

## BIOCHEMICAL AND HAEMATOLOGICAL STUDIES ON CADMIUM CHLORIDE ADMINISTRATION IN RAABITS

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### ABSTRACT

Cadmium chloride (0.5 mg/kg) was given intraperitoneally (i.p.) into mature male rabbits every other day for 5 weeks. The results showed a significant increase in serum total proteins. An increase in  $\beta$  and  $\gamma$ -globulins ( $P < 0.001$ ) was observed. Serum ALT and AST activities were also increased in rabbits treated with cadmium and vaccinated with Clostridia antisera when compared with the vaccinated control group. Whereas,  $\alpha$  globulins and serum urea nitrogen (SUN) were significantly decreased ( $P < 0.001$ ). Blood gases analysis showed significant decreases ( $P < 0.05$ ) in  $PCO_2$ ,  $HCO_3$  and  $TCO_2$  in the vaccinated treated group compared with the vaccinated control group. A marked increase ( $P < 0.001$ ) was also recorded in eosinophils and monocytes counts of the vaccinated treated group compared with the vaccinated control group.

### INTRODUCTION

Cadmium is a toxic metal, were it is wide-spread environmental pollutant. Its industrial use was little until about 50 years ago. Commercial sludge may contain up to 1500 mg of cadmium per kilogram of dry material<sup>(1)</sup>. Meat, fish and fruit contain 1 to 50  $\mu\text{g}/\text{kg}$ , grains contain 10 to 150  $\mu\text{g}/\text{kg}$ . and the greatest concentrations are in liver and kidney of animals. Shell fish accumulate cadmium from the water and then bind to cadmium-binding peptides<sup>(2)</sup>.

Respiratory absorption of cadmium is about 15 to 30%. Workplace exposure to cadmium is particularly hazardous where there are cadmium fumes or airborne cadmium. Most airborne cadmium is respirable<sup>(3)</sup>. A major nonoccupational source of respirable cadmium is cigarettes. One cigarette contains 1-2  $\mu\text{g}$  cadmium, and 10% of the cadmium in a cigarette is inhaled<sup>(1)</sup>. Smoking one or more packettes of cigarettes a day may double the body burden of cadmium. The principal long-term effects of low-level

exposure to cadmium are chronic obstructive pulmonary disease, emphysema and chronic renal tubular disease. There may also be effects on the cardiovascular and skeletal systems<sup>(4)</sup>.

The effects of cadmium on proximal renal tubular function are manifested by increased cadmium in urine, proteinuria, aminaciduria, glucoseuria and decreased renal tubular reabsorption of phosphate<sup>(5)</sup>. Morphological changes are non-specific and consist of tubular cell degeneration in the initial stages, progressing to an interstitial inflammatory reaction and fibrosis. The proteinuria is principally tubular, consisting of low-molecular-weight proteins. The predominant protein is a  $\beta_2$  microglobulin. A number of other low-molecular-weight proteins have been identified in the urine of workers with excessive cadmium exposure such as retinol binding protein, lysozyme, ribonuclease and immunoglobulin light chains<sup>(6)</sup>.

High-molecular-weight proteins have also been observed in the urine, suggest-



ing a glomerular effect of cadmium as well<sup>(5)</sup>. In this context, the present investigation was undertaken to study the possible deleterious effects that could occur in blood and serum constituents of animals exposed to cadmium chloride.

## MATERIAL AND METHODS

Mature male rabbits weighing 1.5-2 kg were divided into 3 equal groups each group of six rabbits. They were acclimated for one week and allowed free access to standard pelleted food and drinking water. One group was given daily i.p. injections of distilled water and kept as control. The other two groups were challenged with Clostridia antisera (0.2 ml/rabbit) given subcutaneously (s.c.) at once as a stress factor and left for one week. One of the groups was i.p. injected every other day with cadmium chloride (BDH, England) in a dose of 0.5 mg/kg for 5 weeks. The remaining group was served as a control for the test group receiving no treatment. At the end of the experiment, all animals were sacrificed by decapitation and blood samples were immediately collected. EDTA (disodium salt) was used as anticoagulant. Blood smears were freshly prepared, air dried, fixed in alcohol and stained with Leishman's stain.

Erythrocyte (RBC) and leucocyte (WBC) counts were determined using the double improved Neubauer chamber<sup>(7)</sup>. Haemoglobin (Hb%) was determined using the acid haematin method<sup>(8)</sup>. Packed cell volume (PCV) was estimated in double capillary tube preparations using a microhaematocrit centrifuge.

Erythrocyte indices were calculated from the values of RBCs, PCV and Hb<sup>(9)</sup>.

Serum biochemical variables were estimated using commercial kits supplied by bioMerieux (France). These included: alanine aminotransferase (ALT) formerly known as GPT and aspartate aminotransferase (AST) formerly known as GOT<sup>(10)</sup>; serum total proteins<sup>(11)</sup>, serum

urea nitrogen (SUN)<sup>(12)</sup>; cholesterol<sup>(13)</sup>; triglycerides<sup>(14)</sup>; bilirubin<sup>(15)</sup>; uric acid<sup>(16)</sup>; creatinine<sup>(17)</sup>; glucose<sup>(18)</sup>, calcium<sup>(19)</sup> and inorganic phosphorus<sup>(14)</sup>.

Serum protein fractions were determined by electrophoresis, using 5  $\mu$ l samples from control and treated groups. These were applied to agarose gel slabs (5401-001 hydragel protein, LKB-Sebia). Electrophoresis was run for 20 minutes using LKB equipment and methods (Sebia 91130, Issy les Moulineaux, France).

Following staining with amidoblack and destaining in 5% acetic acid, the electrophoretograms were scanned for the percentages of albumin,  $\alpha$ ,  $\beta$  and  $\gamma$  globulin fractions and the A/G ratio in LKB-5300 "Preference" densitometer programmed for protein analysis.

Blood gas analysis was done on freshly drawn venous blood collected in heparinized syringes promptly sealed hermetically and injected directly into the blood gas analysis system (2000 Eschweiler, West Germany).

### Statistical analysis:

Data were statistically analysed using paired Student "t" test<sup>(20)</sup>.

## RESULTS

The obtained results revealed that the i.p. administration of cadmium chloride every other day for 5 weeks into mature male rabbits in a dose of 0.5 mg/kg produced the following effects:

Serum total proteins,  $\beta$  and  $\gamma$  globulins, serum ALT and AST were significantly increased ( $P < 0.05$ -  $P < 0.001$ ) in the group treated with cadmium chloride and vaccinated when compared with the control vaccinated and non vaccinated groups (Table 1). Alpha-globulins and serum urea nitrogen (SUN) showed a significant decrease ( $P < 0.001$  &  $P < 0.05$ , respectively) in the same groups. However,  $\beta$ -globulins in the vaccinated control group revealed a significant decrease ( $P < 0.001$ ) when



compared with the nonvaccinated control rabbits.

An insignificant increase in serum albumin, A/G ratio, cholesterol, triglycerides, bilirubin, glucose, inorganic phosphorus and creatinine was recorded. An insignificant decrease in the levels of uric acid and  $\text{Ca}^{2+}$  was also recorded in the vaccinated treated group when compared with the vaccinated non treated and nonvaccinated groups. From the blood picture, blood gas analysis, obviously showed a significant ( $P < 0.001$ ) increase (Table 2) in the eosinophils count which was noted in the vaccinated and treated group when compared with the vaccinated nontreated and nonvaccinated groups. Monocytes showed a marked increase ( $P < 0.001$ ) in the vaccinated treated group when compared with the vaccinated nontreated group.

The  $\text{TCO}_2$ ,  $\text{HCO}_3$  and  $\text{PCO}_2$  showed a significant decrease ( $P < 0.05$ ) when compared with the control vaccinated and nonvaccinated rabbits. The  $\text{PCO}_2$  in the vaccinated nontreated group revealed a significant increase ( $P < 0.05$ ) when compared with the control nonvaccinated group. The  $\text{HCO}_3$  of the vaccinated nontreated group exhibited a moderate increase ( $P < 0.05$ ) when compared with the control nonvaccinated group.

A slight increase in the values of RBCs count, PCV%, MCV, neutrophils count,  $\text{PO}_2$ , BE,  $\text{HCO}_3$  s,  $\text{O}_2$ -SAT% and  $\text{O}_2$ -CT% was recorded in the vaccinated treated group when compared with the other two control groups. An insignificant decrease in WBCs count, Hb%, MCH, MCHC, lymphocytes and pH, was observed in the vaccinated treated group when compared with the other two control groups.

## DISCUSSION

The effects of i.p. administration of cadmium chloride every other day for 5 weeks into mature male rabbits in a dose of 0.5 mg/kg (b.wt.) on the biochemical constituents of serum as well as the

blood picture and blood gas analysis were reported.

The increased serum total proteins deduced by elevated serum albumin may be attributed to dehydration resulting in haemoconcentration<sup>(21)</sup> evidenced in this work by the insignificant increase in RBCs count, PCV% and MCV. Whereas, the decreased  $\alpha$ -globulins seems to be due to acute liver disease evidenced in this study by the elevated serum ALT and AST<sup>(22 & 23)</sup>. It was reported that serum GOT (known now as AST) activity was found to be elevated in early hepatic necrosis in the horse, cow, pig and cat. Moreover, a significant elevation in the plasma GOT have been reported in fatty degeneration of the dog liver<sup>(24)</sup>.

The increase in globulin fractions especially the  $\beta$  and  $\gamma$ -globulins indicates that a state of immunopotentiality has possibly occurred as a result of treatment. The observed hyperglobulinaemia which was accompanied by increased number of eosinophils appears to indicate a state of immune response, possibly a reaction towards tissue damage seen in the liver<sup>(21)</sup>. The increased  $\gamma$ -globulins could partly be accounted for by an increase in IgE due to the increased number of eosinophils, indicating an allergic manifestation<sup>(21)</sup>. Moreover, chronic hepatitis, chronic inflammatory disease and glomerulonephritis are attributing factors.

The decreased serum urea (SUN) seems to be due to liver insufficiency since the liver is the main site of urea synthesis.

It is known that cadmium produces alterations in host resistance and immune function in rodents. Cadmium has been found to alter host susceptibility to bacterial endotoxins<sup>(26)</sup>. However, some cadmium-exposed mice were found to be more resistant to tumor and EMC virus challenges<sup>(1)</sup>.

Table (1): Serum biochemical variables in rabbits treated with cadmium chloride (i.p., 0.5 mg/kg. b.wt.) every other day for 5 weeks.

(Mean values  $\pm$  S.E.) n = 6 Rabbits

Variables	Control non vaccinated	Control vaccinated	Treated vaccinated	Variables	Control non vaccinated	Control vaccinated	Treated vaccinated
Total proteins g/L	64.96 $\pm 6.34$	60.6 $\pm 0.55$	77.9 $\pm 2.12^{***b}$	Urea mg/L	619.7 $\pm 39.14$	697.0 $\pm 10.55$	539 49.1 <sup>b</sup>
Albumin g/L	39.36 $\pm 1.24$	39.05 $\pm 2.83$	47.55 $\pm 2.15^*a$	Creatinine mg/L	3.7 $\pm 0.52$	4.6 $\pm 0.7$	4.8 $\pm 0.32$
$\alpha$ -globulins gm/L	13.03 $\pm 0.59$	9.68 $\pm 0.41^{**}a$	6.87 $\pm 0.26^{***}ab$	Glucose mg/dl	86.07 $\pm 6.98$	82.9 $\pm 13.3$	91.0 $\pm 5.15$
$\beta$ -globulins g/L	6.38 $\pm 0.19$	3.06 $\pm 0.06^{***}a$	6.78 $\pm 0.02^{***}ab$	Uric acid mg/L	93.4 $\pm 1.04$	94.25 $\pm 1.95$	87.16 $\pm 0.94$
$\gamma$ -globulins g/L	6.12 $\pm 0.134$	8.8 $\pm 0.3^{***}a$	16.07 $\pm 0.67^{***}ab$	Phosphorus mg/L	59.9 $\pm 4.39$	59.7 $\pm 4.1$	61.2 $\pm 2.12$
A/G ratio	1.57 $\pm 0.2$	2.165 $\pm 0.59$	1.78 $\pm 0.51$	ALT u/ml.	14.5 $\pm 3.25$	19.62 $\pm 2.37$	32.67 $\pm 2.33^*b$
Cholesterol mg/dL	105.2 $\pm 7.85$	103.16 $\pm 6.87$	125.5 $\pm 17.43$	AST u/ml	62.2 $\pm 2.6$	66.0 $\pm 4.15$	132.0 $\pm 5.8^{***}ab$
Triglycerides g/l	1.9 $\pm 0.26$	2.12 $\pm 0.185$	1.96 $\pm 0.07$	Calcium mg/L	162.5 $\pm 8.54$	155.0 $\pm 2.04$	120.33 $\pm 16.25$
Bilirubin mg/L	37.9 $\pm 2.96$	37.3 $\pm 1.17$	41.1 $\pm 11.15$				

\* P < 0.05  
 \*\* P < 0.01  
 \*\*\* P < 0.001

a = significant from the nonvaccinated control.  
 b = significant from the control vaccinated.



Table (2): Blood picture &amp; blood gas analysis of mature male rabbits given cadmium chloride (0.5 mg/kg. b.wt.) every other day for 5 weeks.

(Mean values  $\pm$  S.E.)

n = 6 in each group

Variables	Control non vaccinated	Control vaccinated	Treated vaccinated	Variables	Control non vaccinated	Control vaccinated	Treated vaccinated
RBCs 10 <sup>6</sup>	6.65 $\pm 0.28$	7.6 $\pm 0.81$	7.37 $\pm 0.49$	Monocytes count	88.0 $\pm 0.0$	85.0 $\pm 0.35^{***a}$	88.0 $\pm 0.0^{***b}$
WBCs No/ $\mu$ l	8830 $\pm 430$	8500 $\pm 650$	8800 $\pm 600$	PO <sub>2</sub> mmHg	50.15 $\pm 3.18$	45.12 $\pm 9.0$	54.6 $\pm 8.33$
HB %	10.2 $\pm 0.16$	9.63 $\pm 0.37$	9.53 $\pm 0.32$	PCO <sub>2</sub> mmHg	98.4 $\pm 25.44$	186.3 $\pm 26.22$	98.8 $\pm 23.15$
PCV %	35.25 $\pm 2.62$	35.5 $\pm 5.55$	42.67 $\pm 3.94$	pH	6.17 $\pm 0.16$	6.35 $\pm 0.1$	6.1 $\pm 0.135$
MCV Cu $\mu$	52.95 $\pm 3.05$	48.35 $\pm 9.89$	59.1 $\pm 9.03$	BE mmol/L	43.85 $\pm 4.7$	33.72 $\pm 4.87$	45.67 $\pm 4.54$
MCH $\mu$ g	15.48 $\pm 0.46$	13.13 $\pm 1.6$	13.06 $\pm 1.07$	HCO <sub>3</sub> A mmol/L	4.15 $\pm 1.77$	11.3 $\pm 2.83$	3.7 $\pm 1.8^{*b}$
MCHC %	29.58 $\pm 2.23$	29.45 $\pm 5.21$	22.63 $\pm 1.71$	HCO <sub>3</sub> S mmol/L	4.93 $\pm 2.76$	21.1 $\pm 5.82$	5.53 $\pm 2.9$
Neutrophils count	4481 $\pm 64.08$	4866 $\pm 262.8$	560.3 $\pm 251$	TCO <sub>2</sub> mmol/L	6.62 $\pm 2.3$	15.95 $\pm 3.32$	5.67 $\pm 1.94^{*b}$
Eosinophils count	88.0 $\pm 0.51$	21.0 $\pm 0.05^{***a}$	176 $\pm 2.02^{***ab}$	O <sub>2</sub> -SAT %	22.9 $\pm 4.54$	24.27 $\pm 7.33$	31.47 $\pm 9.34$
Basophils count	0.0 $\pm 0.0$	0.0 $\pm 0.0$	0.0 $\pm 0.0$	O <sub>2</sub> -CT %	3.05 $\pm 0.62$	3.27 $\pm 0.44$	3.97 $\pm 0.86$
Lymphocytes count	41.72 $\pm 0.75$	3527 $\pm 178.5^{*a}$	3520 $\pm 370$				

\* P < 0.05  
\*\*\* P < 0.001

a = significant with the control nonvaccinated.  
 b = significant with the control vaccinated.  
 HCO<sub>3</sub> A = Actual. HCO<sub>3</sub> S = saturation.  
 O<sub>2</sub> - SAT = saturation. BE = Base excess  
 O<sub>2</sub>-CT = content. TCO<sub>2</sub> = (Total CO<sub>2</sub>).

It was reported that chronic cadmium exposure can result in a decrease in the number of antibody-producing cells and depress serum antibody titers in rabbits<sup>(27)</sup> and mice<sup>(28)</sup>. These findings were in full accordance with our results, since a decrease in lymphocyte count was recorded.

The insignificant increase and decrease in serum phosphorus and decrease in calcium recorded in this study might be attributed to renal affection following administration of cadmium<sup>(29,30)</sup>. There is also an enhanced synthesis in the liver of metal-binding protein, metallothionein<sup>(29,30)</sup>. This compound seems to enhance cadmium nephrotoxicity, possibly because the cadmium - metallothionein complex is taken up by the kidney more readily than when it is unbound. Injury produced by cadmium-metallothionein complex is localized to the first and second segments of the proximal tubule. Aminoaciduria, glucoseuria and decreased tubular reabsorption of phosphate increasing its concentration in the serum was observed<sup>(5)</sup>. Furthermore, cadmium toxicity affects calcium metabolism. Individuals with severe cadmium nephropathy may have calculi and excess excretion of calcium, probably related to increased urinary loss. When chronically exposed, to cadmium urine calcium may be less than normal<sup>(1)</sup>.

A condition of hypoxia was generally noticed among the vaccinated rabbits, together with hypercapnia, accompanied by a parallel significant increase in bicarbonates  $\text{HCO}_3$ . It is of interest to notice a correlation between the increased concentration of carbon dioxide tension and hypercapnia, which clarify the condition of hyperventillation and dyspnea observed clinically among vaccinated rabbits under experimentation.  $\text{CO}_2$  directly stimulates the respiratory center in the medulla and indirectly stimulates chemoreceptors in the aortic arch, and baroreceptors.

The pH value was determined to observe alterations in hydrogen ion concentration of the extracellular fluid, in relation to respiratory diseases. Acidosis arises from the inability of lungs to excrete carbon dioxide,  $\text{PCO}_2$  will be raised, and there will be an increased formation of carbonic acid, and the latter was ionized to produce an increased hydrogen ion concentration and consequently a fall in blood pH.

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## دراسة بيولوجية على حقن كلوريد الكادميوم فى الأرناب

عبد العزيز عباس دياب

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تم فى هذه الدراسة حقن كلوريد الكادميوم داخل غشاء البريتون لذكور الأرناب البالغة فى جرعة مقدارها ٥٠. مجم من وزن الجسم يوم بعد يوم لمدة خمسة أسابيع . وقد أظهرت النتائج حدوث زيادة معنوية فى مستوى بروتينات المصل الكلية متمثلة فى زيادة زلال المصل ، البيتا والجاما جلوبيولينات وكذا فى نشاط إنزيمى الكبد ALT & AST فى المجموعة المعالجة والمحصنة ضد الكلوسترديا وذلك عند مقارنتها بمجموعة الضوابط المحصنة . بينما قلت نسبة الألفا جلوبيولينات والبولينا بدرجة ملحوظة.

أما بالنسبة لصورة الدم فلقد أظهرت النتائج وجود زيادة معنوية فى عدد كرات الدم البيضاء الحامضية وكذا الخلايا الملتهمة الكبيرة فى المجموعة المعالجة والمحصنة عند مقارنتها بمجموعة الضوابط المحصنة.

وقد أظهر تحليل غازات الدم وجود نقص معنوى فى ضغط وتوتر ثانى أكسيد الكربون ، وكذا البيكربونات فى المجموعة المحصنة والمعالجة عندما قورنت بمجموعة الضوابط المحصنة.