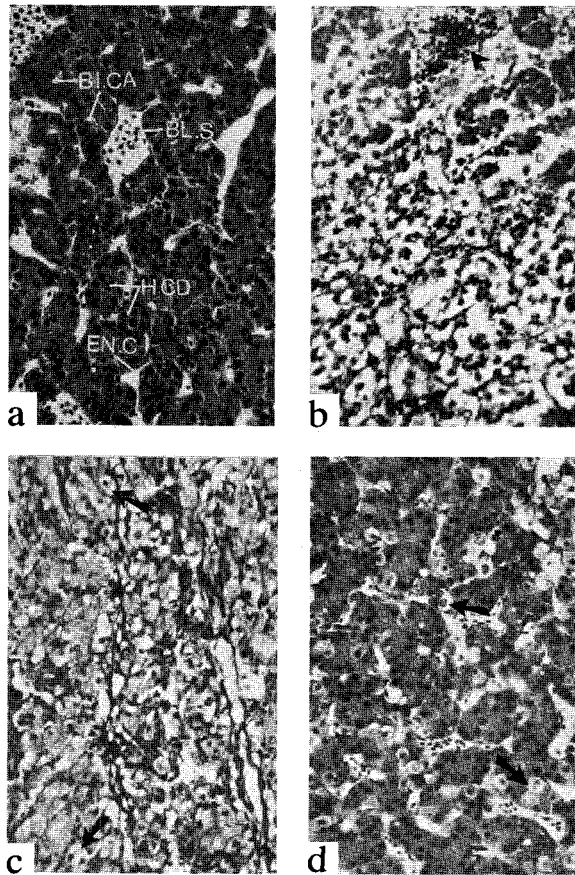


Fig. 1: Sections through the liver of 9-day control and lead-treated chick embryos.

- a. Section through the liver of control chick embryo showing the hepatic cords (H.CD), bile canaliculi (BI.CA) and blood sinusoids (BL.S) lined with flattened endothelial cells (EN.C).
- b. Section through the liver of chick embryo treated with 0.03 mg lead nitrate/egg showing the accumulation of blood corpuscles (arrowhead) inside a destroyed blood sinusoid.
- c. and d. Sections through the liver of chick embryos treated with 0.05 and 0.1 mg lead nitrate/egg respectively showing the vacuolated hepatocytes (arrows).

× 280

The liver of chick embryos treated with 0.03, 0.05 and 0.1 mg lead nitrate/egg showed extensive cell damage affected the endothelial cells lining blood vessels and blood sinusoids. In embryos treated with 0.03 mg lead nitrate/egg, few hepatic cells possessed foamy and granulated cytoplasm (Fig. 2, b). Such cells in embryos treated with 0.05 and 0.1 mg lead nitrate/egg contained vacuolated cytoplasm, small irregular and darkly stained nuclei. In addition, there were many darkly stained particles which represented nuclear and cytoplasmic debris of destructed cells (Fig. 2, c and d).

c. 18-day chick embryos

The liver of 18-day old control chick embryo is composed of polygonal hepatocytes arranged in a series of anastomosing hepatic cords. These hepatocytes are arranged around fine bile canaliculi. The latter unite to form bile ductules and these in turn unite to form the bile duct. The hepatic cords are surrounded and penetrated by a network of fine sinusoids of rather variable width. Many of these sinusoids, however, were only wide enough to allow the passage of a single erythrocyte (Fig. 3, a).

In chick embryos treated with 0.03 mg lead nitrate/egg the liver revealed normal appearance except in few areas where the hepatocytes, blood vessels and blood sinusoids appeared slightly destructed. In chick embryos treated with 0.05 and 0.1 mg lead nitrate/egg, the hepatic cords appeared thin and separated with wide destructed blood vessels and blood sinusoids. The hepatocytes possessed vacuolated or granulated cytoplasm and small faintly stained nuclei (Fig. 3, c and d). Numerous small particles as well as darkly stained pigments were scattered inside blood vessels and sinusoids (Fig. 3, c).

Physiological studies

Liver proteins

The liver protein content of chick embryos treated with the high dose of lead nitrate showed significant increase ($p < 0.02$) after 9 days of treatment. However, the other dosages produced insignificant variations during different stages of development as compared with the corresponding control values (Table 1).

Liver extracts of both control and lead-treated chick embryos revealed 4 anodal migrating protein bands after 9 days of incubation. However, it is clear that, the percentage of protein in these bands of lead-treated embryos varied from those of the controls (Table 2). After 12 days of incubation, the liver extract of control and lead-treated (0.1 mg/egg) embryos revealed also 4 protein bands. In 12-day chick embryos treated with 0.03 and 0.05 mg lead nitrate/egg as well as 18-day control and lead treated ones, only 3 protein fractions were recognized. The percentages of all protein bands of lead-treated embryos during these stages varied markedly from those of corresponding controls (Table 2).

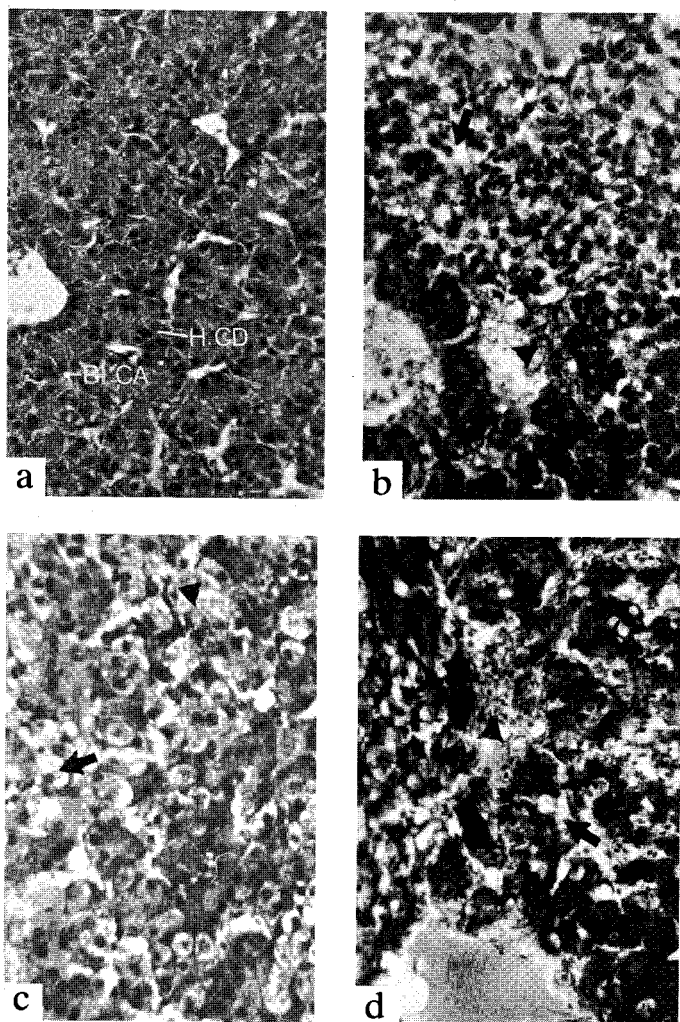


Fig. 2: Sections through the liver of 12-day control and lead-treated chick embryos.

a. Section through the liver of control chick embryo showing hepatic cords (H.CD) and bile canaliculi (BI.CA).

b., c. and d. Sections through the liver of chick embryos treated with 0.03, 0.05 and 0.1 mg lead nitrate/egg respectively showing the vacuolated cells (arrows) and cytoplasmic debris of destroyed cells (arrowheads).

× 280

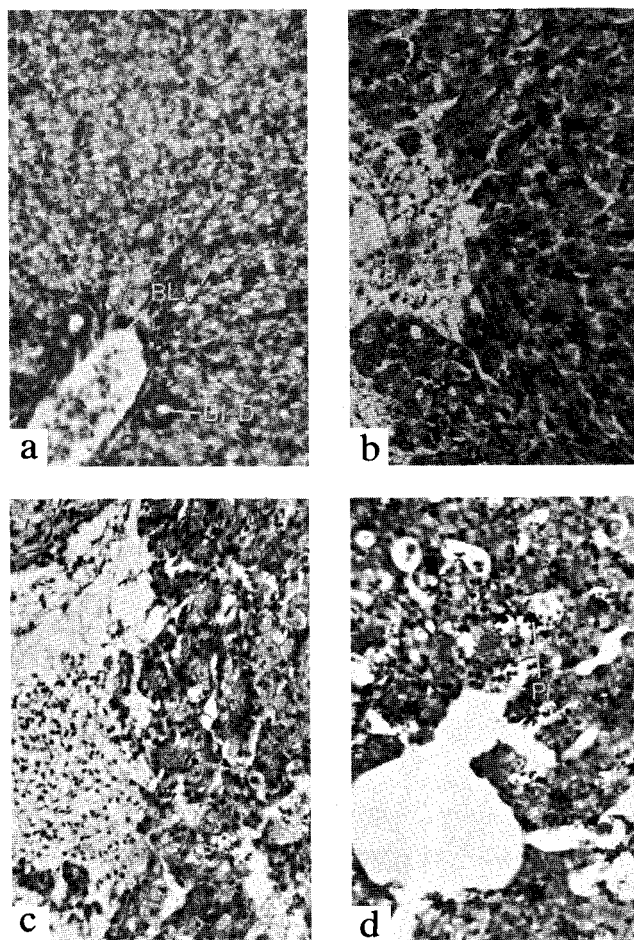


Fig. 3: Sections through the liver of 18-day control and lead-treated embryos.

a. Section through the liver of control chick embryo showing a blood vessel (BL.V) and a bile ductule (BI.D).

b. Section through the liver of chick embryo treated with 0.03 mg of lead nitrate/egg.

c. and d. Sections through the liver of chick embryos treated with 0.05 and 0.1 mg lead nitrate/egg respectively showing numerous small particles and darkly stained pigments (PI) scattered inside the blood sinusoids.

× 280

Table 1

Total liver protein content of control and lead-treated chick embryos at different stages.

	9 days-old chick embryos gm/100 gm of liver				12 days-old chick embryos gm/100 gm of liver				18 days-old chick embryos gm/100 gm of liver			
	Control	leaded embryos			Control	leaded embryos			Control	leaded embryos		
		.03mg/egg	.05mg/egg	.1mg/egg		.03mg/egg	.05mg/egg	.1mg/egg		.03mg/egg	.05mg/egg	.1mg/egg
mean	3.4	3.6	4.8	5.4	5.6	6.2	6.6	6.6	7.4	7.0	6.2	5
S.E.	0.66	0.39	0.51	0.52	0.77	0.62	0.59	0.59	0.79	1.00	0.46	0.68
t-test	—	not sign.	not sign.	P<0.02	—	not sign.	not sign.	not sign.	—	not sign.	not sign.	not sign.

Table 2

The percentage of protein in electrophoretically separated fractions of liver extract of control and lead-treated chick embryos.

Band	9 days chick embryos				12 days chick embryos				18 days chick embryos			
	Control	leaded embryos			Control	leaded embryos			Control	leaded embryos		
		.03mg/egg	.05mg/egg	.1mg/egg		.03mg/egg	.05mg/egg	.1mg/egg		.03mg/egg	.05mg/egg	.1mg/egg
1	13.0	14.8	6.7	10.5	3.2	21.4	50.8	24.6	14.3	10.4	16.4	9.0
2	6.0	19.4	19.2	18.2	73.0	73.7	36.8	51.0	61.8	77.6	77.0	80.1
3	71.0	53.4	65.2	64.7	21.6	4.9	12.4	14.9	23.9	12.0	6.6	10.4
4	9.9	12.0	8.9	6.6	2.2			9.5	—	—	—	—

Acid phosphatase

The mean liver acid phosphatase activity of 9, 12 and 18 day old chick embryos treated with 0.03 mg lead nitrate/egg was insignificantly changed as compared with that of corresponding controls (Table 3). Moreover, the mean liver acid phosphatase activity of embryos treated with 0.05 mg lead nitrate/egg showed insignificant change after 9 and 12 days of incubation and a significant increase after 18 days of incubation compared with corresponding controls (Table 3). A dose of 0.1 mg lead nitrate/egg exhibited a significant increase of mean liver acid phosphatase activity after 9 and 18 days of incubation but not after 12 days of incubation as compared with corresponding controls (Table 3).

Alkaline phosphatase

The mean liver alkaline phosphatase activity of all 9-day lead-treated and that of 12-day chick embryos treated with 0.05 and 0.1 mg lead nitrate/egg was significantly increased compared with corresponding controls (Table 4). In 12-day chick embryos treated with 0.03 mg lead nitrate/egg and all 18-day embryos treated with different doses of lead nitrate, the liver alkaline phosphatase activity was not insignificantly increased compared with corresponding control values (Table 4).

GOT

The mean liver GOT activity of all lead-treated 9-day chick embryos and that of 12-day ones treated with 0.1 mg lead nitrate/egg was significantly increased compared with corresponding controls (Table 5). However, the mean liver GOT activity of 12-day embryos treated with 0.03 and 0.05 mg lead nitrate/egg and that of 18-day embryos treated with all three doses of lead nitrate was not significantly changed (Table 5).

GPT

The mean liver GPT activity of 9-day embryos treated with 0.05 and 0.1 mg lead nitrate/egg, all lead-treated 12-day embryos and 18-day embryos treated with 0.05 and 0.1 mg lead nitrate/egg was significantly increased as compared with the corresponding controls. However, the mean liver GPT activity of 9 and 18-day embryos treated with 0.03mg lead nitrate/egg was insignificantly increased as compared with corresponding controls (Table 6).

LDH

The mean liver LDH activity of all lead-treated chick embryos at different developmental stages was significantly increased as compared with corresponding controls (Table 7).

Table 3

Acid phosphatase activity of liver extract of control and lead-treated chick embryos.

	9 days-old chick embryos (IU/100 gm of liver)				12 days-old chick embryos (IU/100gm of liver)				18 days-old chick embryos (IU/100gm of liver)			
	Control	leaded embryos			Control	leaded embryos			Control	leaded embryos		
		.03mg/egg	.05mg/egg	.1mg/egg		.03mg/egg	.05mg/egg	.1mg/egg		.03mg/egg	.05mg/egg	.1mg/egg
mean	150.1	133.0	146.5	209.7	210.10	218.70	247.7	246.2	330.6	363.8	458.70	507.2
S.E.	5.4	7.7	17.1	16.04	12.06	78.0	15.1	24.4	21.1	32.05	33.5	
t-test	—	not sign.	not sign.	P<0.01	—	not sign.	not sign.	not sign.	—	not sign.	P<0.01	P<0.001

Table 4

Alkaline phosphatase activity of liver extract of control and lead-treated chick embryos.

	9 days-old chick embryos (IU/100 gm of liver)				12 days-old chick embryos (IU/100gm of liver)				18 days-old chick embryos (IU/100gm of liver)			
	Control	leaded embryos			Control	leaded embryos			Control	leaded embryos		
		.03mg/egg	.05mg/egg	0.1mg/egg		.03mg/egg	.05mg/egg	0.1mg/egg		.03mg/egg	.05mg/egg	0.1mg/egg
mean	10.80	20.20	19.8	17.8	33.4	42.0	49.4	44.2	81.0	85.2	92.2	84.8
S.E.	0.85	1.28	3.4	1.5	3.2	3.8	4.8	4.9	7.6	11.3	9.7	6.2
t-test	—	P<0.01	P<0.01	P<0.01	—	not sign.	P<0.01	P<0.01	—	not sign.	not sign.	not sign.

Table 5
GOT activity of liver extract of control and lead-treated chick embryos.

	9 days-old chick embryos (IU/100 gm of liver) lead embryos				12 days-old chick embryos (IU/100 gm of liver) lead embryos				18 days-old chick embryos (IU/100 gm of liver) lead embryos			
	Control	lead embryos			Control	lead embryos			Control	lead embryos		
		0.03mg/egg	0.05mg/egg	0.1mg/egg		0.03mg/egg	0.05mg/egg	0.1mg/egg		0.03mg/egg	0.05mg/egg	0.1mg/egg
mean	3254.8	5930.2	5049.6	5687.2	7022.8	9322	8392.8	9379	7125.2	4531.6	7538.6	10252.6
S.E.	225	363	215	393	1672	1195	1331	1489	1370	614	525	751
t-test	—	P<0.001	P<0.001	P<0.001	—	not sign.	not sign.	P<0.01	—	not sign.	not sign.	not sign.

Table 6
GPT activity of liver extract of control and lead-treated chick embryos.

	9 days-old chick embryos (IU/100 gm liver) lead embryos				12 days-old chick embryos (IU/100 gm liver) lead embryos				18 days-old chick embryos (IU/100 gm liver) lead embryos			
	Control	lead embryos			Control	lead embryos			Control	lead embryos		
		.03mg/egg	.05mg/egg	.1mg/egg		.03mg/egg	.05mg/egg	.1mg/egg		.03mg/egg	.05mg/egg	.1mg/egg
mean	484.5	499.2	1282	1463	318.0	437	479	479.2	262	280	342.4	369
S.E.	49.9	50.8	94	102	7.2	23	51	51.2	23	19.9	43	48
t-test	—	not sign.	P<0.001	P<0.001	—	P<0.001	P<0.01	P<0.01	—	not sign.	P<0.05	P<0.05

Table 7
LDH activity of liver extract of control lead-treated chick embryos.

	9 days-old chick embryos (IU/100 gm liver)				12 days-old chick embryos (IU/100 gm liver)				18 days-old chick embryos (IU/100 gm liver)			
	Control	lead embryos			Control	lead embryos			Control	lead embryos		
		.03mg/egg	.05mg/egg	.1mg/egg		.03mg/egg	.05mg/egg	.1mg/egg		.03mg/egg	.05mg/egg	.1mg/egg
mean	280778	513146	396962	396962	99240	228505	197512	160444	141357	187484	174276	174256
S.E.	26808	43419	23715	23715	9848	16784	21945	99313	5044	6738	6454	6461
t-test	—	0.02	0.02	0.01	—	0.001	0.001	0.001	—	0.001	0.001	0.001

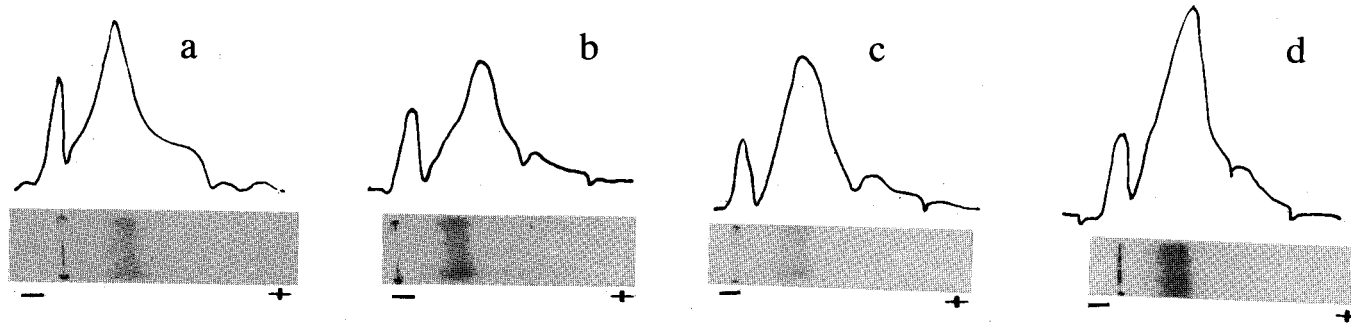


Fig. 4: Electrophoretic patterns and densitometric profiles of liver protein extract of 9-day chick embryos.

a. Control embryo. b., c. and d. Embryos treated with 0.03, 0.05 and 0.1 mg lead nitrate/egg respectively.

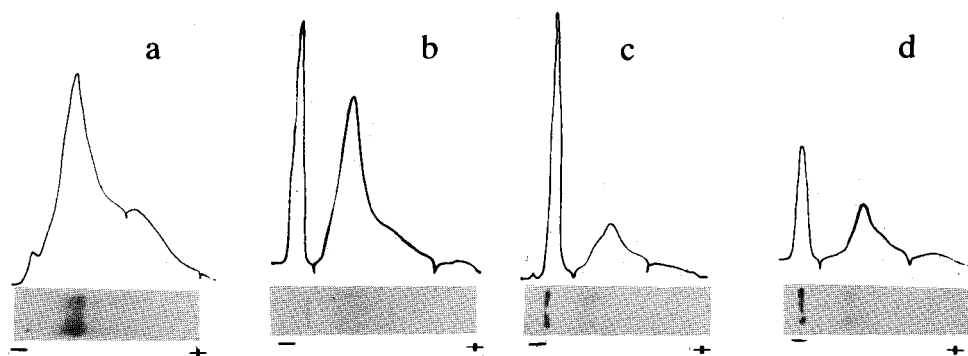


Fig. 5: Electrophoretic patterns and densitometric profiles of liver protein extract of 12-day chick embryos.

a. Control embryo. b., c. and d. Embryos treated with 0.03, 0.05 and 0.1 mg lead nitrate/egg respectively.

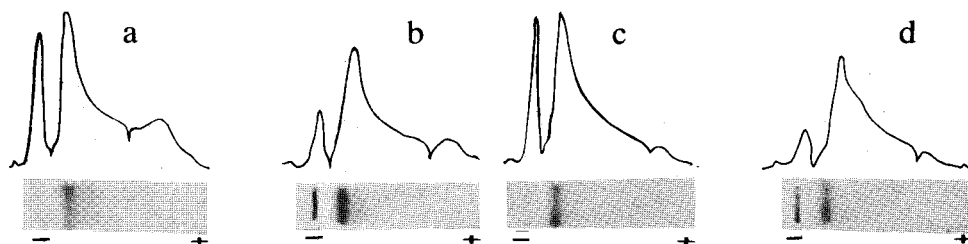


Fig. 6: Electrophoretic pattern and densitometric profiles of liver protein extract of 18-day chick embryos.

a. Control embryo. b., c. and d. Embryos treated with 0.03, 0.05 and 0.1 mg lead nitrate/egg respectively.

DISCUSSION

The liver of developing chick embryos consists of hepatocytes arranged in about 2-cell layered plates. These hepatocytes are arranged around the fine bile canaliculi. Between such plates there are numerous blood sinusoids lined by flattened endothelial cells (Purton, 1969 and Hodges, 1974).

The liver of lead-treated chick embryos showed disorganization of hepatic cords and evidence of extensive cell necrosis affecting both hepatocytes and endothelial cells. The destruction of the liver cells after lead treatment may be due to accumulation of lead in this organ as previously reported by Irwin and Karstad (1972) and Cibulka *et al.* (1985).

The total liver protein of chick embryos treated with 0.1mg lead nitrate/egg was significantly increased after 6 days of treatment. However, insignificant changes of the total liver proteins was noticed after 12 and 15 days of treatment with all doses used. Electrophoretically, the percentage of the 3rd protein fraction, which seems to be an albumin fraction, was markedly decreased compared with irregular changes in the percentages of protein in other bands. This is due to lead intoxication at different incubation periods as compared with controls. The disturbance in the protein fractions of lead-treated chick embryos may be due to inhibition of Na, K ATPase activity as found in nerve cells (Mailman, 1982), red blood cells (Waldron and Stofen, 1974 and Donaldson, 1982) and/or liver failure and reversible renal tubular necrosis (Waldron and Stofen, 1974; Berman, 1980; Clark *et al.*, 1981 and Hodgson and Guthrie, 1982). Moreover a similar disturbance in protein fractions was reported in brain extract of lead-treated chick embryos by El-Shabaka (198).

Normally, liver GOT, acid and alkaline phosphatase activities of control embryos are gradually increased as development proceeds from the 9th to 18th day of incubation. This findings are in accord with those of Romanoff (1988) and King and Liu (1974 a and b). However, the other enzymes are either gradually decreased by incubation (GPT) or decreased up to 12th day then increased again during 18th day of incubation (LDH). These results contradict with those of Solomon (1959, and 1960) and Romanoff (1988).

Lead-treatment caused an increase in the activity of all investigated liver enzymes at most of the developmental stages specially at the highest dose level (0.1 mg lead nitrate/egg). Moreover, the alkaline phosphatase and GOT activities of early embryonic stages appeared to be sensitive to the low doses of lead while those of late stages can tolerate this doses. The increased activities of the investigated enzymes due to the lead intoxication may be attributed to the destruction of lysosomes and endoplasmic reticulum of the hepatocytes. This result has been

reflected in the release of different proteins including enzymes. Further, the present data cannot refer to a direct effect of lead on the enzymes tested. However, previous investigations revealed that lead may inhibit oxidative phosphorylation or other steps of energy metabolism (Mailmon, 1982). Goyer (1971) reported that lead impairs the function of respiratory enzymes in the first part of the electron transport system and pyruvic acid metabolism.

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التأثير السام لنترات الرصاص على أجنة الدجاج

جمال إدريس أبو سنة و حمزة السيد الشبكة و نورة جابر آل حنزاب

تعتبر مركبات الرصاص واحدة من الملوثات البيئية الخطيرة التي تتراكم في جسم الإنسان يوماً بعد يوم . وقد استهدف البحث دراسة تأثير نترات الرصاص على أجنة الدجاج حيث استخدمت ثلاث جرعات هي ٣٠ ، ٥٠ و ١٠٠ ميكروجرام لكل بيضة حقنت بعد ثلاثة أيام من الحضانة .

وقد أوضحت الدراسة تأثير كبد الأجنة المعالجة بنترات الرصاص حيث ظهرت تداع في الخلايا الكبدية وكذلك الخلايا الطلائية المبطنة للجيوب الدموية كما أوضحت الدراسة أيضاً زيادة المحتوى البروتيني للكبد معنوياً فقط في الأجنة المعالجة بجرعة مقدارها ١٠٠ ميكروجرام لكل بيضة بعد مرور ٦ أيام من الحقن . أوضح الفصل الكهربائي لبروتينات الكبد إختلافات في نسب ونظم المكونات البروتينية نتيجة المعالجة بالجرعات المختلفة من نترات الرصاص مقارنة بمثيلاتها في المجموعات غير المعاملة .

لوحظ زيادة النشاط الإنزيمي للفوسفاتيز الحمضي والفوسفاتيز القاعدي وإنزيمي الجلوتامات أكسالو أسيتات ترانس أمينيز وجلوتامات بيرونات ترانس أمينيز والاكاتات دي هيدروجينيز في كبد أجنة الدجاج في معظم المراحل الجنينية التي درست وبدرجات متفاوتة تبعاً للجرعة المستخدمة من نترات الرصاص .