

# Morphological Changes in Duodenal Epithelium of Japanese Quail after Chronic Cadmium Exposure

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Received: 3 June 2009

Accepted: 9 November 2009

## Abstract

Our study investigated morphological changes in enterocytes of adult Japanese quails that were given cadmium ( $\text{CdCl}_2$ ) perorally and individually by tube, dissolved in water at a dose of 0.24 mg Cd per animal per day, for 57 and 118 days. The aim of our study was to observe chronic effects of cadmium on the structure of duodenal epithelium by means of light microscopy (LM) and transmission electron microscopy (TEM). On day 57, following peroral administration of cadmium, necrotizing enterocytes were found in the apical part of intestinal villi and their occurrence was only sporadic. Particularly on day 118 following cadmium administration, we were able to observe clusters of 2-3 necrotizing cells in the apical part of intestinal villi. However, the structure and ultrastructure of goblet cells was normal. The most notable finding in ultrastructure of all enterocytes of treated animals was the damage to cell organelles. Mitochondria and cisternae of the endoplasmic reticulum were more or less damaged and the cytoplasm contained flocculent material, particularly in the basal part of enterocytes. Some enterocytes exhibited signs of necrosis, shrivelled nucleus and damaged organelles within the markedly electrondense cytoplasm. Microvilli on the apical surface of these enterocytes were damaged and disintegrated. Junctions between cells of the intestinal epithelium were disturbed, and a present of intracellular plaques associated with the adhering and occluding junctions was observed. Cadmium caused the formation of gaps within the specialized junctional complexes, and injury to enterocytes results in the breakdown of the intercellular attachments and the sloughing of the injured cells into the intestinal lumen.

**Keywords:** cadmium, small intestine, enterocytes, structure and ultrastructure, Japanese quail

## Introduction

Cadmium is a heavy metal recognized as an industrial and environmental pollutant with characteristic long biological decomposition and cumulative toxic effect on humans and all live organisms. The importance of revealing its toxic effects has increased, particularly in the past decade, as its concentration increases constantly in all components of our environment, i.e. in water, soil and air.

It enters live organisms by inhalation and particularly by the peroral way. Cadmium gets into soil predominantly due to the application of phosphate fertilizers, some pesticides, wastes and wastewater treatment sludge. From the contaminated soil it passes particularly to leafy plants [1]. Extremely high levels of this metal were found in mushrooms. This results in its subsequent increased uptake by live systems and incorporation into the food chain of animals and man. New types of sources of this heavy metal have been discovered increasingly, particularly in industrially advanced countries. Acute intoxication with cadmium

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is rare, more frequently we observe its chronic cumulative effect on organisms resulting frequently in metabolic disorders and, subsequently, to pathological changes some organs [2].

Cadmium can damage a number of organs, including the gastrointestinal tract. When taken up perorally, it is only little absorbed in the mouth cavity and the stomach [3]. However, it is absorbed well in the intestine, particularly in the duodenum and less in the jejunum and ileum [4-6]. Thus the intestinal single-layered epithelium is the first barrier against absorption of cadmium.

The mechanism of absorption of Cd in the gastrointestinal tract is still unknown. Cellular injury induced by Cd is dependent on a number of factors, including dose, route of exposure, and duration of exposure. However, its absorption is affected by a number of factors, particularly the presence or deficiency of certain compounds in the feed. Intestinal absorption of cadmium rapidly increases iron depletion, resulting in expression of divalent metal transporters (DMT1). Enterocytes have been shown to be present in the luminal plasma membrane. These divalent transporters of metals are non-specific transporters as they may transport not only iron but also other metals [6-8]. Although the specific mechanism(s) involved in the absorptive transport of Cd is/are not known presently, there is a growing body of evidence indicating that DMT1 likely plays a key role in transporting Cd into enterocytes. DMT1 is a proton-coupled, membrane potential-sensitive transport protein that is capable of transporting a number of divalent cations. The level of expression of this transporter decreases along the length of the intestinal tract, with the highest levels expressed in the proximal duodenum and the lowest levels expressed toward the distal colon. According to Min et al., [9] essential metals can affect the metabolism of nonessential metals. It has been suggested that Fe deficiency increases intestinal absorption of Cd via DMT1. They studied the effect of nutritional status of Ca, Cu, Mg, Zn, and Fe that is most often ingested by humans at levels below recommended dietary allowances on tissue accumulation of orally administered Cd. Hepatic Cd accumulation was significantly increased after oral Cd administration in all essential metals deficient diets mice, but not in any essential metals-supplemented mice. These results suggested that DMT1 is not the sole transporter of Cd, and that Cd is absorbed and accumulated through multiple pathways. Therefore, essential metals nutritional status is a risk factor for increasing hepatic accumulation of ingested Cd.

According Zalusps and Ahmad [10] one of the potential mechanisms in the uptake of Cd by enterocytes is endocytosis of proteins, including metallothionein (MT), to which Cd is bound. However, there is very little information about the role of endocytosis in the luminal uptake of Cd by enterocytes. Following ingestion of a protein-containing meal, oligopeptides and amino acids are formed in the lumen of the small intestine by pancreatic enzymes and enzymes localized on the luminal plasma membrane on enterocytes. Amino acid and oligopeptide transport may be involved in the absorptive transport of Cysteine-S-conjugates of Cd

along the small intestine. When Cd binds to DMT1, through some form of ionic homology or "mimicry", it is transported into the cytosolic compartment of enterocytes. Recent findings indicate that the luminal uptake of Cd can also occur through one of the luminal transporters of zinc – ZTL1. It is also possible that some forms of Cd may "leak" from the luminal compartment into the basolateral compartment through the relatively leaky junctional complexes between adjacent enterocytes. Intercellular leak of Cd is also likely to occur when enterocytes begin to become intoxicated by Cd. As the intracellular pool of Cd accumulates within the enterocytes, this pool can interact with various intracellular components and compartments within the cells. Some of the Cd ions induce the transcription of the genes for metallothionein (MT). Increases in the cellular content of MT protein result from the translation of the increased amounts of mRNA for MT that is induced following exposure to Cd. The induced MT protein serves as a sink to bind some of the intracellular Cd, which results in increased retention of Cd within the enterocytes. If the intracellular pool of exchange Cd increases beyond what the protective elements inside the enterocyte can handle, oxidative stress is induced, which in turn can alter mitochondrial respiratory activity and lead to lipid peroxidation in the plasma membrane and other perturbations in cellular metabolism. All of these effects lead to the induction of cell death by either necrosis or apoptosis.

Foulkes and colleagues [11-13] have proposed a two-step process for the absorptive movement of Cd ions from the intestinal lumen into enterocytes. Using everted sacs formed from portions of the jejunum, they showed that the absorption of Cd into enterocytes was preceded by the movement of Cd ions into a compartment that was accessible to chelators, but was insensible to temperature. The Cd associated with this compartment likely represented non-specific binding of Cd to the luminal plasma membrane. The second step in the absorptive process appeared to involve the slower movement of Cd into a temperature-sensitive compartment that was not accessible to chelators. This second step likely represented the actual movement or transport of Cd across the luminal plasma membrane into the enterocytes.

The high accumulation of Cd in different target organs has often been associated with high metallothionein (MT) concentrations found in this organ [14]. Metallothionein are low molecular weight intracellular proteins that are characterized by their inducibility upon exposure to heavy metals, and their extremely high cysteine residue content and metal-binding activity. They are also antioxidant proteins induced in cells under oxidative stress conditions. MT that is induced in enterocytes plays an important role in the retention of Cd within the mucosa of the small intestine after the ingestion of Cd [15]. Retention of Cd by enterocytes would reduce the amount of Cd entering into systemic circulation, which in turn would decrease the load of Cd delivered to target organs, such as the kidneys and liver [16].

Cadmium ions exhibit high affinity to sulphydryl groups and thiol anions present in intracellular and extracellular components [17]. Well known is an interaction

Table 1. Composition of complete mixed feed HYD-10.

Fed composition	Minerals		Vitamins		
Metabolizable energy	11.5 MJ·kg <sup>-1</sup>	Ca	40.0 g·kg <sup>-1</sup>	A	8.000 IU·kg <sup>-1</sup>
Ash	160.0 g·kg <sup>-1</sup>	P	5.0 g·kg <sup>-1</sup>	D <sub>3</sub>	1.600 IU·kg <sup>-1</sup>
Fiber	60.0 g·kg <sup>-1</sup>	Na	2.3 g·kg <sup>-1</sup>	E	10 mg·kg <sup>-1</sup>
Crude protein	153.0 g·kg <sup>-1</sup>	Zn	60.0 mg·kg <sup>-1</sup>	B <sub>2</sub>	4 mg·kg <sup>-1</sup>
Lysine	7.0 g·kg <sup>-1</sup>	Mn	40.0 mg·kg <sup>-1</sup>	B <sub>12</sub>	10 µg·kg <sup>-1</sup>
Methionine + cysteine	6.0 g·kg <sup>-1</sup>	Fe	40.0 mg·kg <sup>-1</sup>		
Methionine	3.5 g·kg <sup>-1</sup>	Cu	4.0 mg·kg <sup>-1</sup>		
Linoleic acid	15.0 g·kg <sup>-1</sup>				

between ions of cadmium and calcium. ATP controls the calcium transport across cytoplasmic membranes and even low concentrations of cadmium are capable of inhibiting calcium transport into intestinal epithelium. Interaction between Cd<sup>2+</sup> and Ca<sup>2+</sup> on the plasma membrane could lead to reduced Cd influx if both cations were present extracellularly [18]. Theoretically, extracellular calcium would be capable of binding to the same sites to which cadmium was bound. Calcium and cadmium could have an affinity for the same chemical groups on the enterocyte membrane. This property could affect the intestinal absorption of amino acids. Several studies have shown that an important relationship exists between the toxic effects of cadmium and the level of calcium in the diet [19].

The primary sites of cadmium action are biological membranes because cadmium ions react easily with membrane phospholipids. Cadmium can penetrate into the cell and disturb the synthesis of nucleic acids that is the basis of its mutagenic effect, chromosomal aberrations, carcinogenic and teratogenic effects and necrosis. Cadmium is one of the heavy metals showing pronounced immunosuppressive effects [20, 21] and its carcinogenic effects also have been established [22, 23].

The object of this experimental work was to study morphological changes in enterocytes and goblet cells of adult Japanese quails that were given cadmium (CdCl<sub>2</sub>) perorally and individually by tube, dissolved in water at a dose of 0.24 mg Cd per animal and day, for 57 and 118 days. The aim of our study was to observe chronic effects of cadmium on the structure of duodenal epithelium by means of light microscopy (LM) and transmission electron microscopy (TEM).

## Experimental Procedures

### Animals

This study used 3-week-old male Japanese quails (n=50). After a week of acclimatization, the animals were divided into groups.

The first experiment was conducted on 15 experimental (n=15) and 10 control (n=10) quails. The experimental quails were daily administered individually by tube CdCl<sub>2</sub> dissolved in water at a dose of 0.24 mg Cd/head/day for 57 days. The mean weight of control animals was 180.0 g, and experimental animals 172.3 g.

Fifteen experimental quails (n=15) from the second group were administered cadmium for 118 days, and an additional 10 quails (n=10) served as a control. The mean weight of control animals was 169.7 g, and experimental Japanese quails 160.1 g.

The birds were fed complete mixed feed HYD-10, throughout the experiment (Table 1). Feed and water was provided *ad libitum*. The mean consumption of 1 quail was about 40 g feed per day. The composition of the diet was in compliance with the Regulation of the Government of the Slovak Republic No. 440/2006. The maximum allowed limit for Cd concentration in feeds according to the Regulations of the Government of Slovak Republic Nos. 347 and 438/2006 is 0.5 mg/kg at 12% moisture. The Cd content in feed (0.007 mg·kg<sup>-1</sup>) and water (0.001 mg·l<sup>-1</sup>) used in the experiment and for watering the birds varied below the limit value. Therefore, the birds were not exposed to an excessive supply of cadmium by feed and water. The Japanese quails were kept in cages under microclimatic conditions favourable for their growth and welfare. The experiments were approved by the local ethical commission and the State Veterinary and Food Agency (ŠVPS SR Č. k. Ro-7879/04-220/3).

After experiments the birds were euthanized with ether, decapitated and samples were withdrawn from the duodenum for light and electron microscopic examination.

## Methods

Excisions for light microscopy (LM) and transmission electron microscopy (TEM) were fixed by immersion in 3% glutaraldehyde for 3 hours and subsequently postfixed in 1% osmium oxide in phosphate buffer (pH 7.4), dehydrated in acetone and propylenoxide and embedded in Durcupan ACM (Fluka). The semithin sections for LM

were stained with toluidine blue and observed under a Jenamed light microscope. Ultrathin sections for TEM were contrasted with uranyl acetate and lead citrate and examined under a transmission electron microscope (TEM) Tesla BS 500.

## Results

### Light Microscopic Observations

Examination of the duodenal epithelium by light microscopic (LM) of semithin sections from birds exposed to cadmium for 57 days showed no marked morphological changes except for scattered vacuolization of cytoplasm in some enterocytes (Fig. 1). Necrotizing enterocytes were found in the apical part of intestinal villi and their occurrence was only sporadic. The brush border on the surface of

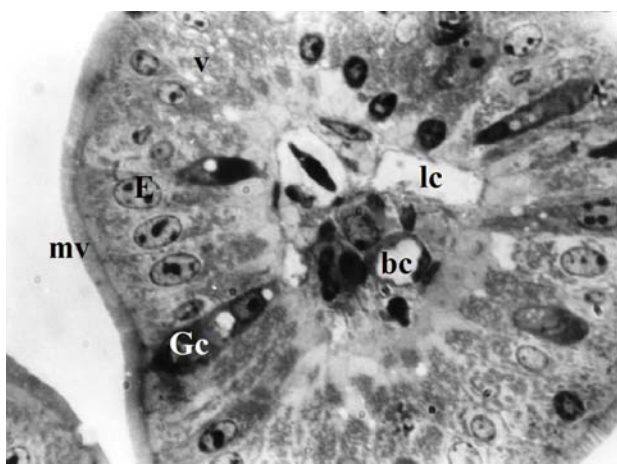


Fig. 1. Cross-section of an intestinal villus – 57-day peroral administration of cadmium (semithin section, 400 x). E – enterocytes; mv – microvilli (brush border); v – vacuoles; Gc – goblet cell; lc – lymph capillary; bc – blood capillary.

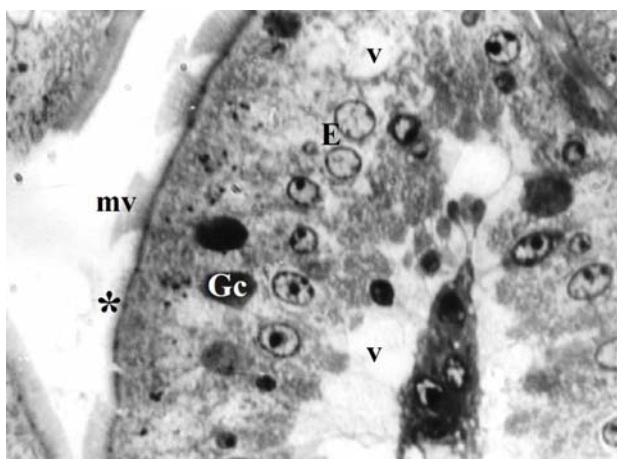


Fig. 2. Cross-section of an intestinal villus – 118-day peroral administration of cadmium (semithin section, 400 x). E – enterocytes; mv – microvilli (brush border); v – vacuoles; asterisk – damaged microvilli (brush border); Gc – goblet cell.

enterocytes, consisting of numerous microvilli, was continuous. The structure of mucin-producing goblet cells was normal and they were dispersed relatively evenly throughout the intestinal lining membrane. Endothelial cells in blood vessels and lymph capillaries, observed in the central part of intestinal villi, showed no signs of damage.

Following the 118-day peroral administration of cadmium, brush border on the surface of intestinal villi was disturbed and many intestinal cells showed signs of necrotization (Fig. 2). The presence of flocculent material in enterocyte cytoplasm was observed, particularly in the basal part of the cells.

### Electron Microscopic Observations

The most notable finding in ultrastructure of all enterocytes of treated animals was the damage to cell organelles.

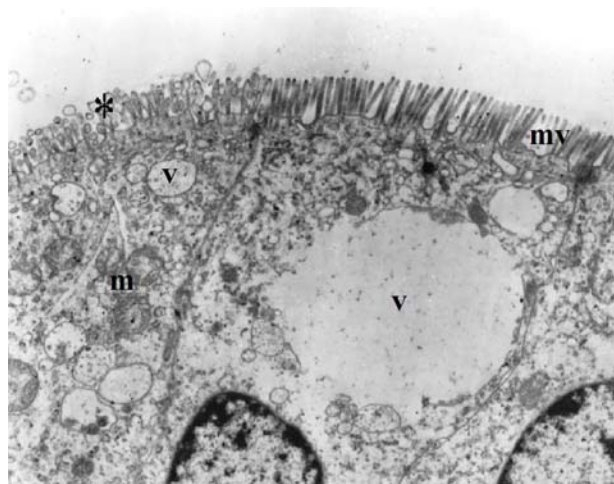


Fig. 3. Apical part of the enterocytes – 57-day peroral administration of cadmium (electronmicrograph, 10 000 x). mv – microvilli; m – mitochondria; v – vacuoles; asterisk – damaged microvilli.

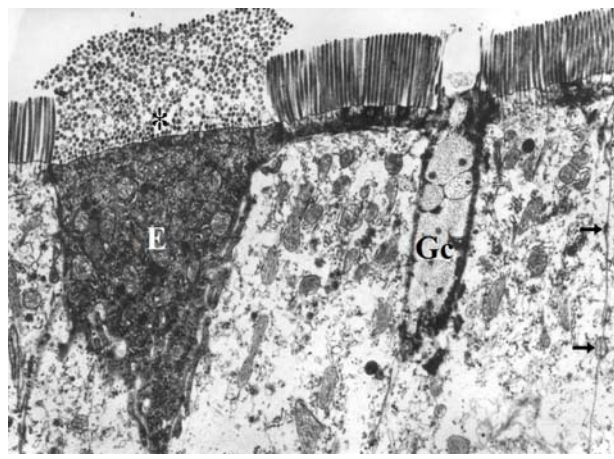


Fig. 4. Necrotizing enterocyte with electron-dense cytoplasm and damaged microvilli – 118-day peroral administration of cadmium (electronmicrograph, 8 000 x). E – necrotizing enterocyte; asterisk – damaged microvilli; Gc – goblet cell; arrows – intercellular junctions.

Mitochondria and cisternae of the endoplasmic reticulum were more or less damaged in experimental quails from both groups and the cytoplasm, particularly that in the basal part, contained flocculent material (Figs. 3 and 4). Some enterocytes exhibited signs of necrosis, shrivelled nucleus and damaged organelles within the markedly electrondense cytoplasm. Microvilli on the apical surface of these enterocytes were damaged and disintegrated. Junctions between cells of the intestinal epithelium were disturbed. Cadmium caused the formation of gaps within the specialized junctional complexes. Initial disruption of cell-cell junctions coincided with a present of intracellular plaques associated with the adhering and occluding junctions. However, the ultrastructure of goblet cells was normal. In the central part of intestinal villi lymph capillaries with irregular lumen but preserved structure were observed. The blood capillaries were continuous and for birds typical nucleated erythrocytes and liquid with some flocculent material in their lumen was observed (Fig. 5).

### Discussion

Over the last 15 years, birds have been used extensively as monitors for pollutants. They are easy to identify and study, which make them ideal for monitoring [24]. Japanese quails (*Coturnix coturnix japonica*) are kept for egg and meat production. Quails have been used for investigation of physiological processes in the body of the fowl and appear suitable for observation of interactions between essential chemical elements and xenobiotics under in vivo conditions.

However, only a few studies have shown that orally administered cadmium can cause tissue damage in the gastrointestinal tract in birds. Enteropathy of the small intestine after cadmium feeding in Japanese quail has been reported by Richardson et al. [25]. The mechanism of induced damage in the intestinal mucosa due to cadmium administration is still unknown.

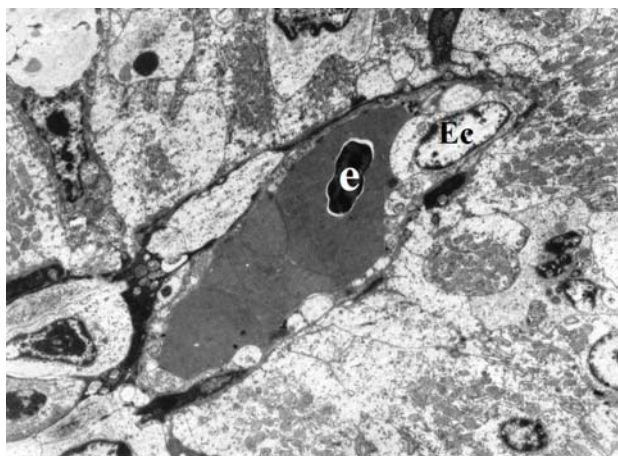


Fig. 5. Basal part of enterocytes and blood vessel with erythrocytes – 118-day peroral administration of cadmium (electron-micrograph, 8,000 x). Ec – endothelial cell; e – erythrocytes.

The purpose of this study was to describe structural and ultrastructural changes in the Japanese quail enterocytes and goblet cells after an experimental administration of cadmium chloride. From birds exposed to cadmium for 57 days by light microscopy, necrotizing enterocytes were found in the apical part of intestinal villi; however, their occurrence was only sporadic and indicated physiological replacement of these cells. The life span of enterocytes is relatively short and their replacement depends on functional load. When using electron microscopy, the most notable finding in all treated animals was the presence of damaged mitochondria in the cytoplasm of enterocytes. Other morphological alterations included cell vacuolization, decreased number of cytoplasmic organelles and a decrease in the number of surface microvilli.

On the other hand, in enterocytes of experimental birds exposed to cadmium for 118 days, many enterocytes exhibited signs of necrotization. Ultrastructural analysis showed that the most notable finding in ultrastructure of all enterocytes of treated animals was the damage to cell organelles. Mitochondria and cisternae of the endoplasmic reticulum were damaged. Flocculent material was found in the cytoplasm of damaged enterocytes, particularly in their basal part. We assume that its presence in the cytoplasm of enterocytes may be related to the production of metallothioneine (MT), capable of binding metals and therefore also cadmium, and cumulating them in enterocytes. The cumulated cadmium subsequently damages the cell organelles, particularly mitochondria and cisternae of endoplasmic reticulum. Elsenhans et al. [4] reported that low doses of perorally administered cadmium induce production of intestinal metallothioneine and cadmium-methalothioneine complexes responsible for gradual accumulation of cadmium in kidneys, particularly at chronic intoxications. According to Berzina et al. [26] it is likely that the accumulated cadmium in the intestinal mucosa exceeds the binding capacity of MT, which cannot prevent the induction of lipid peroxidation by ligand-free cadmium. Under these circumstances oxidative damage of intestinal mucosa can compromise the epithelial barrier by inducing premature enterocytes apoptosis and desquamation. Heavy metal induced oxidative stress and changes in physiological processes via free radicals [27].

In this study, microvilli on the apical surface of necrotizing enterocytes were damaged and disintegrated, and junctions between cells of the intestinal epithelium were disturbed. The structure of blood and lymph capillaries was not disturbed. We failed to observe any changes in the ultrastructure of endothelial cells and pericytes. The lumen of blood capillaries contained nucleated erythrocytes and liquid with flocculent material. According Druizer et al. [28], cadmium disturbs the connection between cells of the intestinal mucosa, which makes it easier for this metal to pass across the walls of the digestive system. The idea that the vascular endothelium is an important target of Cd toxicity stemmed from an observation by Alsberg and Schwartz (1919) [29] when they reported that acute exposure to subcutaneously administered Cd in rats caused purple discoloration of the testes. Later studies showed that Cd

produced this effect by causing the breakdown of the junctions between the endothelial cells of the testicular capillaries and venules, resulting in an increase in vascular permeability, followed by edema, hemorrhage, and testicular necrosis. A great deal of morphologic and biochemical evidence indicated that these effects of Cd on microvascular permeability resulted from direct actions of Cd on the endothelial cells in this particular vascular bed. However, this also raised the intriguing question as to why the endothelial cells in the testis were sensitive to this effect of Cd, whereas the endothelial cells in most other vascular beds were not affected [30]. Recent studies have provided new insights into the mechanisms by which metals can influence vascular function. Expression of the ZIP8 metal ion transporter appears to be a key factor contributing to the selective toxicity of Cd in the endothelial cells of organs such as the testes and kidneys. Microvascular effects of Cd involve alterations in the function of the Ca-dependent cell adhesion molecule VE-cadherin (vascular endothelial cadherin). E-cadherin is the dominant cadherin expressed in most epithelial cells. Both are single pass transmembrane proteins that are usually localized at adherens-type cell-cell junctions.

The finding that the cadherins might be targets of Cd toxicity arises from a series of observations by Prozialek and coworkers [31, 32], who found that exposing cultured renal epithelial to Cd caused the cells to separate from each other. This effect coincided with the loss of E-cadherin from the cell-cell contacts and a reorganization of the actin cytoskeleton. Cd can disrupt junctional complexes between epithelial cells by altering the Ca-dependent, E-cadherin/beta-catenin system that is part of the zonula adherens of the junctional complexes. Disruption of intercellular junctions would clearly provide a path for Cd-MT or other forms of Cd to pass between adjacent enterocytes and enter the lamina propria of the intestinal mucosa.

Ultrastructural analysis showed that cadmium caused the formation of gaps within the zonulae adherens between LLC-PK1 cells (porcine renal epithelial cell line) [33]. This effect increased with time of exposure and paralleled an increase in the space between cells and a change in the shape of the cells from squamous to round. These results indicate that Cd has relatively specific damaging effects on the adhering and occluding junctions, and that these effects may involve the disruption of cytoskeletal actin filaments.

According to Prozialek and Edwards [34] changes in cell adhesion molecule function also appear to play important roles in the signalling cascades and alterations in gene expression that can lead to apoptotic or necrotic death of the injured cells. These junctional complexes are necessary for restriction of permeability, the establishment of epithelial polarity, traffic of membrane proteins to either the apical or the basolateral cell surface and, ultimately, the normal transport of solutes and electrolytes across the enterocytes. Our study provides further evidence in support of this.

Morphological changes in kidney and testes vessels resulting from chronic cadmium exposure have been observed by many investigators, and adverse effects of various xenobiotics were reported [35, 36]. Cadmium passes

from the intestine into organs copiously supplied with blood, particularly kidneys and liver, [37-41] but also to reproductive organs of both males and females, resulting in subsequent adverse effects on reproduction and others. During chronic exposure to cadmium, the kidneys are usually the most critically affected organs. The metal accumulates in renal cortex and leads to renal tubular dysfunction. Increased apoptosis was seen in the proximal tubules epithelium, and degeneration of the endothelial cells in peritubular capillaries was increased [41]. The injury affects the main resorptive part (proximal convoluted tubules) and partially the filtering part (glomeruli) of the nephron [42, 43]. There are also various effects on reproduction that cause follicular atresia [44], degenerative alterations in testes, and decreases in spermatozoa motility [45-47].

## Conclusions

Our study investigated the chronic effects of cadmium on structure and ultrastructure of duodenal epithelium of Japanese quail. We observed an adverse effect of cadmium on enterocytes which showed more or less intensive damage and, for some of them, even necrosis or apoptosis. However, the structure and ultrastructure of goblet cells was normal, and they were interspersed between the absorptive cells-enterocytes. Particularly on day 118 following peroral administration of cadmium, we were able to observe clusters of 2-3 necrotizing cells in the apical part of intestinal villi. Damage to brush border on the surface of intestinal villi and the presence of flocculent material in enterocyte cytoplasm was observed. A very important finding of this study is that cadmium caused the formation of gaps within the specialized junctional complexes. Junctions between cells of the intestinal epithelium were disturbed. Initial disruption of cell-cell junctions coincided with the presence of intracellular plaques associated with the adhering and occluding junctions. The sloughing of the injured cells into the intestinal lumen was observed.

The mechanism of absorption of Cd in the enterocytes is still unknown, and we believe this initial study may provide for further more detailed studies of the chronic effects of cadmium on the structure and ultrastructure of duodenal epithelium cells.

## References

1. BARANOWSKA I., SROGI K., WLOCHOWICZ A., SZCZEPANIK K. Determination of heavy metal contents in samples of medicinal herbs. *Polish J. Environ. Stud.* **11**, 467, **2002**.
2. HUGHES M. R., SMITS J. E., ELLIOT J. E., BENNETT D. C. Morphological and pathological effects of cadmium ingestion on Peking ducks exposed to saline. *J. Toxicol. Environ. Health*, **A61**, 591, **2000**.
3. EWERS U., SCHLIPKÖTER H. W. Chronic toxicity of metals and metal compounds. In: MERIAN E.: *Metals and their compounds in the environment*. Weinheim: VCH, pp. 591-603, **1991**.

4. ELSENHANS B., STRUGALA G. J., SCHAFER S. G. Small-intestinal absorption of cadmium and the significance of mucosal metallothionein. *Hum. Exp. Toxicol.* **16**, 429, **1997**.
5. ELSENHANS B., HUNDER G., STRUGALA G., SCHUMANN K. Longitudinal pattern of enzymatic and absorptive functions in the small intestine of rats after short-term exposure to dietary cadmium chloride. *Arch. Environ. Contam. Toxicol.* **36**, 341, **1999**.
6. REEVES P. G., CHANEY R. L., SIMMONS R. W., CHERIAN M. G. Metallothionein induction is not involved in cadmium accumulation in the duodenum of mice and rats fed diets containing high-cadmium rice or sunflower kernels and a marginal supply of zinc, iron, and calcium. *J. Nutr.* **135**, 99, **2005**.
7. PARK J. D., CHERINGTON N. J., KLAASSEN C. D. Intestinal absorption of cadmium is associated with divalent metal transporter 1 in rats. *Toxicol. Sci.* **68**, 288, **2002**.
8. SMITH M. V., SHENOY K. B., DEBNAM E. S., DASHWOOD M. R., CHURCHILL L. J., SRAI S. K. Divalent metal inhibition of non-haem iron uptake across the rat duodenal brush border membrane. *Br. J. Nutr.* **88**, 51, **2002**.
9. MIN K. S., UEDA H., KIHARA T., TANAKA K. Increased hepatic accumulation of ingested Cd is associated with upregulation of several intestinal transporters in mice fed diets deficient in essential metals. *Tox. Sci.* **106**, 284, **2008**.
10. ZALUPS R. K., AHMAD S. Molecular handling of cadmium in transporting epithelia. *Toxicol. Appl. Pharmacol.* **186**, 163, **2003**.
11. FOULKES E. C. Interactions between metals in rat jejunum: implications on the nature of cadmium uptake. *Toxicology* **37**, 117, **1985**.
12. FOULKES E. C. On the mechanism of transfer of heavy metals across cell membranes. *Toxicology* **52**, 263, **1988**.
13. FOULKES E. C. Transport of toxic heavy metals across cell membranes. *Proc. Soc. Exp. Biol. Med.* **223**, 234, **2000**.
14. WEBB M. Role of metallothionein in cadmium metabolism. In: FOULKES E. C. (Ed.) *Cadmium*, Springer - Verlag, Berlin/New York, pp. 281-337, **1986**.
15. FOULKES E. C., Mc MULLEN D.M. Endogenous metallothionein as determinant of intestinal cadmium absorption: a reevaluation. *Toxicology* **38**, 285, **1986**.
16. MIN K. S., ONOSAKA S., TANAKA K. Role of intestinal metallothionein in absorption and distribution of orally administered cadmium. *Toxicol. Appl. Pharmacol.* **109**, 7, **1991**.
17. VERBOST P. M., SENDEN M. H., VAN OS C. H. Nanomolar concentrations of Cd<sup>2+</sup> inhibit Ca<sup>2+</sup> transport systems in plasma membranes and intracellular Ca<sup>2+</sup> stores in interstitial epithelium. *Biochim. Biophys. Acta* **902**, 247, **1987**.
18. MESONERO J.E., RODRIGUES YOLDI M.C., RODRIGUES YOLDI M.J. Calcium-cadmium interaction on L-threonine intestinal transport. *Reprod. Nutr. Dev.* **35**, 319, **1995**.
19. WASHKO P. W., COUSINS R. J. Effect of low dietary calcium on chronic cadmium toxicity in rats. *Nutr. Rep. Int.* **11**, 113, **1975**.
20. SANT' ANA M.G., MORAES R., BERNARDI M.M. Toxicity of cadmium in Japanese quail: evaluation of body weight, hepatic and renal function, and cellular immune response. *Environ. Res.* **99**, 273, **2005**.
21. NAD P., PISTL J., SKALICKA M., KORENEKOVA B., DEMETEROVA M., BINDAS L., MALA P. Effect of oral cadmium and zinc application on leucocytes biological activity in Japanese quails (*Coturnix coturnix japonica*). *Berl. Münch. Tierärztl. Wochenschr.* **122**, 364, **2009**.
22. WAISBERG M., JOSEPH P., HALE B., BEYERSMANN D. Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicology* **192**, 95, **2003**.
23. WAALKES M. P. Cadmium carcinogenesis. *Mutat. Res. Fundam. Mol. Mech. Mutagen.* **533**, 107, **2003**.
24. FASOLA M., LEONZIO C. Birds as monitor of environmental contamination in Asia. *Acta Zool. Sinica* **52**, 32, **2006**.
25. RICHARDSON M. E., SPIVEY FOX M. S., FRY B. E. Pathological changes produced in Japanese quail by ingestion of cadmium. *J. Nutr.* **104**, 323, **1974**.
26. BERZINA N., MARKOV J., ISAJEV S., APSITE M., SMIRNOVA G. Cadmium-induced enteropathy in domestic cocks: a biochemical and histological study after subchronic exposure. *J. Compilation Nordic Pharmacological Society. Basic, Clinical Pharmacology, Toxicology* **101**, 29, **2007**.
27. KAMIŃSKI P., KURHALYUK N., SZADY-GRAD M. Heavy metal-induced oxidative stress and changes in physiological process of free radicals in the blood of white stork (*Ciconia ciconia*) chicks in polluted areas. *Polish J. Environ. Stud.* **16**, 555, **2007**.
28. DUIZER E., GILDE A. J., VERSANTVOORT CH., GROTEN J. P. Effects of cadmium chloride on the paracellular barrier function of intestinal epithelial cell lines. *Toxicol. Appl. Pharmacol.* **155**, 117, **1999**.
29. ALSBERG C. L., SCHWARTZE E. W. Pharmacological action of cadmium. *J. Pharmacol. Exp. Ther.* **13**, 504, **1919**.
30. PROZIALECK W. C., EDWARDS J. R., NEBERT D. W., BARCHOWSKY A., ATCHISON W. D. The vascular system as a target of metal toxicity. *Tox. Sci.* **102**, (2), 207, **2008**.
31. PROZIALECK W. C. Evidence that E-cadherin may be a target for cadmium toxicity in epithelial cells. *Toxicol. Appl. Pharmacol.* **164**, 231, **2000**.
32. PROZIALECK W. C., NIEWENHUIS R. J. Cadmium (Cd<sup>2+</sup>) disrupts intercellular junctions and actin filaments in LLC-PK1 cells. *Toxicol. Appl. Pharmacol.* **107**, 81, **1991**.
33. NIEWENHUIS R. J., DIMITRIU C., PROZIALECK W. C. Ultrastructural characterization of the early changes in intercellular junctions in response to cadmium (Cd<sup>2+</sup>) exposure in LLC-PK1 cells. *Toxicol. Appl. Pharmacol.* **142**, 1, **1997**.
34. PROZIALECK W. C., EDWARDS J. R. Cell adhesion molecules in chemically – induced renal injury. *Pharmacol. Ther.* **114**, (1), 74, **2007**.
35. DANKO J., LESNIK F., JENCA A. Xenobiotics and their relation to health. University of Veterinary Medicine, Kosice, pp. 107, **2005** [In Slovak].
36. FLESAROVA S. Angiogenesis and hemangiogenesis. In: LESNIK F., JENCA A., DANKO J. *Angiogenesis and lymphangiogenesis*. University of Veterinary Medicine, Kosice, pp. 201-209, **2005** [In Slovak].
37. KRAMAROVA M., MASSANYI P., SLAMECKA J., TARTARUCH F., JANCOVA A., GASPARIK J., FABIS M., KOVACIK J., TOMAN R., GALOVA J., JURCIK R. Distribution of cadmium and lead in liver and kidney of some wild animals in Slovakia. *J. Environ. Sci. Health* **A40**, 593, **2005**.
38. KRAMAROVA M., MASSANYI P., JANCOVA A., TOMAN R., SLAMECKA J., TARTARUCH F., KOVACIK J., GASPARIK J., NAD P., SKALICKA M., KORENEKOVA B., JURCIK R., CUBON J., HASICIK P. Concentration of cadmium in the liver and kidneys of some wild and farm animals. *Bull. Vet. Inst. Pulawy* **49**, 465, **2005**.

39. KIMAKOVA T., KORENEKOVA B., BERNASOVSKA K., SKALICKA M., NAD P., PORACOVA J. Study of the protective effect of selenium on organism of Japanese quail after experimental exposure to cadmium. *Slov. Vet. J.* **31**, 32, **2005** [In Slovak].
40. HOLOVSKA K., CIGANKOVA V., ALMASIOVA V., SOBEKOVA A., NAD P., SKALICKA M. Ultrastructural and biochemical changes in the turkeys liver after the chronic cadmium exposure. *Acta Veter. Beograd* **59**, 167, **2009**.
41. KUKNER A., COLAKOGLU N., KARA H., ONER H., OZOGUL C., OZAN E. Ultrastructural changes in the kidney of rats with acute exposure to cadmium and effect of exogenous metallothionein. *Biological Trace Elements Research*, Humana Press Inc., pp. 137-146, **2007**.
42. BRZÓSKA M. M., KAMIŃSKI M., SUPERNAK-BOBKO D., ZWIERZ K., MONIUSZKO-JAKONIUK J. Changes in the structure and function of the kidney of rats chronically exposed to cadmium. I. Biochemical and histopathological studies. *Arch. Toxicol.* **77**, 344, **2003**.
43. BRZÓSKA M. M., KAMIŃSKI M., DZIKI M., MONIUSZKO-JAKONIUK J. Changes in the structure and function of the kidney of rats chronically exposed to cadmium. II. Histoenzymatic studies. *Arch. Toxicol.* **78**, 226, **2004**.
44. NAD P., MASSANYI P., SKALICKA M., KORENEKOVA B., CIGANKOVA V., ALMASIOVA V. The effect of cadmium in combination with zinc and selenium on ovarian structure in Japanese quails. *J. Environ. Sci. Health* **A42**, 2017, **2007**.
45. LUKACINOVA A., BENACKAR., LOVASOVA E., RACZ O., NISTIAR F. Reproduction parameters in low dose chronic exposure with heavy metals in rats. *Polish J. Environ. Studies* **17**, 911, **2008**.
46. MASSANYI P., TRANDZIK J., NAD P., SKALICKA M., KORENEKOVA B., LUKAC N., FABIS M., TOMAN R. Seminal concentration of trace elements in fox and relationship to spermatozoa quality. *J. Environ. Sci. Health* **A40**, 1097, **2005**.
47. LUKAC N. Trace elements and the quality of sperms. Nitra, SPU, pp. 1-118, **2007** [In Slovak].