52 Robertson, D. H., Paganelli, R., Dinwiddie, R., and Levinsky, R. J., Milk antigen absorption in the preterm and term neonate. Archs. Dis. Childh. *57* (1982) 369–372.
53 Svedberg, J., Dettaas, J., Leimenstoll, G., Paul, F., and Teschemacher, H., Demonstration of β-casomorphin immunoreactive materials in in vitro digests of bovine milk and in small intestine contents after bovine milk ingestion in adult human. Peptides *6* (1985) 825–830.
54 Smith, E. M., Menamin, D. H., and Blalock, J. E., Lymphocyte production of endorphins and endorphin-mediated immunoregulatory activity. J. Immun. *135* (1985) 779–781.
55 Umbach, M., Teschemacher, H., Praetorius, K., Hirshhäusser, R., and Bostedt, H., Demonstration of a β-casomorphin immunoreactive material in the plasma of newborn calves after milk intake. Reg. Pept. *12* (1985) 223–230.
56 Wybran, J., Appelboom, T., Famacy, J. P., and Govaerts, A., Suggestive evidence for receptors for morphine and methionine-enkephalin on normal human T lymphocytes. J. Immun. *123* (1979) 1068–1070.
57 Yachie, A., Miyawaki, T., Nagaoki, T., Yokoi, T., Mikio, M., Uwadana, N., and Taniguchi, N., Regulation of B cell differentiation by T cell subsets defined with monoclonal OK T4 and OK T8 antibodies in human cord blood. J. Immun. *127* (1981) 1314–1317.
58 Yoshikawa, M., and Chiba, H., Abstracts of the Annual Meeting of Agric. chem. Soc. Jap., Sendai (1983) 574.
59 Yoshikawa, M., Yoshimura, T., and Chiba, H., Opioid peptides from human β-casein. Agric. Biol. Chem. *48* (1984) 3185–3187.
60 Yvon, M., and Pelissier, J. P., Characterization and kinetics of evacuation of peptides resulting from casein hydrolysis in the stomach of the calf. J. Agric. Food Chem. *35* (1987) 148–156.
61 Zioudrou, C., Streaty, R. A., and Klee, W. A., Opioid peptides derived from food proteins. The exorphins. J. biol. Chem. *254* (1979) 2446–2449.

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# Full Papers

## Cadmium-induced changes in avian renal morphology

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*Summary.* The effects of i.m. administered cadmium on growth rate and nephromorphology were studied in young pullets. The growth rate of pullets treated with 0.6 mg $Cd^{2+}$/kg at 48-h intervals was severely retarded, reaching only 50% of normal growth by 21 days. Such a decrease in growth rate was prevented when cadmium was given with either ferric or magnesium EDTA chelate. Electron micrographs of kidney tissue from cadmium intoxicated birds revealed massive intracellular disorganisation of proximal tubular cells, showing increased vacuolation and dilated endoplasmic reticulum. Mitochondria were few and swollen with reduced cristae. Some disorganisation was noted in the group treated with MgEDTA in conjunction with cadmium, with normal morphology observed in the group treated with FeEDTA plus cadmium.
In general, glomerular morphology of intoxicated pullets appeared normal, except that a 25% increase in thickness of the glomerular basement membrane was evident. No such membrane thickening was observed in any of the chelate treated groups.
These findings indicate that both chelates can provide certain levels of protection, in terms of growth rate and morphology, from cadmium intoxication. The possible mechanisms by which chelates offer protection have been discussed, but many questions remain unanswered.
*Key words.* Cadmium treatment; avian nephromorphology; growth rate; chelate.

### *Introduction*

Several reports have described the pathological, physiological and biochemical effects of cadmium intoxication in humans and other mammals[1–6]. In general, the intoxication has revealed some common features, such as demineralisation of bone and hypercalcaemia, leading ultimately to bone fragility, massive retention of the body burden of cadmium, mainly in liver and kidney tissue, and interference in mitochondrial activity and membrane bound enzymes.
It has been shown in long-term studies that rats treated with low levels of cadmium (2.1 μg/day, orally) accumulate approximately 80% of the metal in the cytosol of renal cells with 7, 4 and 3% appearing in the mitochondria, nuclei and lysosomes respectively[7]. It is possible that this large retention of cadmium, either in the bound thionein complex or in the free form, could lead to changes in renal ultrastructure. Indeed, it has been stated[4] that biochemical and ultrastructural alterations in proximal tubules appear to parallel each other.
Samarawickrama[4] has suggested that in the kidney, pathological changes resulting from cadmium intoxication are essentially the same irrespective of the route of administration. When cadmium is administered orally, however, no valid conclusion can be made about its absorption. Estimates of absorption of ingested cadmium have been suggested to be approximately 2% in laboratory animals, though values of about 6% have been recorded in humans[8].
On administration, cadmium is initially stored in the liver, and subsequently transported to other organs, predominantly the kidney[9]. In rats, a single i.v. injection (2.5 mg/$Cd^{2+}$/kg) resulted in dilatation of ER, mitochondrial swelling and areas of degenerated cytosol in liver cells. Over this short period, no such disruption was observed in renal tissue, except that there was occasional pyknosis of nuclei in proximal tubular cells[10]. These findings suggest that kidney changes follow those seen in the liver.

In chronic exposure, however, (0.6 mg $Cd^{2+}$/kg s.c. daily for 24 days) changes were observed [11] in proximal tubule cells, the mitochondria showing a reduction in numbers of cristae. Mitochondrial changes in proximal tubule cells were also observed in a series of experiments on rats given 50 or 300 mg/l $Cd^{2+}$ in drinking water, for periods ranging from 6 to 40 weeks. Swelling of mitochondria, a decrease in density of the matrix and occasional rupture of the outer membrane were recorded [12].

In a study where cadmium was administered at various levels to rats in drinking water (estimated intake 5 µg, 50 µg, 0.5 mg and 2 mg per day) for 6 or 12 weeks, dose related changes in peritubular capillaries were seen. The basal lamina of peritubular capillaries appeared to be increased, and there was irregular thickening of the glomerular capillary basal lamina [13]. These changes were seen at all dose levels and at both time periods. Moderate thickening of glomerular basal lamina has also been seen in rats ingesting cadmium (50 or 300 mg/l in drinking water) over periods of up to 24 weeks [12], and in rabbits given s.c. injections of 1.53 mg/$Cd^{2+}$/kg weekly for 11–29 weeks marked cell vacuolation and desquammation in proximal tubules was observed. Mild glomerular alterations (thickening of the basal membrane) were also evident in these animals [14].

In other studies, rabbits provided with cadmium in drinking water (98 mg $Cd^{2+}$/l; 9 mg $Cd^{2+}$/kg/d) over a long period (200 d) [15] showed dilated ER in proximal cells, and glomeruli were found to have deposits of collagen between mesangial cells and the basement membrane.

In mice exposed to s.c. injected cadmium at 0.112 mg $Cd^{2+}$/72 h for 30–39 days (estimated at 4.50 mg $Cd^{2+}$/kg/injection) severe cell necrosis, mitochondrial swelling and nuclear pyknosis were reported in proximal tubule cells, and in general terms, similar observations were made in proximal cells of starlings following an identical dosing schedule [16].

Associated with cadmium intoxication, it has been reported that the cadmium cation displaces functional endogenous ions such as magnesium, zinc, iron and cadmium from their action sites [5]. Such displacement and alteration in metabolism could produce a range of functional abnormalities. With respect to cadmium, for example, it has been reported that a low dietary cadmium intake increases renal deposition of cadmium and enhances bone demineralisation in rats [17, 18], and aggravation of abnormal skeletal changes has been noted in cadmium-treated calcium deficient rats [19]. In the quail, it is known that continual dietary exposure to cadmium decreases calcium retention, though the workers suggested that this might be due to iron deficiency or reduced food intake [20]. Experiments on quail fed with cadmium resulted in iron deficiency and poor bone mineralisation [21], which presumably may result from an increased secretion of parathyroid hormone.

In birds, there is a high rate of calcium turnover, associated with early growth and subsequent egg laying activity. A drain on body calcium could lead to profound alterations in calcium metabolism, including such processes as medullary bone deposition in young pullets and induction of massive renal loss of calcium. In the present series of experiments, pullets were provided with a high calcium diet prior to and during studies on the effects of i.m. injected cadmium on growth rate and on changes in renal ultrastructure over a 3-week period. In addition, the use of two heavy metal chelates, ferric monosodium EDTA (FeEDTA) and magnesium disodium EDTA (MgEDTA) (both supplied by BDH Ltd.) in braking or protecting the birds from the effects of cadmium intoxication was investigated.

As the sodium salt of EDTA forms a very stable complex with calcium ions in the serum [22], it is often administered as the calcium salt. This has been used in mice [23], which were partly protected against a range of single, i.v. doses of cadmium chloride (from 2.28 to 7.60 mg $Cd^{2+}$/kg). In the current study, however, two other compounds of EDTA were used, the ferric and magnesium salts, the cations of which could dissociate from the EDTA and become available to supply any deficiency and in so doing increase the efficiency of EDTA in chelating cadmium. Indeed, enhancement of cadmium uptake has been noted in iron deficient rats [24] and mice [25]. An additional supply of iron could therefore be advantageous in maintainance of normal metabolism. Similar arguments might also be applied to the magnesium chelate, for example the maintainance of structural integrity of cell membranes is associated with metal ions including magnesium [26]. Additional magnesium may therefore assist in this respect.

*Materials and methods*

Twenty-eight Rhode Island Red cross Light Sussex (RIR × LS) female pullets, of age six weeks and average weight 191.1 ± 11.8 g were used in these experiments. They were maintained on a high calcium commercial diet, containing 4.1% calcium and 0.9% phosphorous. Food and water were supplied ad libitum. Prior to experimentation, the pullets were randomly separated into groups comprising 5 birds per group, except for two groups where 4 birds per group were used. After allowing a 'settling in' period of 7 days, each pullet was given i.m. injections at 48-h intervals for the duration of the experiment (21 days).

Group 1 pullets were injected with 0.5 ml of isotonic saline (160 mM sodium chloride). Group 2 pullets were given 0.5 ml of the ferric monosodium salt of EDTA (FeEDTA) at 27.2 mM (26.2 mg/kg) dissolved in saline; group 3 animals were given 0.5 ml of the magnesium disodium salt of EDTA (MgEDTA) at 27.9 mM (26.2 mg/kg) also dissolved in saline and those in group 4 were administered with 0.5 ml of 54.6 mM cadmium chloride in saline (approximately 0.6 mg $Cd^{2+}$/kg per injection). Birds in group 5 were treated with 0.25 ml 109.2 mM cadmium chloride (approximately 0.6 mg $Cd^{2+}$/kg per injection), and additionally with 0.25 ml 54.4 mM FeEDTA, and those in group 6 with 0.25 ml 109.2 mM cadmium chloride and with 0.25 ml 55.8 mM MgEDTA. In those animals given both chelate and cadmium chloride, separate injections were made, in the right and left breast muscle respectively. The total volume of injected fluid was kept constant in all animals, hence the doubling of the concentrations in groups 5 and 6, where two test substances were administered. The amount of chelate and of cadmium chloride given to animals in groups 5 and 6, however, was the same as in the other groups.

All pullets were weighted at regular intervals, and their general appearance observed and recorded. At day 21 the experiment was terminated and gross macroscopic anatomy examined at autopsy. Samples of renal tissue for subsequent examination by electron microscopy were taken from all individuals. The kidneys were located and immediately flooded with 2.5% gluaraldehyde (made up from the standard, approximately 25% solution for electron microscopy, supplied by BDH) in 0.1 M cacodylate buffer at pH 7.2, made up in 0.25 M sucrose. The cranial divisions were rapidly removed, and 1-$mm^3$ blocks of renal tissue were cut and immersed in fresh, cold (4 °C) glutaraldehyde fixative for 3 h. The specimens were then rinsed with 0.1 M cacodylate buffer (pH 7.2) containing 0.25 M sucrose. Post fixation was in 1% (39.34 mM) osmium tetroxide in cacodylate buffer/sucrose for 1 h (at room temperature) after which the blocks were rinsed in buffer and dehydrated through a series of ethanol, with en bloc staining at the 70% ethanol stage. After dehydration, the samples were rinsed in propylene oxide (2 changes), followed by a 1 : 1 mixture of propylene oxide and resin

(araldite). After transfer to fresh resin, the blocks were embedded in araldite and polymerised at 60 °C for 24 h.
Ultrathin sections were cut using an LKB III ultramicrotome and were collected on uncoated 300 mesh copper grids. The sections were then stained in saturated uranyl acetete (1 min) followed by Reynolds' lead citrate (1 min).
Sections derived from all individuals in the experiment were examined using a Jeol 100 S TEM, and photographs taken at a range of magnifications. The resulting electron-micrographs were then analysed using the technique described by Loud [27], in which the relative areas occupied by cytoplasmic structures such as mitochondria, and by the cytoplasm itself may be calculated. Measurements of cells were made on electron micrographs using a grid overlay. By measuring the length of the lines of the grid that were superimposed on various structures, for example mitochondria, it is possible, after suitable calculations, to determine the area occupied by that structure in the section of the cell studied. Student's t-test was used for statistical evaluations of these results.

## *Results*

### *General observations*

Pullets treated with saline solution (group 1) or with ferric monosodium (group 2) or magnesium disodium (group 3) salts of EDTA (FeEDTA or MgEDTA) showed no visible signs of ill health in the course of the experiment. In contrast, from day 14 those pullets treated with cadmium alone (group 4) appeared to show increasing signs of ill health as indexed by reduced food and water intake. No similar changes were observed amongst those cadmium-treated pullets that were additionally administered with either FeEDTA or MgEDTA (groups 5 and 6 respectively).
Internally, fluid accumulation was observed in both the thoracic and abdominal cavities of pullets treated with cadmium alone. The adrenal glands of these birds were enlarged and appeared somewhat swollen, compared with those observed in all other groups investigated. Macroscopic examination of the liver revealed the presence of white blotches on one or more lobes in all cadmium-treated pullets, and also in a few animals treated with cadmium and MgEDTA. In those treated with cadmium and FeEDTA, however, the liver was of normal appearance. The kidneys of cadmium-treated pullets showed a highly lobulated appearance with white superficial deposits, perhaps suggestive of either renal necrosis or deposition of insoluble urates in the renal parenchyma. No such changes were noted in the saline control or chelate-treated groups.

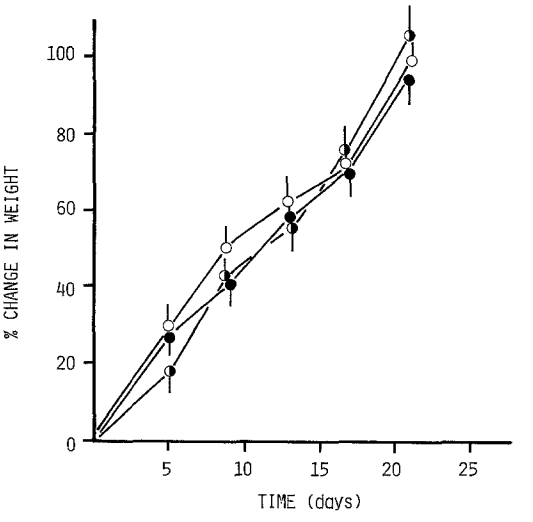


Figure 1. Growth of pullets (mean ± SEM) treated with saline (group 1: n = 4, ●), FeEDTA (group 2: n = 4, ◑) and MgEDTA (group 3: n = 4, ○).

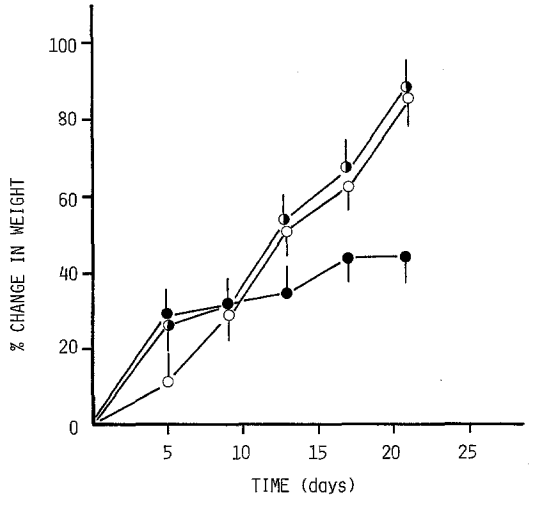


Figure 2. Growth rate of pullets (mean ± SEM) treated with $Cd^{2+}$ in saline (group 4, n = 5, ●), $Cd^{2+}$ plus FeEDTA in saline (group 5, n = 5, ◑) and $Cd^{2+}$ plus MgEDTA in saline (group 6, n = 5, ○). In groups 5 and 6 the cadmium and chelate were administered separately.

### *Effects of cadmium on growth rate of pullets*

Figure 1 shows the pattern of response of pullets treated with saline (group 1) and ferric and magnesium salts of EDTA (groups 2 and 3). There was a progressive increase in weight from day 5 in groups 1, 2 and 3 pullets. By day 21, these increases were 187 ± 8.8 g (95 ± 5%), 216 ± 15 g (105 ± 6%) and 200.5 ± 5.5 g (100 ± 3%) respectively. No significant differences were seen when the growth rate of saline-treated pullets was compared with birds treated with FeEDTA or MgEDTA alone, indicating that chelate administration alone did not markedly affect the growth rate of these pullets.
In the group of cadmium-treated pullets, however, growth rate appeared to be severely restricted, the weight increase being 65 ± 1 g (45 ± 5%) by day 21, compared with increases of 196 ± 22.4 g (88 ± 7%) and 149 ± 5 g (85 ± 6%) for cadmium-treated pullets given additionally either FeEDTA or MgEDTA respectively (fig. 2). Statistical appraisal (Student's t-test) of these results revealed that the growth rates of groups 5 and 6 pullets (cadmium plus FeEDTA and cadmium plus MgEDTA respectively) were highly significantly increased ($p < 0.001$) between days 15 and 21, relative to those observed in birds treated with cadmium alone (group 4).
In addition, comparison of the results obtained from groups 2 and 3 pullets (chelate alone) with those from groups 5 and 6 (cadmium plus chelate) showed no significant difference at day 21. In general, these results indicate that both chelates act to protect the pullets, in terms of growth rate, from the deleterious effects associated with cadmium intoxication.

### *Renal ultrastructural changes associated with cadmium intoxication*

On examination, the electron-micrographs of renal tissue derived from pullets treated with cadmium alone revealed small histological changes in glomeruli and extensive disor-

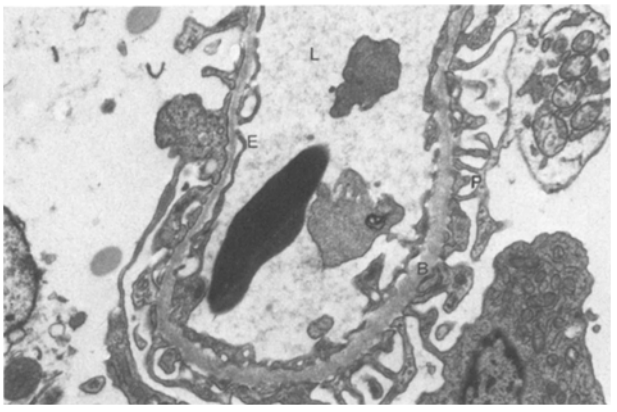


Figure 3. Transmission electron-micrograph of a glomerular capillary loop from a cadmium-treated pullet illustrating endothelium (E), basement membrane (B), epithelium with podocytes (P) and capillary lumen (L). × 7000.

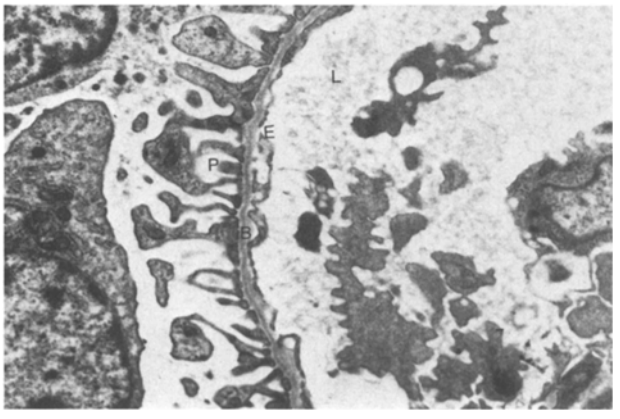


Figure 4. Transmission electron-micrograph of a normal glomerular capillary loop from a saline-treated pullet illlustrating endothelium (E), basement membrane (B), epithelium with podocytes (P) and capillary lumen (L). × 7000.

ganisation of cellular components, particularly with respect to the proximal and distal convoluted tubules.

In pullets treated with cadmium alone, glomerular morphology appeared to be essentially normal, when compared to that in either saline or chelate-treated animals. In all the glomeruli from cadmium-treated birds investigated, however, there was an approximate 25% increase ($43 \pm 0.49$ nm) in the thickness of the basal lamina (fig. 3), compared to that seen in saline or chelate-treated animals. The increased thickness was not seen in those birds treated with either ferric or magnesium chelate in conjunction with cadmium (fig. 4). It is likely that the increased thickness of the glomerular basement membrane may militate against glomerular filtration of both endogenous and exogenous filtrands. This effect has also been noted in ischaemia and hypertension[28], though in these cases the change is supposedly due to shrinkage of the glomerulus, the basal lamina material being accommodated in a smaller volume[29].

Examination of proximal and distal tubular cells revealed vacuolation of the cytosol, and marked dilatation of endoplasmic reticulum (ER) in cadmium-treated birds (fig. 5). This was not seen in the saline-treated control group (group 1) or in any of the groups treated with chelate, either alone (groups 2 and 3) or in conjunction with cadmium (groups 5 and 6).

Based on examination of a number of proximal tubular cells ($n = 40$), absolute numbers of mitochondria seen in cell sections were found to be markedly ($p < 0.02$) reduced in cadmium-treated birds ($58 \pm 4$) compared with those in saline-treated animals ($90 \pm 8$). Calculation of mitochondrial-cytoplasmic ratios (MCrs) in proximal tubule cells, using Loud's method[27] revealed values of 1 : 2.8 in saline-treated birds and 1 : 4.7 in cadmium-treated animals. These findings indicate a reduction in numbers of mitochondria in proximal tubule cells of cadmium-treated individuals. The ratios determined from pullets treated with cadmium and either FeEDTA or MgEDTA were found to be 1 : 2.3 and 1 : 3.2 respectively, indicating no marked differences between the relative efficacy of the two chelates in this respect. Moreover, these MCrs are comparable with the values obtained from proximal tubule cells derived from saline-treated birds. Measurements taken from electron micrographs of proximal tubule cells revealed no overall cell enlargement when cadmium-treated birds were compared with saline-treated animals. In addition to the reduced numbers of mitochondria in cadmium-treated pullets, many of the remaining mitochondria appeared to be somewhat swollen, and occasional cavitation

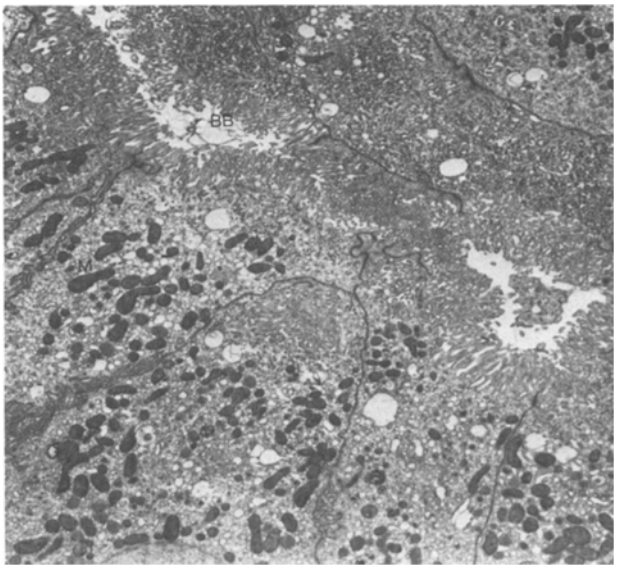


Figure 5. Transmission electron-micrograph of proximal tubule cells from a cadmium-treated pullet, illustrating brush border (BB) and mitochondria (M). × 5000.

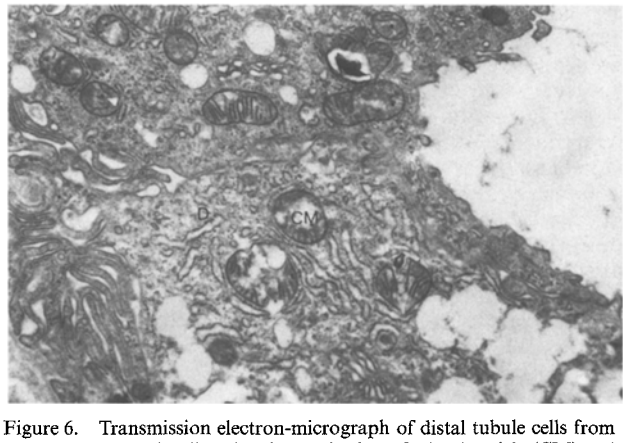


Figure 6. Transmission electron-micrograph of distal tubule cells from a cadmium-treated pullet, showing cavitation of mitochondria (CM) and dilatation of ER (D). × 14,000.

of the matrix, swelling of the cristae and rupture of the outer membrane was observed (fig. 6). Hydropic mitochondria have been seen in numerous conditions[30] and are symptomatic of injury to the cell[29]. The numbers of cristae were also reduced in the mitochondria of proximal cells derived from cadmium-treated animals.

In tissues derived from cadmium-treated pullets, intra-mitochondrial granules were seen, whereas in those derived from the saline-treated control birds (group 1) and from FeEDTA, MgEDTA and cadmium plus chelate groups (2, 3, 5 and 6), intra-mitochondrial granules were not observed.

In general, the cells of the distal tubules of cadmium-treated pullets appeared to be highly vacuolated, compared with control animals. The reduction in numbers of mitochondria, however, was less marked than that seen in the proximal tubules (saline-treated, 42 ± 3; cadmium-treated, 26 ± 2.7), nevertheless there was a pronounced dilatation of ER.

*Discussion*

The present investigation has demonstrated that cadmium intoxication in growing pullets profoundly affects the rate of growth. This restriction in growth rate has also been observed in mammals. In young rats, s.c. injection of 0.75 mg $Cd^{2+}$/kg at either 48-h intervals[31] or three times per week[1] for 8 weeks did not affect weight gain, but injections of 1.5 mg $Cd^{2+}$/kg/d induced a reduction in body weight in rabbits from the second day[32]. Rabbits injected with 1 mg $Cd^{2+}$/kg s.c. for 5 days per week for 8 weeks lost weight, whereas untreated controls increased in weight[33]. In oral administration of cadmium in drinking water at 50 and 300 mg/l, there was a depression in growth rate in rats[12], and in day-old quail, growth retardation was observed when they were fed 75 mg $Cd^{2+}$/kg for 2- or 4-week periods[21]: however in the cases of oral administration, the amount of cadmium absorbed is uncertain[4].

In general terms, it is probable that the reduced growth rate may be attributed to disorganisation of cellular components, combined with a loss of appetite. In pullets treated with either ferric or magnesium salts of EDTA in conjunction with cadmium, however, no significant reduction in growth rate was observed, but the mechanism by which such protection is effected remains unanswered.

It is probable that in the cadmium-treated pullets the pollutant exerted its effects by altering kidney function, effectively preventing its excretion by hepatic and/or renal routes. Such a retention would result in interference with the metabolic processes of the cells and could lead to profound alterations in renal cellular activity involving the maintainance of functions related to excretion.

Massive renal retention of cadmium ultimately resulted in development of renal tubular lesions and atrophy of renal tubules in rats, mice and rabbits[8]. In addition, when rabbits were injected i.p. with a range of doses of cadmium, from 2.5 to 7 mg $Cd^{2+}$/kg/d for 21 days, it was found that up to 3 mg $Cd^{2+}$/kg/d there was no detectable effect at light microscope level, but by 4 mg $Cd^{2+}$/kg/d, extensive hydropic swelling and necrosis of tubular epithelium was produced. Free fluid was also noted in the peritoneal cavity[34], an event that was seen in the current experiments.

In our study, using the lower dose of 0.6 mg $Cd^{2+}$/kg/48 h for 21 days, no major morphological (ultrastructural) changes in the glomerulus were observed, the only change being an increase in thickness of the basement membrane. Similar findings in rats have been reported[13] at a range of oral doses of cadmium chloride (estimated intakes being 3.07 µg, 30.66 µg, 0.31 mg or 1.23 mg $Cd^{2+}$/d for 6 or 12 weeks; the amount of cadmium given per kg body weight, and the amount absorbed from the intestine, however, was unknown). It is possible that an increase in membrane thickness could act to reduce the glomerular filtration rate, and result in the retention of cadmium, unless compensated for by an increased hydrostatic pressure. In short-term studies, it has been shown that cadmium causes a 40% reduction in glomerular filtration rate[35], but whether the reduction could be ascribed to a change in the thixotropic nature of the membrane, or a redistribution of blood away from the kidney remains unexplored. In long-term studies in rats[36] treated with a low oral dosage, a thickening in renal arterial walls and glomerular changes characteristic of the associated hypertension was observed, and in an EM study, the presence of thromboses in glomerular and peritubular capillaries, and collagen deposits in the glomeruli were demonstrated[37]. These previous studies appear to give support to the possibility of retention not only of the pollutant, but also of fluid, the latter leading to oedema, a condition observed in cadmium-treated pullets in the current study. The development of oedema, however, was not evident in birds treated with a combination of cadmium and either of the chelates.

In addition to membrane thickening in the cadmium-treated pullets, extensive cellular disorganisation was seen. The reduction in numbers of mitochondria, together with the presence of degenerated cristae in the remaining, somewhat swollen mitochondria would seriously affect those energy-requiring processes involved in tubular reabsorption of glomerular filtrands. It is likely that the cadmium displaces the iron moiety from metallo-flavoprotein enzymes, and in so doing inhibits the activity of those enzymes involved in ATP production. It has been pointed out that mitochondria that are swollen and otherwise damaged would lead to a depression in ATP production, inducing failure of the sodium pump at the cell membrane[29]. This in turn could cause flooding with water, and may partly explain the pronounced vacuolation of the cytosol and dilatation of the ER observed in cadmium-treated individuals, and the fluid accumulation noted at autopsy.

It is known that mitochondrial swelling can be of two types, passive (due to osmosis) and active, which is electron-transport dependent[29]. The latter may be induced by various substances such as calcium, mercury and phosphorous ions; fatty acids, hydrocortisone, insulin, oxytocin, thyroxine and vasopressin[38, 39], and from various experiments it has been demonstrated that cadmium affects mitochondrial structure in mammalian and avian renal tissue[4, 12, 16]. It has been reported that in many pathological instances, it is likely that active swelling occurs initially, and after cell flooding, passive swelling will ensue[29]. It must be admitted, however, that artefactual and pathological swelling can be very difficult to differentiate.

The disruption of mitochondrial activity by displacement of functional endogenous cations has been suggested[40] as a biochemical basis for the toxic effects of cadmium on nerve membranes, mitochondria and kidney tubules in mammals, and it is known that cadmium has a greater affinity than calcium for phospholipid monolayers of biomembranes[41]. It seems likely, therefore, that both structural and biochemical alterations result from cadmium toxicity, as has previously been suggested[4]. It may be that the intra-mitochondrial granules seen in the proximal cells of birds treated with cadmium alone are symptomatic of mitochondrial functional disruption.

In vitro studies have revealed that certain mitochondrial enzymes (such as keto-acid dehydrogenase) are inhibited by cadmium[42, 43], and it has been suggested[44] that this susceptibility to inhibition may be due to the high metal chelating qualities of the reduced forms of the lipoic acid coenzymes, which indirectly are deactivated by cadmium[45]. It has been shown that even very small cadmium concentrations disrupted mitochondrial function in vitro (e.g. 1.6 µM $Cd^{2+}$ causes 50% uncoupling of succinate oxidation)[46]; however,

whether cadmium inhibits mitochondrial functions in vivo will depend on its intracellular concentration.

In pullets treated with cadmium and FeEDTA, there was significantly less disruption of cell integrity, in terms of mitochondrial number, damage to ER and vacuolation, than that seen in birds treated with cadmium alone. It is suggested that the ferric moiety of the EDTA salt would compete successfully with cadmium for available anionic sites on iron-requiring flavo-protein and other mitochondrial enzymes, thereby protecting mitochondrial activity. In addition, the presence of EDTA would provide available sites for cadmium attachment, which could facilitate excretion of the heavy metal toxin. Provision of a 'pool' of iron could also be of significance in preventing the anaemia and reduction in haematocrit that has been observed not only in rabbits[33], rats[47], growing rats[48] and chicks[49] but also in the domestic fowl exposed to i.p. injections of 1.078 mg $Cd^{2+}$/kg/d for 15–22 days[50] and in quail fed with 75 mg $Cd^{2+}$/kg for periods of 1–4 weeks[51]. Parenteral or dietary addition of iron has been shown to prevent cadmium-induced anaemia in mice and rats[52].

The use of the magnesium chelate may similarly provide a pool of magnesium, which could be of significance in maintaining the structural integrity of cell membranes. It has been reported[26] that such structural integrity is frequently determined by the presence of metallic ions, principally calcium and magnesium[53, 54] which are loosely bound, and therefore potentially displacable by other cations (for example cadmium).

In conclusion, this study suggests that the two chelates (ferric monosodium and magnesium disodium salts of EDTA) are effective in protecting pullets from the effects of cadmium intoxication. The mechanism by which the chelates exert their protective influence, however, remains to be fully explained.


1 Faeder, E. J., Chaney, S. Q., King, L. C., Hinners, T. A., Bruce, R., and Fowler, B. A., Toxic. appl. Pharmac. *39* (1977) 473.
2 Kazantis, G., Envir. Health Perspect. *28* (1979) 155.
3 Lauwreys, R., in: The Chemistry, Biochemistry and Biology of Cadmium, p. 433. Ed. M. Webb. Elsevier/North Holland, Amsterdam 1979.
4 Samarawickrama, G. P., in: The Chemistry, Biochemistry and Biology of Cadmium, p. 341. Ed. M. Webb. Elsevier/North Holland, Amsterdam 1979.
5 Webb, M., in: The Chemistry, Biochemistry and Biology of Cadmium, p. 285. Ed. M. Webb. Elsevier/North Holland, Amsterdam 1979.
6 Fassett, D. W., in: Metals in the Environment, p. 61. Ed. H. A. Waldron. Academic Press, New York 1980.
7 Sabbioni, E., Marafante, E., Pietra, R. Amantini, L., and Ubertalli, L., Proc. Cadmium Symposium, Jena 1978.
8 Friberg, L., Piscator, M., Nordberg, G. F., and Kjellstrom, T., in: Cadmium in the Environment, 2nd edn. C.R.C. Press, Cleveland 1974.
9 Piscator, M., in: Cadmium, Handb. exp. Pharmac., vol. 80, p. 179. Ed. E.C. Foulkes, Springer-Verlag, Berlin 1986.
10 Hoffman, E. O., Cook, J. A., DiLuzio, N. R., and Coover, J. A., Lab. Invest. *32* (1975) 655.
11 Gonick, H., Indraprasit, S., Neustein, H., and Rosen, V., Curr. Probl. clin. Biochem. *4* (1975) 111.
12 Nishizumi, K., Archs envir. Health *24* (1972) 215.
13 Fowler, B. A., Jones, H. S., Brown, H. W., and Haseman, J. K., Toxic. appl. Pharmac. *34* (1975) 233.
14 Axelsson, B., Dahlgren, S. E., and Piscator, M., Archs envir. Health *17* (1968) 24.
15 Stowe, H. D., Wilson, M., and Goyer, R. A., Archs Path. *94* (1972) 389.
16 Nicholson, J. K., Kendal, M. D., and Osborn, D., Nature *304* (1983) 633.
17 Larsson, S. E., and Piscator, M., Israel J. med. Sci. *7* (1971) 495.
18 Piscator, M., and Larsson, S. E., Proc. 17th int. Congr. Occup. Health, Buenos Aires 1972.
19 Itokowa, Y., and Tanaka, S., Arch envir. Health *26* (1973) 241.
20 Jacobs, R. M., Fox, M. R. S., and Fry, B. E., Fedn Proc. *31* (1972) 2724.
21 Fox, M. R. S., Fry, B. E., Harland, B. F., Schertel, M. E., and Weeks, C. E., J. Nutr. *101* (1971) 1295.
22 Jones, M. M., and Pratt, T. H., J. chem. Educ. *53* (1976) 342.
23 Cantilena, L. R., and Klaassen, C. D., Toxic. appl. Pharmac. *53* (1980) 510.
24 Ragan, H. A., J. Lab. clin. Med. *90* (1977) 700.
25 Hamilton, D. L., and Valberg, L. S., Am. J. Physiol. *227* (1974) 1033.
26 Reynolds, J. A., Ann. N.Y. Acad. Sci. *195* (1972) 75.
27 Loud, A. V., J. Cell Biol. *15* (1962) 481.
28 Jenis, E. H., and Lowenthal, D. T., Kidney Biopsy Interpretation. F. A. Davis, Philadelphia 1977.
29 Ghadially, F. N., Ultrastructural Pathology of the Cell and Matrix, 2nd edn. Butterworths, London 1982.
30 David, H., Submicroscopic Ortho and Pathomorphology of the Liver: translated by H. G. Epstein. Akademie-Verlag, Berlin; Pergamon Press, Oxford 1964.
31 King, L. C., Clark, V., and Faeder, E. J., Bull. envir. Contam. Toxic. *16* (1976) 572.
32 Nomiyama, K., Sato, C., and Yamamoto, A., Toxic. appl. Pharmac. *24* (1973) 625.
33 Berlin, M., Fredricsson, B., and Linge, G., Archs envir. Health *3* (1961) 176.
34 Bonnell, J. A., Ross, J. H., and King, E., Br. J. ind. Med. *17* (1960) 69.
35 Prashad, D. N., Hawkins, E. A., Blackburn, R., and Haslam, J., Experientia *42* (1986) 389.
36 Kanisawa, M., and Schroeder, H. A., Expl molec. Path. *10* (1969) 81.
37 Berry, J. P., Path. Biol. *20* (1972) 401.
38 Lehninger, A. L., Physiol. Rev. *42* (1962) 467.
39 Lehninger, A. L., The Mitochondrion. Molecular Basis of Structure and Function. W. A. Benjamin Inc., New York 1965.
40 Vallee, B. L., and Ulmer, D. D., A. Rev. Biochem. *41* (1972) 91.
41 Suzuki, Y., and Matsushita, H., Ind. Health *6* (1968) 128.
42 Sanadi, D. R., Langley, M., and White, F., J. biol. Chem. *234* (1959) 183.
43 Webb, M., Biochim. biophys. Acta *89* (1964) 431.
44 Webb, M., in: Clinical Chemistry and Chemical Toxicology of Metals, p. 51. Ed. S. S. Brown. Elsevier/North Holland, Amsterdam 1977.
45 Stein, A. M., and Stein, J. H., J. biol. Chem. *246* (1971) 670.
46 Jacobs, E. E., Jacob, M., Sanadi, D. R., and Bradley, L. B., J. biol. Chem. *223* (1956) 147.
47 Pindborg, E. V., Pindborg, J. J., and Plum, C. M., Acta pharmac. toxic. *2* (1946) 302.
48 Pond, W. G., and Walker, E. F., Nutr. Rep. Int. *5* (1972) 365.
49 Hill, C. H., Matrone, G., Payne, W. L., and Barber, C. W., J. Nutr. *80* (1963) 227.
50 Sturkie, P. D., Avian Dis. *17* (1973) 106.
51 Jacobs, R. M., Fox, M. R. S., and Aldridge, M. H., J. Nutr. *99* (1969) 119.
52 Bunn, C. R., and Matrone, G., J. Nutr. *90* (1966) 395.
53 Tidball, C. S., Am. J. Physiol. *206* (1964) 243.
54 Burger, S. P., Fujii, T., and Hanahan, D. J., Biochemistry *7* (1968) 3681.


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