NEUROPATHOLOGIC CHANGES AND BIOCHEMICAL INDICES IN LEAD-EXPOSED QUAIL EMBRYO.

By

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Abstract: *This study was conducted to determine the influence of lead acetate (Pb) injection into fertile quail eggs on embryonic growth, some hematological and biochemical indices, histopathological changes and hatchability. Seven hundred eggs were injected on the 6th day (d) of incubation with different levels of Pb: 0.01,0.02,0.03 and 0.04 ppm. The results showed that embryo weight and hatchability were decreased, while mortality rate was increased in the treated groups. In most embryos visible signs of Pb injuries were noted. Pb at the level of 0.03 and 0.04 ppm increased (P≤ 0.01) serum glutamate pyruvate transaminase (GPT) in newly hatched chicks compared with the control group .*

Clumps of blood cells and areas of necrosis were observed in the spinal cord and degeneration and necrosis in cerebral hemisphere in embryos injected with Pb as compared to control. It was evident that Japanese quail eggs that were injected with Pb on the 6th day of incubation exhibited marked malformation in the developing embryos associated with low hatchability.

INTRODUCTION

Lead (Pb) is a heavy metal that is spread almost every where in nature. Presently, the National Research Council (NRC, 1994) considered that most of the cases of accidental poisoning in domestic animals world–wide is attributed to Pb. The susceptibility to the effects of Pb can vary considerably from species to species. **Edens and Garlich (1983)** reported that Japanese quail was more susceptible to reproductive properties of Pb than chicken hen. Additionally, the bird is apparently different from some mammals in the manner of handling Pb. For example in rats, Pb causes mobilization of bone calcium and consequently, increased blood calcium but in birds blood calcium decreases after Pb treatment.

Lead toxical effect on living systems is through inhibition of the activity of almost all enzymatic mechanisms of heme biosynthesis process

such as delta aminolevulinic acid dehydrase (ALAD) in Japanese quail **(Stone *et al.*, 1977)**. the results from different studies related to the changes of blood transaminase activity in the case of Pb intoxication are inconsistent. Some authors found an increase in the level of this class of enzymes **(Rozhaja *et al.*, 1983)**, while the other did not report any changes in their level **(Waldron, 1964)**.

In mice, the impact of teratogenic effect of Pb uptake is usually prominent during the early embryonic stages of developing organisms that include structural malformation, growth retardation and perhaps lead to embryonic death **(Hubermont *et al.*, 1976)**. This teratogenic effect depends on several important factors such as dose level, timing of exposure and genetic variations in susceptibility between individuals **(Stine and Brown, 1996)**. There is little published information on the effects of Pb intoxication in avian embryo. **De Gennaro (1978)** demonstrated that 0.5 to 1.5 ppm Pb significantly decrease growth in White Leghorn chick embryo at 10 d of incubation. The Pb- treated embryos failed to hatch and the embryos died before the 21st d of incubation. Recent study by **Vodela *et al.*, (1997)** reported a significant increase (68.84 %) in embryonic mortality of broiler breeders after drinking water containing (7.0 ppm Pb) compared with (16.16 %) for control bird.

The present study therefore was conducted on Japanese quail:

1. To determine the effect of Pb injection into fertile eggs on embryonic growth and hatchability.
2. To examine the morphological changes of the embryos following lead injection.
3. To evaluate, histologically, the central nervous system (CNS) of the quail embryo.

MATERIALS AND METHODS

1. Materials:

Seven hundred Japanese quail eggs (*Coturnix coturnix japonica*) were obtained from 120 quail females 12 wk old, each one was individually caged with a male. Birds were maintained at 16: 8 light dark cycle throughout the experiment and fed *ad libitum* on a breeder ration containing 20.06 % crude protein, 2920 K cal M. E./Kg. diet. Eggs were gathered daily for a period of one wk and stored in plastic plates in refrigerator at 10 C° prior to setting. Eggs were placed in a forced draft incubator operated at a temperature of 99.5 ° F and 60 % RH and were manually turned every 6 h.

2. Treatments:

On the 6 th d of incubation, eggs were taken out of the incubator and were randomly divided into seven groups of 100 eggs each, 3 controls and 4 experimental treatments. Each egg was candled and unfertilized eggs were discarded, and only fertile eggs were utilized. The air cell position was identified by a marker pencil. Eggs of the first group were uninjected and served as untreated control (C1), group 2 (Sham) was pierced with the injected needle, no solution was injected (C2), while eggs of group 3 were injected with 0.04 ml distilled water/egg (C3). For the fourth, fifth, sixth and seventh groups, eggs were injected with Pb acetate solution at a dose of 0.01, 0.02, 0.03 and 0.04 ppm Pb /0.04 ml distilled water, respectively. The injection was into the air cell by using insulin syringe. The injection site was Immediately sealed with non-toxic glue stick. All eggs were returned to the incubator. Ten eggs were taken at random from each of the seven groups for morphological examination at 2 d after injection and at 2 d intervals afterwards and photographed. On the 14 th day of incubation four embryos from each of the four experimental treatments and the three control groups were weighed using digital electronic and their weights were recorded to the nearest gram and as a percentage of egg weight.

3. Histological technique for light microscope:

At autopsy, embryos were examined for gross lesions, and tissue samples from spinal cord and whole brain were collected and fixed in 10% natural buffered formalin, embedded in paraffin, sectioned at 4μm and stained with hematoxylin and eosin according to **Carleton *et al.*, (1980)**. Cross sections were examined using light microscope and photographed.

4. Blood samples preparation:

Hatched quail chicks from each treatment was decapitated and blood samples were obtained and pooled. The blood samples were allowed to clot at room temperature for 20 min. The coagulated blood was centrifuged at the speed of 2500- rpm for 15 min. Sera samples were decanted and frozen at a temperature of $-20C^0$ until the biochemical analyses were carried out.

5. Biochemical assay:

5.1. Serum total protein (STP):

Serum total protein (g/dl) determination was based on Biuret method using enzymatic methods with commercial kits. Spectrophotometer at wave length of 540 nm was used and STP (g/dl) was calculated as:

$$\text{STP (g/dl)} = \frac{A_{\text{sample}}}{A_{\text{standred}}} \times 10$$

5.2. Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT):

As mentioned before, SGPT and SGOT (μ/L) determination were performed by Spectrophotometer at wave length of 540 nm and the activity of SGPT and SGOT were obtained by linear relationship using the absorbance reading.

6. Statistical analyses:

Statistical analysis was performed according to one-way analysis of variance of the traits studied using SAS package, SAS/sat user's guide (1994). In order to determine significant differences between all possible mean comparisons, Duncan's New Multiple Range Test (1955) was applied to the data. Statements of significance are based on $p< 0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

a. Body weight:

The average weights (expressed as a percentage of egg weight) of Japanese quail embryos injected on the 6 th d of incubation with different levels of Pb are presented in Table 1. After 2 d of injection, 0.01 and 0.02 ppm Pb doses significantly decreased average embryo relative weight than control, while 0.03 ppm Pb caused a significant increase in embryo relative weights. However such results could be disputed due to the abnormal large head formation, which contributed to such unexpected increase in weight. At 4 d post injection, these differences between toxicated and control disappeared, However, after 6 and 8 d of injection, in other words at 12 – 14 d-old, quail embryos treated with Pb at the levels of 0.01, 0.02, 0.03 and 0.04 ppm weighed significantly ($P<0.01$) less than controls, that may be due to the Pb toxicity in CNS and other organs which caused retardation of embryonic development in treated embryos as compared to those untreated

control. These results are in agreement with the findings of **De Gennaro (1978)** who found that in White Leghorn chick embryos that were injected with 0.5 to 1.5 ppm Pb nitrate at 10 d of incubation, weighed less than the control weight at the later stages of incubation and the most toxicity was ascribed to its action on proteins, enzyme activity and other sites.

b. Morphological observation:

Japanese quail embryos taken out of incubated eggs that have been injected with different doses of Pb at the 6 th d of incubation were examined visibly at 2 d after injection and at 2 d intervals afterwards and photographed (Figures 1, 2, 3 and 4).

It is clearly noted that the head and some parts of the body of the Pb intoxicated embryos were dark red in color than the controls (Figure 1 and 2), which may be due to accumulation of blood cells throughout the brain and spinal cord tissues as a result of hemolysis through damage to the RBC cell membrane. These results are in agreement with the findings of **De Gennaro (1978)**.

Embryos that were treated with Pb at 0.02 and 0.03 ppm were poorly feathered than controls and this effect was noticed at 6 d after injection (Figure 3). The deleterious effect of the injected Pb was also noted in the presence of curled toes on one or both legs and it was most conspicuous at 8 d after injection (Figure 4). **Thomas and Thomas (1974)** in ultrastructural studies believe that the first effects of lead are seen in the derangement of organelles within the capillary endothelium. The consequence of such damage is then increased permeability and eventually edema of the nervous tissue. Also, the radioactive lead became localized in the walls of blood vessels in the cerebellum of neonatal rats **(Thomas *et al.* 1973)**.

c. Histological changes:

Histopathological examination of the brain of quail's embryo intoxicated with Pb showed that the cells of cerebral hemisphere appeared to be necrotic probably due to decreasing blood supply or flow and destruction of the cells (Figure 5).

Further observations revealed deleterious effects of Pb in the developing spinal cord, which was noticeably different from that of the controls. A section from lumbosacral region of 14 d lead quail embryos shows numerous accumulations of blood cells throughout the tissue (Figure 6).

Remarkable degeneration was also observed in white matter of the Spinal cord of leaded embryos (Figure 7). On the other hand, there were no detectable effects of Pb poisoning such as lesions in liver of 14 d-old leaded

embryos compared to controls. **Ignacio *et al.*, (1996)** reported that lesions in children's brains occur in both gray and white matters, particularly in cerebral molecular layer, and consist of necrotic vessels, hemorrhages, foci of necrosis and edema. The nervous system is a vulnerable target for toxicants due to critical voltages which must be maintained in cells and the all or none responses when voltages reach threshold levels. In addition, the role of the nervous system in directing many critical physiological operations means that any damage may lead to significant functional consequences (**Stine and Brown, 1996**). In terms of Pb effects on the central nervous system, it is probably damaging the neuron.

d. Hatchability:

The data in Figure (8) show a significant negative effect of Pb level and hatchability percent. Eggs injected with 0.01 and 0.02 ppm Pb exhibited significantly ($P<0.01$) lower hatchability with proportionally higher embryonic mortality than controls. Whereas, increasing Pb dose to either 0.03 or 0.04 ppm caused sharp decrease in hat chability by approximately 90 percent compared to the hatchability of control eggs (Figure 8).

The low hatchability of Pb-treated quail embryos compared with controls in the present study indicates that Pb poisoning was associated with the damage which occurred in cerebral hemisphere and spinal cord as obviously shown in the histopathological examination (Figure 5, 6 and 7) and consequently death occured. The failure of leaded chick embryos to hatch is most likely due to injuries of brain and spinal cord resulting in the loss of behavioral reflexes and coordinated movements necessary for that complexed process **(Oppenheim, 1973).**

e. Serum protein and enzymes:

Effect of Pb injection into fertile eggs on serum total protein (STP) and enzymes of Japanese quail chicks are presented in Table 2. STP and SGOT concentrations of one day-old quail chicks that were hatched from eggs which injected with 0.01 to 0.04 ppm Pb at 6 th d of incubation were similar to those of chicks which hatched from controls. However, marked elevation in SGPT level of chicks which hatched from intoxicated eggs with Pb was reported as level of Pb administrated is increased. The results of this study indicate that the level of only SGPT was markedly affected by the Pb toxicity. However, slight variation occurred with respect to STP and SGOT. In the present study it could be considered that the level of Pb at 0.02 ppm is the threshold above which marked elevation of SGPT occurred (Table 2). It is known that GPT is a non-functional plasma enzyme. Its normal level in plasma is lower than in tissue, but since after tissue destruction (which in

the present study was due to Pb) GPT is liberated from tissue to plasma and its level is increased **(Murray *et al.* 2000)**. Moreover GPT is found in most tissues, but relative values in liver is high. Therefore, in the present study, Pb that has been injected into quail fertile eggs caused an increase in SGPT level of hatched chicks than control, which indicates liver diseases, as liver damage or cirrhosis.

In conclusion it is evident that the results of the present study clearly indicated that injecting Pub to Japanese quail embryos at the 6 th d. of incubation caused deleterious effects on their normal development, increased the injurious effects and lowered the hatchability.

Table 1. - Effect of Pb acetate injected at the 6 th d of incubation on of embryo weights* (means ± S. E) of Japanese quail.

	Days post injection			
Treatment (ppm)	2	4	6	8
Control	6.15 ^{b} ±0.15	12.52 ±0.29	30.30 ^{a} ±0.74	48.84 ^{a} ±0.57
Sham	6.31 ^{b} ±0.07	12.73 ±0.20	29.31 ^{a} ±0.79	48.98 ^{a} ±1.57
Distilled water	6.39 ^{b} ±0.09	11.83 ±0.44	28.66 ^{a} ±0.34	47.38 ^{a} ±0.59
0.01	5.15 ^{d} ±0.15	12.15 ±0.57	26.43 ^{b} ±0.32	39.56 ^{b} ±1.21
0.02	5.64 ^{c} ±0.12	12.33 ±0.53	23.50 ^{c} ±0.85	40.66 ^{b} ±2.69
0.03	6.95 ^{a} ±0.26	12.95 ±0.26	24.46 ^{bc} ±0.44	39.92 ^{b} ±1.51
0.04	6.47 ^{ab} ±0.19	11.75 ±0.38	23.17 ^{c} ±1.15	37.79 ^{b} ±1.53
Probability	**	NS	**	**

Means with the same letter within column are not significantly different.
* Embryo weight (g) as a percentage of egg weight.
NS= Non-significant.
** $P \leq 0.01$.

Table 2 .- Means± S.E of serum total protein g/dL (STP), GOT (μ/L) and GPT (μ/L) of Japanese quail chicks hatched from lead treated eggs at the 6 th d of incubation.

	Traits		
Treatment (ppm)	STP	SGOT	SGPT
Control	1.86	89.00	10.16 ^{a}
	±0.03	±0.57	±0.16
Sham	1.89	85.75	10.98 ^{a}
	±0.09	±3.59	±1.12
Distilled water	1.74	84.75	10.25 ^{a}
	±0.08	±4.58	±0.62
0.01	1.97	93.00	12.50 ^{ab}
	±0.09	±2.22	±1.50
0.02	1.85	89.75	13.33 ^{ab}
	±0.03	±0.25	±0.55
0.03	1.87	88.66	15.33 ^{b}
	±0.05	±0.33	±1.20
0.04	1.79	93.00	14.50 ^{b}
	±0.05	±5.00	±1.50
Probability	NS	NS	**

Means with the same letter within column are not significantly different.
NS= Non-significant.
** $P \leq 0.01$

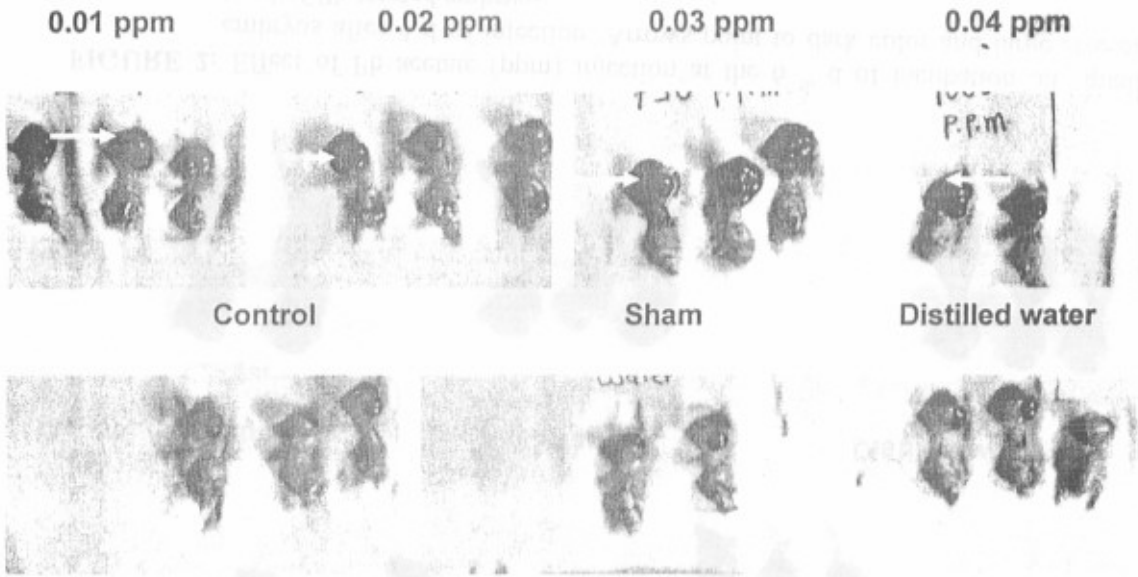

FIGURE 1: Effect of Pb acetate (ppm) injection at the 6 th d of incubation on quail embryos after 2 d of injection. Arrows point to dark color and large size of head of Pb-treated embryos.

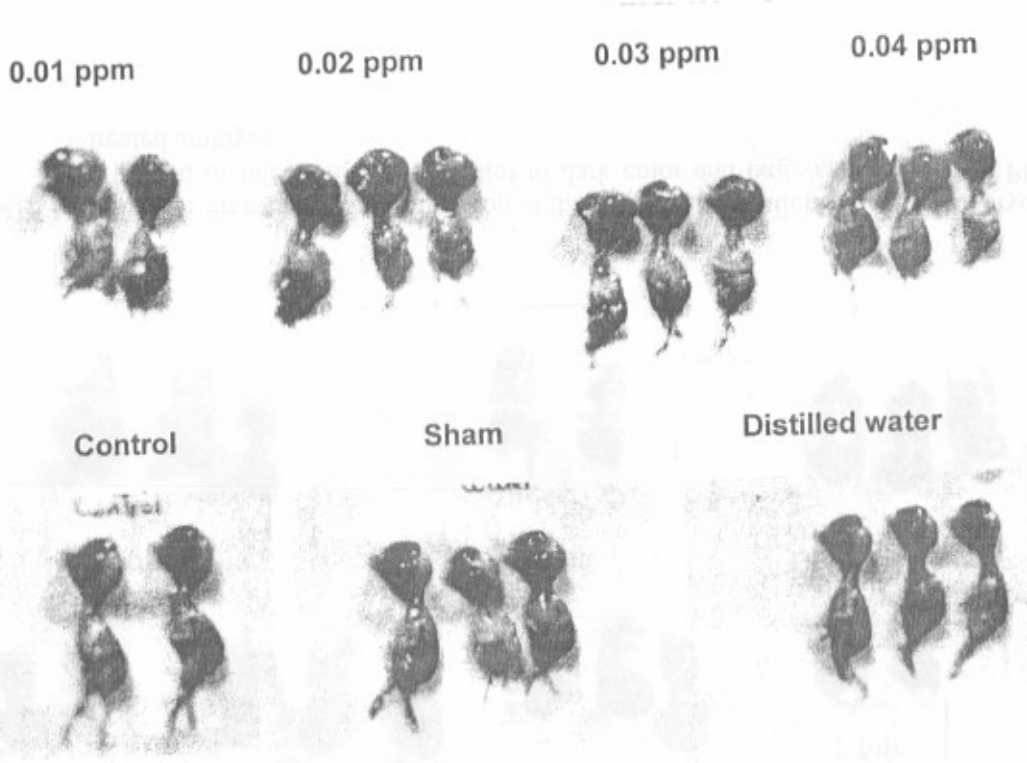

FIGURE 2: Effect of Pb acetate (ppm) injection at the 6 th d of incubation on quail embryos after 4 d of injection. Arrows point to dark color and large size of head of Pb-treated embryos.

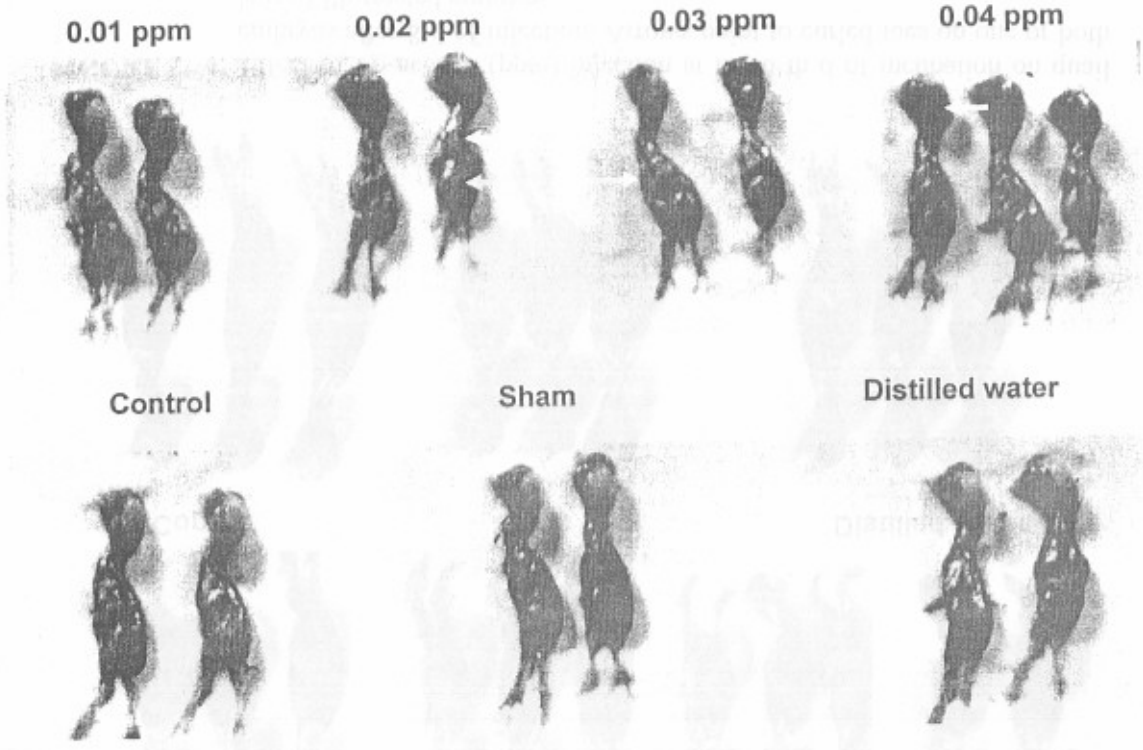

FIGURE 3: Effect of Pb acetate (ppm) injection at the 6 th d of incubation on quail embryos after 6 d of injection. Arrows point to dark color of head and poor feathered of Pb-treated embryos.

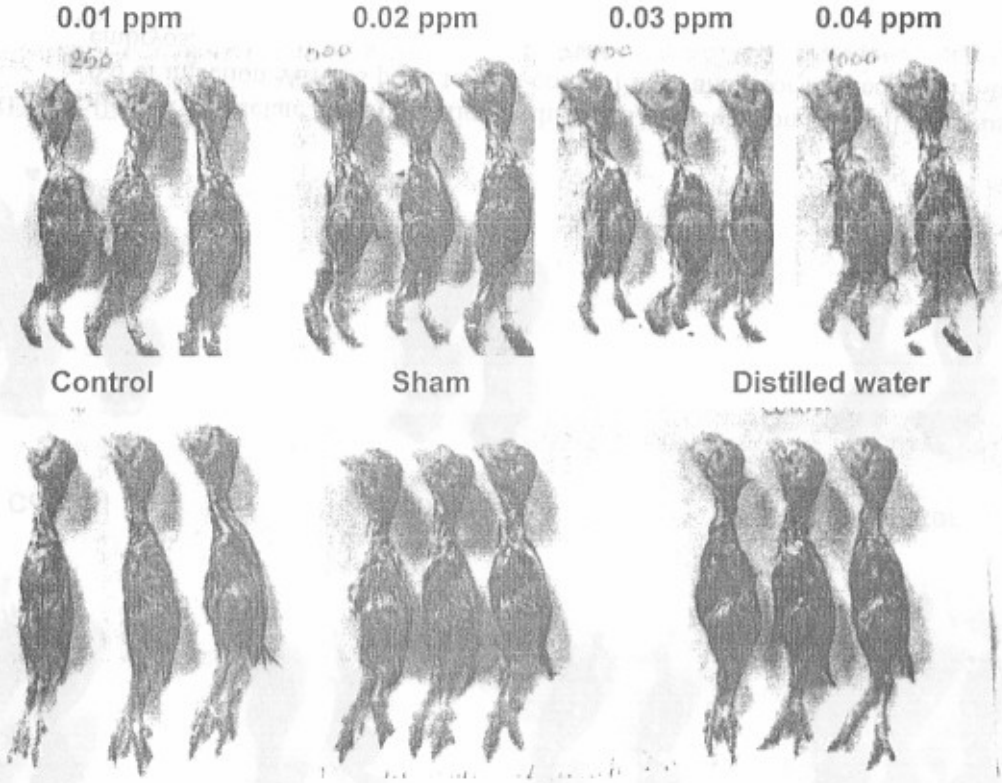

FIGURE 4: Effect of Pb acetate (ppm) injection at the 6 th d of incubation on quail embryos after 8 d of injection. Arrows point to curled toes on one or both legs of Pb-treated embryos.

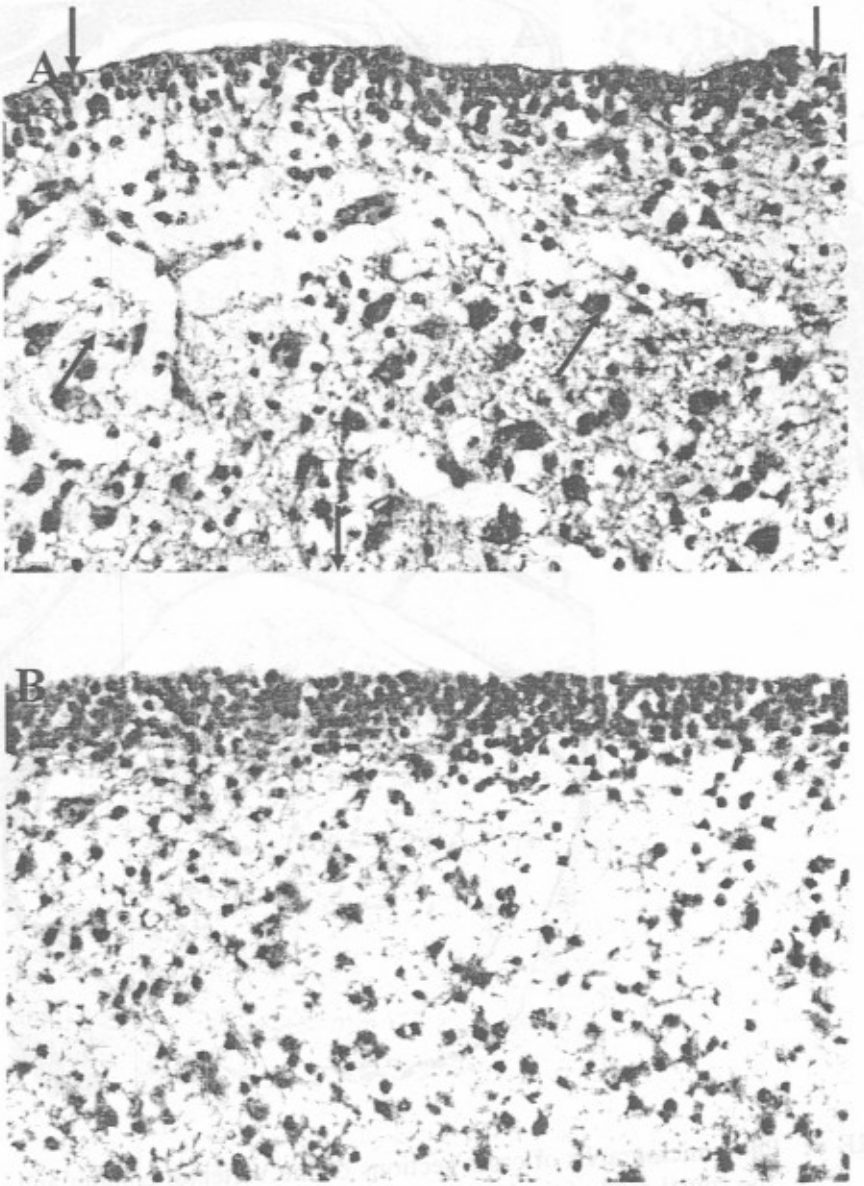

FIGURE 5: Photomicrographs of cross sections of the cerebral hemisphere of 14 d leaded embryo (A) and control (B). Arrows point to degeneration and necrosis in gray matter (Pia matter and molecular layer). H & E. X 400.

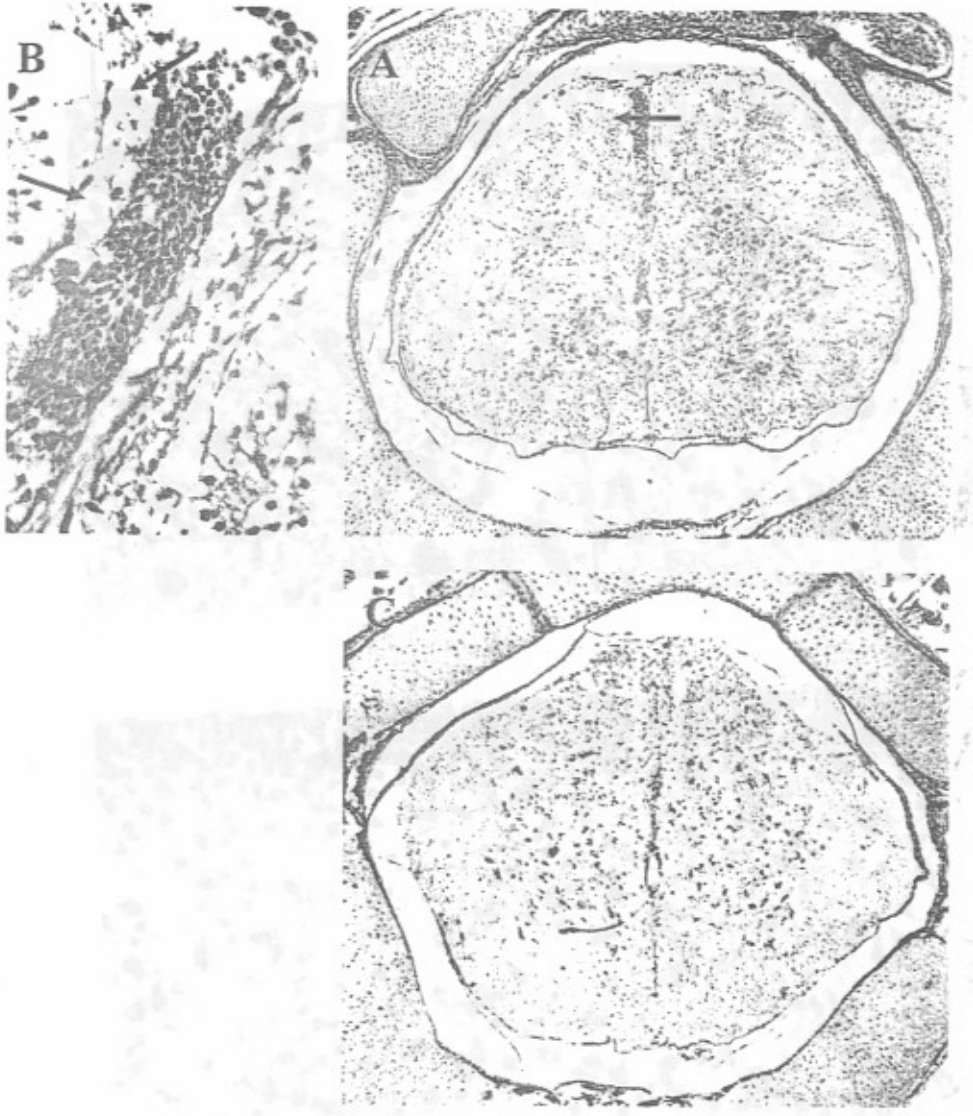

FIGURE 6: Photomicrographs of cross sections of the vertebral column and the spinal cord of the lambosacral region of 14 d leaded embryo (A) and control (C). Arrows point to large clump of blood cells (A and B). H & E. X 40 (A and C). X 400 (B).

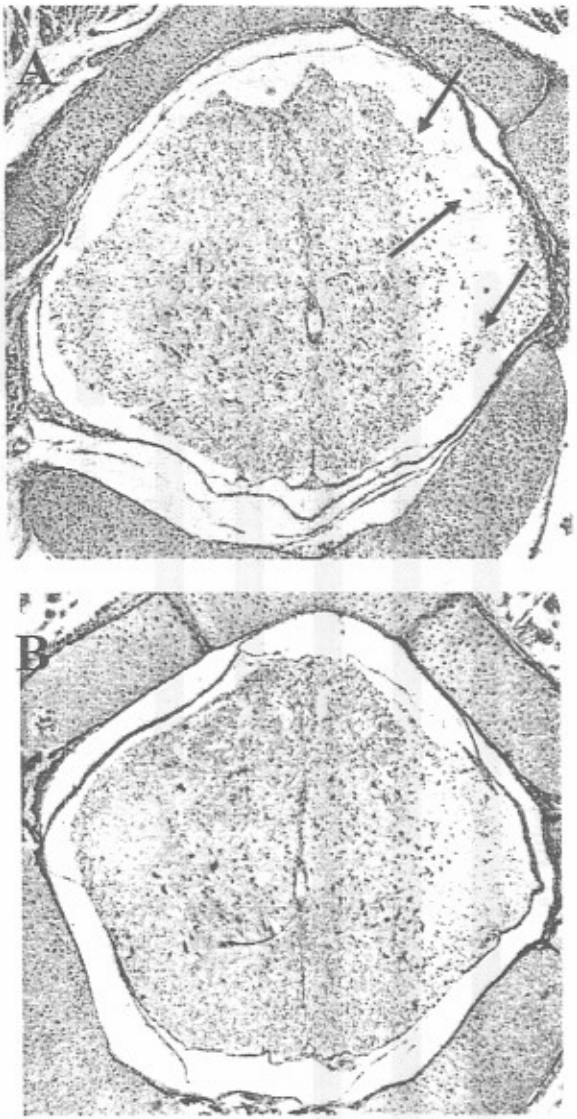

FIGURE 7 : Photomicrographs of cross sections of the vertebral column and the spinal cord of the lambosacral region of 14 d leaded embryo (A) and control (B). Arrows point to degeneration and necrosis in white matter of the spinal cord. H & E. X 40.

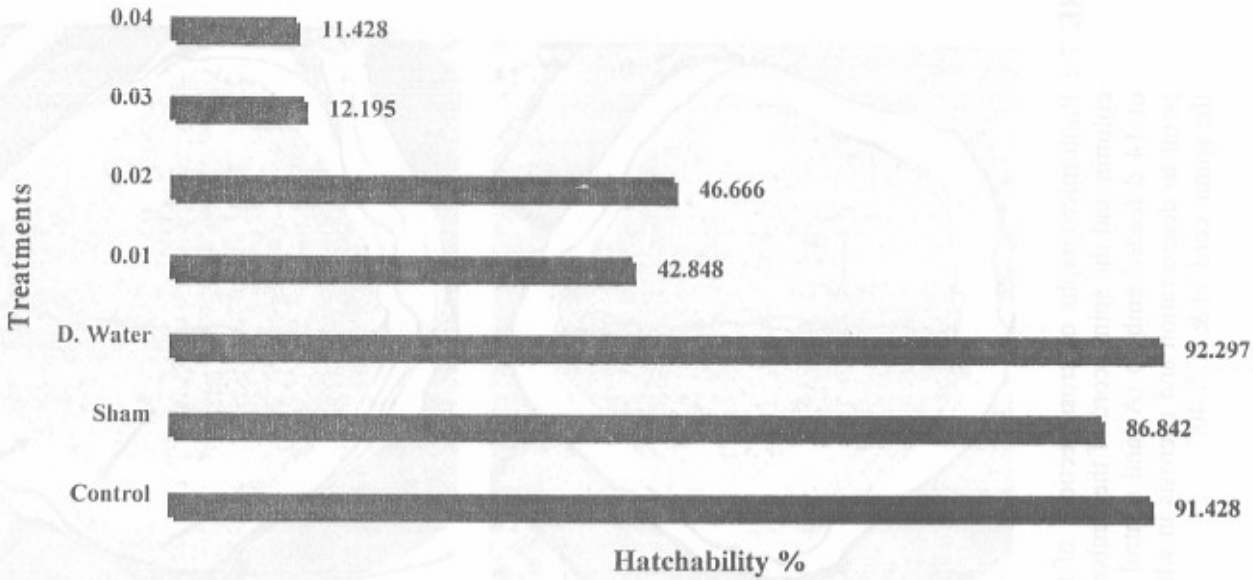

FIGURE 8: Effect of Pb acetate (ppm) injected at the 6 th d of incubation on hatchability percent of Japanese quail fertile eggs.

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الملخص العربي

التغيرات العصبية الباثولوجية والمؤشرات الكيميائية الحيوية في أجنة السمان المعرض للتلوث بالرصاص

فيصل بيومي عبد السلام بدري ، يسري محمد الحمصاني ، نعمة الله جمال الدين علي، مي فؤاد علي

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أجريت هذه الدراسة في معمل الفسيولوجي قسم إنتاج الدواجن ــ كلية الزراعة ــ جامعة عين شمس بهدف دراسة تأثير الحقن بالرصاص Lead acetate (Pb) على نمو الأجنة ونسبة الفقس والتغييرات المورفولوجية والبيوكيماوية والهستولوباثولوجية الناتجة عن هذا التلوث بالرصاص.

تم استخدام عدد ٧٠٠ بيضة سمان ياباني قسمت عشوائياً إلى ٧ مجاميع منهم ٣ مجاميع كنترول (مجموعة غير معاملة ــ مجموعة Sham ومجموعة تم حقنها بالغرفة الهوائية بالماء المقطر ٠,٠٤مل/بيضة) وباقي المجاميع حقنت بالرصاص عند المستويات ٠,٠١ ، ٠,٠٢، ٠,٠٣، ٠,٠٤ جزء في المليون .

عند اليوم الثاني، الرابع، السادس، الثامن من الحقن تم أخذ عدد ١٠ بيضات من كل مجموعة، وزنت ثم فتحت وتم وزن الجنين وفحص أي تغيرات في الشكل الظاهري للجنين. عند اليوم الثامن من الحقن تم أخذ عينات طازجة من المخ ، الحبل الشوكي و الكبد لفحص التغييرات والهستولوباثولوجية.

عند الفقس تم أخذ عينات دم من الكتاكيت الفاقسة لكل معاملة وتقدير مستوى البروتين الكلي، GOT، GPT في السيرم.

وكانت أهم النتائج المتحصل عليها كما يلي :

١. الأجنة التي تم حقنها عند اليوم السادس بالرصاص كانت أقل معنوياً فى متوسط وزن الجسم مقارنة بالمجموعات الكنترول بعد ٦ و٨ أيام من الحقن .

٢. انخفاض نسبة الفقس فى الأجنة المحقونة بالرصاص انخفاض معنوي عن الكنترول.

٣. زيادة مستوى GPT فى سيرم الأجنة المحقونة بالمستويات العالية من الرصـــاص (٠,٠٣ و٠,٠٤ جزء فى المليون) معنوياً عن الكنترول بينما لا يتأثر مستوى البروتين الكلي أو GOT

٤. حدوث تشوهات فى الأجنة المحقونة بالرصاص خاصة فى منطقة الرأس التي أخذت اللـــون الأحمر الداكن نتيجة وجود تجمعات دموية مع زيادة حجم الرأس بالنسبة للجسم وكذلك حـــدوث التواء فى أصابع الأرجل.

٥. وجود تجمعات دموية وموت موضعى للخلايا (تليف) فى أنسجة الحبل الشوكى وأنسجة المخ فى الأجنة المعاملة بالرصاص مقارنة بالكنترول.