ToxEHS

Hemotoxic Effects of Cadmium in Normal and Protein Malnourished Rats

Syed Saleem Husain[1]

[1]Faculty of Applied Medical Sciences, Jazan University, Saudi Arabia
Correspondence and requests for materials should be addressed to S. S. Husain (drsaleemsaim@gmail.com)

Received 22 April 2015 / Received in revised form 27 May 2015
Accepted 11 June 2015
DOI 10.1007/s13530-015-0231-3

Abstract

Cadmium is chemically related to zinc and found wherever zinc occurs in nature. Cadmium is emitted to air and water by mines, metal foundries, viz. brass and industries using Cadmium in alkaline accumulators, alloys, paints and plastics. Cadmium exposure results in increase of concentration of a protein, called metallothionein, which plays an important role in detoxification of heavy metals. In persons not exposed to high amounts of cadmium, the concentration of cadmium in blood, bound mainly to red blood cells, is very small, less than 1 µg/100 mL whole blood. Gontzea and Popescu[1] revealed that rats reared on low protein (casein 8.8%) diet were more susceptible to the adverse effects (blood and liver enzyme parameters) of injected Cd, than control given a diet with 17.8% casein. The present study was conducted on rats maintained on normal (21% protein) and protein malnourished (8% protein) diets. Exposure to cadmium in growing rats induced structural anomalies in the erythrocytes and leukocytes. Microcytic hypochromic anemia with erythrocytopenia and leukocytopenia were evident. Protein malnutrition appears to enhance the vulnerability of the animals to these changes.

Keywords: Cadmium toxicity, Protein malnourishment, Hematology

Introduction

The effects of chronic exposure to cadmium toxicity, under protein undernourished state, were studied by Syed Saleem Husain: hepatotoxicity[2] and behavioral aberrations were reported, both in Fo and F1 generations[3].

The effects of chronic cadmium toxicity on the hemostatic system were studied by Kocak and Akcil[4]. The investigators concluded that chronic cadmium toxicity sets the stage for hyper coagulation and hence increases the risk of thrombosis.

Wilson *et al*.[5] induced anemia in rats with a cadmium diet. Increase in eosinophils and reticulocytes with hyper plastic bone marrow were also found. Friberg[6] demonstrated that the anemia induced by cadmium intoxication is iron sensitive, that is, the anemia was correctable with supplementary iron administration and therefore might have resulted in part from iron deficiency.

OGNJANOVIĆ *et al*.[7] studied the effects of acute exposure to cadmium (Cd) on the blood antioxidant defense system, lipid peroxide concentration and hematological parameters, as well as the possible protective role of vitamin E. Red blood cell counts, hematocrit value and hemoglobin concentration were significantly decreased in the blood of Cd-treated rats. Intoxication with cadmium was also followed by significantly increased lipid peroxide concentrations. Kowalczyk *et al*.[8] assessed the effect of long-term uptake of cadmium chloride on selected biochemical parameters and oxidative stress biomarkers in animal models. Long-term intoxication with cadmium chloride elevated blood serum concentration of urea, creatinine, glucose, AspAT and A1AT activity as well as TBARS and protein carbonyl group concentrations. Microscope studies showed marked anisocytosis, with many microcytic, hypochromic red blood cells and polychromasia, with 8 to 10 nucleated red blood cells per 100 white blood cells. Berlin and Friberg[9] further showed an increased destruction of red blood cells without any alteration in the utilization of iron for hemoglobin synthesis indicating that cadmium creates only a state of iron deficiency (decreased intestinal uptake) without any blockage in hemoglobin synthesis or erythropoietin activity.

The concept that cadmium-induced anemia may be resulted from decreased intestinal absorption of iron was further supported by the experiments of Fox and Fry[10] and Fox *et al*.[11] who demonstrated that ascorbic acid, which increased intestinal iron absorption, also prevented the occurrence of anemia. Prevention of

Table 1. Effect of Cd exposure (120 days) on Serum enzyme activity in normal and protein malnourished rats (values in parentheses represent % change).

Treatment	n mole hydrazones formed/m/mg protein		n mole p-nitro phenol formed/m/mg protein	
	GPT	GOT	Alkaline phosphatase	Acid phosphatase
Normal diet	32.44 ± 1.32	90.39 ± 1.08	43.07 ± 1.23	40.35 ± 0.49
Normal diet + Cd	50.79 ± 0.56 (↑ 57)[a] ***	108.92 ± 0.86 (↑ 9)[a] **	55.75 ± 2.19 (↑ 16)[a] *	45.14 ± 1.45 (↑ 5)[a] NS
Low protein diet	30.56 ± 1.22	83.25 ± 2.23	40.86 ± 1.71	39.73 ± 0.52
Low protein diet + Cd	56.89 ± 1.67 (↑ 86)[b] ***	123.59 ± 2.17 (↑ 48)[b] ***	63.10 ± 1.02 (↑ 54)[b] ***	58.45 ± 1.98 (↑ 47)[b] ***

Values represent mean ± SE of six rats; Statistical evaluation by one-way ANOVA followed by LSD comparison; a = Compared to normal diet control, b = Compared to low protein diet control; p** = < 0.05; *** = < 0.01; ↓ = decrease; NS = Non-significant.

cadmium-induced anemia by a prophylactic injection of iron was also demonstrated by Pond and Walker[12].

Stowe *et al*.[13] treated male rabbits with 160 ppm Cd through drinking water, for 200 days. Cadmium ingestion caused retarded growth, anemia, neutrophilia, lymphopenia, hypoalbuminemia, elevated alpha-1, beta-2 globulins, splenomegaly, cardiomegaly and renal enlargement.

An increase in plasma volume[14] and ahaptoglobinemia[15] were found in animals intoxicated with cadmium, suggesting hemolytic anemia. A large quantity of cadmium was stored in the erythrocytes as cadmium-metallothionein in complex[16].

Although there are only a handful of articles that have endeavored to investigate the role of dietary protein in modifying the toxicity of Cd, the first such study was published by Fitzhugh and Meiller[17] who demonstrated that a low protein diet increased Cd toxicity. Fox *et al*.[11] reported that the type of dietary protein in purified diets significantly affected the severity of Cd-induced toxicity. For instance, when either casein gelatin or soy isolate was used as the source of dietary protein for Japanese quail, given 75 ppm Cd from 7 to 14 days of age, the animals developed anemia and low levels of liver, iron, tibia, zinc and total ash. In contrast, when dried egg white was the protein source, Cd toxicity was markedly reduced, presumably because of better utilization of dietary iron and zinc. Gontzea and Popescu[1] revealed that rats reared on low protein (casein 8.8%) diet were more susceptible to the adverse effects (blood and liver enzyme parameters) of injected Cd, than control given a diet with 17.8% casein.

Results

Serum Enzymes

A significant increase in the serum GPT, GOT, alkaline and acid phosphatase activities was observed in both the dietary groups. The effects on these enzymes were more marked in the protein malnourished animals (Table 1).

Table 2. Effect of Cd exposure (120 days) on Serum enzyme activity in normal and protein malnourished rats (values in parentheses represent % change).

Treatment	mg/dL	g/dL
	Blood glucose	Serum albumin
Normal diet	38.470 ± 0.59	3.776 ± 0.05
Normal diet + Cd	50.773 ± 1.25 (↑ 32)[a] ***	3.383 ± 0.04 (↓ 10)[a] ***
Low protein diet	42.833 ± 2.15	3.303 ± 0.03
Low protein diet + Cd	54.609 ± 1.43 (↑ 27)[b] ***	2.954 ± 0.09 (↓ 11)[b] ***

Values represent mean ± SE of six rats; Statistical evaluation by one-way ANOVA followed by LSD comparison; a = Compared to normal diet control, b = Compared to low protein diet control; p** = < 0.05; *** = < 0.01; ↑ = increase; ↓ = decrease.

Blood Glucose

Cd exposure resulted in as significant increase in blood glucose levels, of more or less equal magnitude, in both the diet groups (Table 2).

Serum Albumin

A significant reduction of the same magnitude in serum albumin level was observed in the Cd-exposed animals of both the diet groups (Table 2).

Cd, Zn, Cu and Fe Levels in Blood

The blood Cd levels were significantly elevated in the Cd exposed animals in both dietary groups. The Zn, Cu and Fe levels were decreased in the Cd-exposed animals of both the dietary groups but the effects on Zn and Fe were more pronounced in the malnourished

Table 3. Effect of Cd exposure on the Blood metal levels in normal and low protein diet fed, Fo-growing male rats.

Group	Blood level, µg/100 mL			
	Cd	Zn	Cu	Fe
Normal protein diet	0.023 ± 0.006	126.24 ± 9.7	39.41 ± 1.7	194.31 ± 22.00
Normal protein diet + Cd	7.960 ± 0.093*** a ↑ (346 folds)	92.17 ± 4.1** a ↓ (27%)	21.75 ± 1.31** a ↓ (45%)	162.52 ± 9.31* a ↓ (17%)
Low protein diet	0.058 ± 0.004	137.41 ± 7.4	32.74 ± 1.78	236.46 ± 18.00
Low protein diet + Cd	6.62 ± 0.39*** b ↑ (114 folds)	86.22 ± 2.6** b ↓ (37%)	24.15 ± 0.86* b ↓ (26%)	142.39 ± 8.62** b ↓ (42%)

Values represent mean ± SE of six rats; Statistical evaluation by one-way ANOVA followed by LSD comparison; a = Compared to normal protein diet control, b = Compared to low protein diet control; p* = < 0.05; ** = < 0.01; ***0.001, NS = Not Significant; ↑ = Increase; ↓ = Decrease.

Table 4. Effect of oral cadmium exposure on total erythrocyte and leukocyte counts in normal and low protein diet fed rats.

Treatment	Erythrocyte × 10^6/mm^3				Leukocytes × 10^3/mm^3			
	Day of Cd exposure				Day of Cd exposure			
	30	60	90	120	30	60	90	120
Normal protein diet	7.59 ± 0.45	8.37 ± 0.44	8.45 ± 0.39	8.76 ± 0.21	16.95 ± 1.5	17.75 ± 1.48	16.5 ± 1.5	17.75 ± 1.48
Normal protein diet + Cd	7.39^{aNS} ± 0.5	7.97^{aNS} ± 0.5	7.23^{a*} ± 0.4	6.99^{a**} ± 0.52	16.35^{aNS} ± 1.51	15.02^{aNS} ± 1.46	13.4^{a*} ± 1.45	14.02^{a**} ± 1.46
Low protein diet	6.95 ± 0.42	8.43 ± 0.41	8.47 ± 0.39	6.15 ± 0.14	17.01 ± 0.9	17.16 ± 0.87	16.32 ± 0.9	17.158 ± 0.87
Low protein diet + Cd	6.70^{bNS} ± 0.55	7.94^{bNS} ± 0.52	7.2b* ± 0.48	5.40^{b*} ± 0.19	16.89^{bNS} ± 1.8	14.44^{b*} ± 1.6	12.59^{b**} ± 1.5	12.53^{b**} ± 1.60

Values represent mean ± SE of six rats; Statistical evaluation by one-way ANOVA, followed by LSD comparison; a = Compared to normal protein diet control; b = Compared to low protein diet control; p values, * = < 0.05; ** = 0.01; *** = < 0.001; NS = Non-significant

animals whereas the effect on Cd and Cu level was more marked in the normal protein diet-fed group (Table 3).

Morphological Anomalies in Blood Cells

A marked anisocytosis and hypochromia were observed in the Cd exposed animals of both the diet groups from day 60 onwards. A few target cells were also spotted in the exposed animals of both the diet groups at day 90 of exposed, more frequently in the malnourished animals. Microcytic hypochromic anemia was fully established on day 90 of exposure in the malnourished rats and on day 120 in the normal diet fed animals. A few spur cells (acanthocytes) appeared from day 60 of exposure in the malnourished group and on day 120 in the normal diet fed animals. Marked degeneration on cell walls in erythrocytes was observed in both of the experimental groups at 60 days.

Total Erythrocyte and Leukocyte Counts

Significant reductions in the total erythrocyte and leukocyte counts were observed in the Cd-exposed animals of both the dietary groups. The erythrocytopenia was evident on days 90 and 120 of Cd exposure in both the diet groups but the effect was more marked on the normal protein diet fed animals on day 120 of exposure. In these animals, leukocytopenia was evident from day 90 onwards whereas in the protein malnourished, it occurred earlier, i.e. from day 60 (Table 4).

Hemoglobin

A significant reduction in hemoglobin (Hb) level, of more or less equal magnitude, was observed in the Cd-exposed animals of either dietary group on day 60 and 90 of exposure while the effect was more marked in the normal protein diet fed animals on day 120 of exposure (Table 5).

Differential Leukocyte Count

An increase (18%) in the eosinophils was observed only in the Cd-exposed, protein malnourished animals. The rest of the leukocyte counts were not affected in either dietary group at any time of the exposure (Table 5).

Table 5. Effect of oral cadmium exposure on hemoglobin content and eosinophil counts in normal and low protein diet fed rats.

Treatment	Hb (g/dL)				Eosinophil count
	Day of Cd exposure				Day of Cd exposure
	30	60	90	120	120
Normal protein diet	12.4 ± 0.19	12.5 ± 0.18	13.0 ± 0.20	13.5 ± 0.20	2.83 ± 0.31
Normal protein diet + Cd	12.0^{aNS} ± 0.13	11.6^{a**} ± 0.14	12.0^{a**} ± 0.15	10.5^{a***} ± 0.41	3.17 ± 0.17^{aNS}
Low protein diet	11.5 ± 0.40	11.77 ± 0.30	11.9 ± 0.40	10.7 ± 0.44	2.67 ± 0.21
Low protein diet + Cd	11.2^{bNS} ± 0.20	10.8^{b**} ± 0.19	10.5^{b**} ± 0.20	9.6^{b*} ± 0.16	$3.17 \pm 0.12^{b*}$

Values represent mean ± SE of six rats; Statistical evaluation by one-way ANOVA, followed by LSD comparison; a = Compared to normal protein diet control; b = Compared to low protein diet control; p values, * = < 0.05; ** = 0.01; *** = < 0.001; NS = Non-significant

Discussion

The present results show that exposure to cadmium in growing rats induces structural anomalies in the erythrocytes as well as the cells of the leukocytic lineage. Microcytic hypochromic anemia with erythrocytopenia and leukocytopenia were evident. Protein malnutrition appears to enhance the vulnerability of the animals to these changes.

Following absorption, Cd is initially bound to a high molecular weight protein in plasma. Within a few hours most of this Cd moves to red blood cells where again it is protein bound. Following uptake of plasma protein-bound Cd by the liver and the induction of metallothionein in that organ, a small concentration of metallothionein-bound Cd has been identified in the plasma within a few days of initial exposure. Thus following repeated exposure, Cd is present at low concentration in plasma, partly bound to albumin and other high molecular weight proteins and a part, bound to metallothionein, with the major part bound to proteins within red cells.

More than 90% of the blood-Cd is found in the erythrocytes where it is bound to hemoglobin[9]. The same authors pointed out that increased destruction of erythrocytes might be attributed to diminution of their resistance after they had taken up Cd.

Hypochromic, microcytic anemia is one of the most sensitive parameters of oral cadmium intoxication in humans and animals. In rats, dietary exposure levels as low as 3 μg/g, are known to induce anemia[5,18,19]. Reduction in Hb has been observed in rats after 2 weeks of exposure to 50 μg Cd/mL in drinking water, whereas 10 μg/mL did not induce anemia even after one year of exposure[20]. In the present study, anemia occurred from day 60 of exposure onwards. The magnitude of the effect was more or less the same in normal and protein malnourished rats on days 60 and 90 but the effect was more marked in the normal protein diet fed animals on day 120 of exposure. Protein malnutrition itself had resulted in a significant reduction in the Hb level at this stage and this might be the reason for the observed decrease in the effect of Cd on Hb levels in these animals.

One of the most important nutritional requirements for production of red cells is an adequate supply of first class protein in the diet which is essential to supply amino acids for synthesis of the protein globin of hemoglobin. Diets low in proteins and high in carbohydrates have previously been reported to induce anemia[21]. Therefore, protein deficiency in man impairs hemoglobin synthesis.

Cd altered the metabolism of Fe when administered orally through water, as low as 4.3 μg Cd/mL[21,22]. Therefore, it could be stated that a marked deficiency of iron was responsible for less synthesis of hemoglobin. Therefore protein under nutrition predisposes the animal to a more deleterious degree of anemia caused by Cd-toxicity.

Experimental evidence suggests that anemia may be linked, at least in part, to a decrease in intestinal iron absorption, leading to decreased serum iron levels[12,23,24]. The decrease in body iron is directly related to cadmium ingestion and not secondary to reduced diet consumption[25]. The increased destruction of erythrocytes under cadmium intoxication may also be a contributing factor[9,26].

Decreased iron absorption, distortion of erythropoiesis and hemolysis of red blood cells, resulting decrease in synthesis of hemoglobin are the manifestation of cadmium toxicity[27,28]. Structural anomalies in the erythrocytes and leukocytes due to cadmium exposure have been reported in rats, fishes and higher vertebrates including humans[29-32].

In the present study damaged erythrocytes, anisocytosis, appearance of target and spur cells and abnormalities in the leukocytes were observed. Some of these aberrations such as the appearance of target and spur cells which are indicative of liver damage occurred more frequently in the protein malnourished animals. The deterioration of the cell walls of erythrocytes may be due to the action of cadmium on the -SH groups which are critical in the maintenance of intact erythro-

cytes[33].

Variable effects of cadmium on the differential blood counts, in different animal species, have been reported earlier. Thus no effect[34-36], higher neutrophils and lower lymphocyte counts[14] increase in large lymphocyte counts[31,32,37] have been observed. In the present study, the differential cell counts did not reveal any significant alteration except an increase in the eosinophils (only in the malnourished cadmium exposed animals) which is in agreement with the observations of Wilson *et al*.[5], and Friberg[6]. The variations in the actions of cadmium on the differential cellular elements might be due, in part, to differences in inter-species tolerance limits as well as the experimental designs employed.

The above findings, while characterizing the hematological effects of chronic cadmium exposure in rats, reveal the enhanced susceptibility of protein malnourished animals to these effects. The chronological sequence of appearance of these changes has also been revealed.

Materials and Methods

Nutritional deficiencies are known to alter the response of the organism to the environmental toxicants in a manner different to that observed in the nutritionally adequate state. Hence, the present study was conducted on rats maintained on normal (21% protein diet) and protein malnourished (8% protein diet).

Experimental groups	Treatment schedule
I	21% Casein diet (normal protein diet) + drinking water (CONTROL)
II	21% Casein diet + Cd, 50 ppm in drinking water.
III	8% Casein diet (low protein diet) + drinking water (CONTROL)
IV	8% Casein diet + Cd, 50 ppm in drinking water.

The hematological effects were also assessed in order to understand the toxic effects of cadmium in a more elaborated from. The following parameters were carried out: Total erythrocyte count, total leukocyte count (TLC), differential leukocyte count (DLC), hemoglobin, morphology of erythrocytes, micro hematocrit, and different erythrocyte indices (MCV, MCHC, MCH).

Monthly evaluation of hematological parameters was also done. The parameters studied are as follow: Total erythrocyte count, total leukocyte count, differential leukocyte count, hemoglobin, morphology of erythrocytes, and erythrocyte indices (Micro hematocrit, MCV, MCHC, MCH).

At 120 day the animals were sacrificed by decapitation. ACP, ALP, GPT, GOT and albumin were estimated in serum and glucose was done in whole blood. Monthly evaluation of hematological parameters was also done. The parameters studied are as follow: Total erythrocyte count, total leukocyte count, differential leukocyte count, hemoglobin, morphology of erythrocytes, and erythrocyte indices (Micro hematocrit, MCV, MCHC, MCH).

Hematological Parameters

Whole blood, obtained by cutting tip of the tail, was used for preparation of blood smears, total erythrocyte, leukocyte and differential leukocyte counts and hemoglobin (Hb), at 30 days interval during the experimental period. Hematocrit (Hct) estimation was done at the end of the exposure period, i.e. day 120 using blood drawn by heart puncture. Other erythrocytic indices such as mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were estimated based on the Hb and Hct values obtained at day 120 of exposure.

Morphological Aberrations

Blood smears, two slides per rat, were prepared from tail blood, air dried, fixed in methanol and stained with Leishman stain[38]. Blood corpuscles were examined under an oil immersion objective.

Blood Cell Counts

Blood cell counts were made manually using standard techniques. Whole blood 5 µL was added to 1.0 mL saline and mixed gently. A Hemocytometer field was flooded with the suspension and the cells were counted at 40X. Simultaneously total leukocytes were counted using Turk's diluting fluid. Percentage counts of granulocytes (neutrophils, eosinophils and basophils) and agranulocytes (lymphocytes and monocytes) were made using two slides per animal.

Erythrocytic Indices

Hemoglobin was estimated by the Sahli-Helliege method[39]. Hematocrit was determined using microhematocrit capillary tubes, centrifuged at 15,000 rpm for 3 min.

$$\text{MCV}\,(\text{mm}^3) = \frac{\text{Hematocrit}\,(\%) \times 10}{\text{Erythrocytes}\,(\text{millions/mm}^3)}$$

$$\text{MCH}\,(\text{pg}) = \frac{\text{Hemoglobin}\,(\text{g/dL}) \times 10}{\text{Erythrocytes}\,(\text{millions/mm}^3)}$$

$$\text{MCHC}\,(\%) = \frac{\text{Hemoglobin}\,(\text{g/dL}) \times 10}{\text{Hematocrit}\,(\%)}$$

Acknowledgements

The author acknowledges with thanks the financial grant by Indian Council of Medical Research, New Delhi, India and cooperation of Dr M. M. Ali, Assistant Director, Indian Institute of Toxicology Research, Lucknow, India.

References

1. Gontzea, I. & Popescu, F. The effect of body protein supply on resistance to cadmium. *Brit. J. Ind. Med.* **35**, 154-160 (1978).
2. Husain, S. S. Studies on hepato and renal toxicity of cadmium on normal and protein malnourished rats. *J. Material Sci. Eng.* **2**, 129 (2013).
3. Husain, S. S. Behavioral toxicity of cadmium in normal and protein malnourished rats (Two-Generation Study). *Biochem. Physiol.* **3**, 124 (2014).
4. Kocak, M. & Akcil, E. The effects of chronic cadmium toxicity on the hemostatic system, Pathophysiol. *Haemost. Thromb.* **6**, 411-416 (2006).
5. Wilson, R. H., Deeds, F. & Cox, A. J. Dietary cadmium and the development of Anaemia. *J. Pharmacol. Exp. Ther.* **71**, 222-227 (1941).
6. Friberg, L. Health hazards in the manufacture of alkaline accumulators with special reference to chronic cadmium poisoning; a clinical and experimental study. *Acta Med. Scand.* **138**, 1-124 (1950).
7. OGNJANOVIĆ, B. I. *et al*. Protective influence of Vitamin E on antioxidant defense system in the blood of rats treated with cadmium. *Physiol. Res.* **52**, 563-570 (2003).
8. Kowalczyk, E. *et al*. effect of long-term cadmium intoxication on selected biochemical parameters in experimental animals. *Pol. J. Environ. Stud.* **11**, 599-601 (2002).
9. Berlin, M. & Friberg, L. Bone marrow activity and erythrodestruction in chronic cadmium poisoning. *Arch. Environ. Health* **3**, 176-179 (1961).
10. Fox, M. R. S. & Fry, B. E. Cadmium toxicity decreased by dietary ascorbic acid supplements. *Science* **169**, 989-990 (1970).
11. Fox, M. R. S. *et al*. Effects of ascorbic acid on cadmium toxicity in the young coturnix. *J. Nutr.* **101**, 1295-1298 (1971).
12. Pond, W. G. & Walker, E. F. Jr. Cadmium induced anemia in growing rats, prevention by parenteral iron. *Nutrition and Reproduction Int.* **5**, 356-370 (1972).
13. Stowe, H. D., Wilson, M. & Goyer, R. A. Clinical and morphological effects of oral cadmium toxicity in rabbits. *Arch. Pathol.* **94**, 389-405 (1972).
14. Berlin, M. & Piscator, M. Blood volume in normal and cadmium poisoned rabbits. *Arch. Environ. Health* **2**, 576-583 (1961).
15. Axelsson, B. & Piscator, M. Renal damage after prolonged exposure to cadmium: an experimental study. *Arch. Environ. Health* **12**, 360-373 (1966a).
16. Nordberg, G., Friberg, L. & Piscator, M. in Cadmium in the Environment, 2nd Edn (CRC Press, Cleveland, Ohio, 1971).
17. Fitzhugh, O. G. & Meiller, F. H. "Sub acute toxicity," Appraisal of the Safety of Chemicals. *JPET* **72**, 15 (1941).
18. Cousins, R. J., Barber, A. K. & Trout, J. R. Cadmium toxicity in growing swine. *J. Nutr.* **103**, 964 (1972).
19. Freeland, J. H. & Cousins, R. J. Effect of dietary cadmium on anemia, iron absorption, and cadmium binding protein in the chick. *Nutr. Rep. Int.* **8**, 337-347 (1973).
20. Decker, L. E. *et al*. Chronic toxicity studies. I. Cadmium administered in drinking water to rats. *AMA. Ind. Health* **18**, 228-231 (1958).
21. Petering, H. G., Murthy, L. & Cerklewski, F. L. in *Biochemical Effects of Environmental Pollutants* (eds Lee, S.D. Ann.) 365 (Arbor Science, Ann. Arbor, MI. 1977).
22. Umoli, I. B., Maduagwu, E. N. & Amole, A. A. Fate of ingested linamarin in malnourished rats. *Food Chem.* **20**, 1-9 (1986).
23. Petering, H. G., Choudhary, H. & Stemmer, K. L. Some effects of oral ingestion of cadmium on zinc, copper, and iron metabolism. *Environ. Health Persp.* **28**, 97-106 (1979).
24. Freeland, J. H. & Cousins, R. J. Effect of dietary cadmium on anemia, iron absorption, and cadmium binding protein in the chick. *Nutr. Rep. Int.* **8**, 337-347 (1973).
25. Prigge, E. Early signs of oral and inhalative cadmium uptake in rats. *Arch. Tox.* **40**, 231-247 (1978).
26. Washko, P. W. & Cousins, R. J. Role of Dietary Calcium and Calcium Binding Protein in Cadmium Toxicity in Rats. *J. Nutr.* **107**, 920-928 (1977).
27. Axelsson, B. & Piscator, M. Renal damage after prolonged exposure to cadmium: An experimental study. *Arch. Environ. Health* **12**, 360-373 (1966).
28. Horiguchi, H. *et al*. Hypoproduction of erythropoietin contributes to anaemia in chronic cadmium intoxication: clinical studies on itai-itai disease in Japan. *Arch. Toxicol.* **68**, 632-636 (1994).
29. Horiguchi, H. *et al*. Long term cadmium exposure induces anaemia in rats through hypoinduction of erythropoietin in the kidney. *Arch. Toxicol.* **71**, 11-19 (1996).
30. Pindborg, E. V., Pindborg, J. J. & Plum, C. M. Studies on incisor pigmentation in relation to liver iron and blood picture in the white rat. *Acta Pharmacology and Toxicology* **2**, 302 (1946).
31. Buckley, J. A. Heinz body hemolytic anemia in coho salmon (Oncorhynchus kisutch) exposed to chlorinated seawater. *J. Fish. Res. Board Can.* **34**, 215-224 (1977).

32. Gill, S. T. & Pant, J. C. Erythrocytic and leucocytic response to cadmium poisoning in fresh water fish Puntius conchonius. *Environ. Res.* **36**, 327 (1985).
33. Benesch, R. E. & Benesch, R. Relation between erythrocyte integrity and sulfhydryl groups. *Arch. Biochem. Biophys.* **48**, 38-42 (1954).
34. Loeser, E. & Lorke, D. Semi chronic oral toxicity of cadmium. I. Studies on rats. *Toxicology* **7**, 215-224 (1977).
35. Loeser, E. & Lorke, D. Semi chronic oral toxicity of cadmium. II. Studies on dogs. *Toxicology* **7**, 225-232 (1977).
36. Itokawa, Y., Abe, T., Tabei, R. & Tanaka, S. Renal and skeletal lesions in experimental cadmium poisoning. *Arch. Environ. Health* **28**, 149-154 (1974).
37. Oshawa, M. & Kawai, M. Cytological shift in lymphocytes induced by cadmium in mice and rats. *Environ. Res.* **24**, 192-200 (1981).
38. WINTROBE, M. M. *et al.* in Clinical Hematology 8th Edn (Lee & Febiger, Philadelphia, 1981).
39. Hesser, E. F. Methods for routine fish haematology. *Prog. Fish Cult.* **22**, 104-171 (1960).