DISTRIBUTION OF HEAVY METALS AND THEIR ULTRAHISTOCHEMICAL DETERMINATION IN THE ORGANS OF CALVES

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Abstract

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The distribution of Cd, Zn, Se and Cu in the tissues of animals receiving different total doses of these metals for different time periods was studied at the submicroscopical level. In experiment I, 32 calves of both sexes were given heavy metals singly (Cd and Zn) or in combination (Cd, Zn; Cd, Se) from 2 to 14 weeks of age. Experiment II included 21 bulls which received Cd, or Cd and Cu or Cd and Zn in a diet from 3 to 15 months of age. For ultrahistochemical examination, samples of liver, kidney, pancreas, testis or ovary and diaphragm were collected in experiment I and those of liver, kidney, pancreas and testis in experiment II.

The ultrahistochemical method used was based on the detection of metal sulphides using a hydroquinone developer with silver nitrate added. The foci of reduced silver on sulphide molecules were identified as dark granules in cell cross-sections.

In experiment I, after combined administration of Cd and Zn, the highest amounts of reduced silver granules were seen in the cytoplasm of hepatocytes and cells of the proximal and distal renal tubules and the lowest amounts were found in glandular cells of the pancreas. Administration of Cd and Se resulted in the presence of large numbers of granules in the nuclei and nucleoli of spermatogonies. In experiment II, ingestion of Cd and Zn in feed led to the appearance of highest amounts of granules in the nucleoli, nuclei and cytoplasm of cells in testes, kidneys and pancreas. Following Cd intake, the highest accumulation of granules was observed in the nucleoli of hepatocytes and cells of the proximal and distal renal tubules. Combined Cd and Cu produced the highest numbers of granules in cells of the proximal and distal renal tubules and in the nucleoli and nuclei of germinal epithelium.

Xenobiotics, Cd, Zn, Se and Cu administration, detection, distribution, cells

The content of heavy metals in and their effects on animal and human tissues have been assessed first by biochemical, histological and histochemical methods and later by techniques working at the ultrastructural and ultrahistochemical levels. These methods have been able to reveal changes in the morphology, structure, including those at the subcellular level, and biochemistry of tissues and organs and have permitted quantitative assessments of heavy metals in the specimens examined but failed to localize heavy metals in the cell. The circulation of xenobiotics in the environment and some of their effects on living organisms have been reviewed in the monograph by Cibulka et al. (1991). Gradually, histochemical methods for localization of heavy metals in cells have been developed; the first of them, published by Timm in 1958, was subsequently modified by several authors (Danscher 1981; Danscher et al. 1994) to be eventually adapted to suit the requirements

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of electron microscopy (Kodama et al. 1993). This method, in combination with biochemical determination of heavy metal content, can serve to detect precisely the distribution of these elements in the cell and their accumulation in cell organelles, including nuclei and nucleoli. and thus facilitate estimation of a possible extent of damage caused to each cell compartment. While most of the information based on histochemical findings was published in the 1970s and 1980s, the ultrahistochemical approach has been used less frequently and only in the last decade. It is important to note that the majority of these experiments have employed small laboratory animals (Condron et al. 1994; Jagetia and Adiga 1994; Murthy and Holovack 1991; Watanabe and Endo 1991; Wildman et al. 1994) and only few data are related to farm animals (Abdelrahman et al. 1994; Cigánková et al. 1994), birds (Chishti and Rotkiewics 1993; Maretta et al. 1995) and dogs (Hamada et al. 1991; Patra and Bose 1990).

Since only minimum information based on studies in large mammals is available on the localization and distribution of heavy metals in cells and their accumulation in organelles and/or intercellular matter, we developed a modified method for the ultrastructural localization of selected heavy metals in organs of the calves which had been given increased doses of either one or more heavy metals. The results were compared with the quantitative data obtained by biochemical examinations of these tissues.

Materials and Methods

The presence, distribution and localization of heavy metals in the cells of some organs and tissues of calves were studies in two experiments. Calves of the Holstein-Friesian breed were housed unrestrained in individual pens. The heavy metals studied were given in milk which was one component of the daily feed ration consisting of 4 liters of milk, calf grain mixture and water ad libitum. Zindep and Selevit were used at preventive doses and cadmium was administered at 1 mg, 2 mg and 5 mg doses.

I. The first experiment included 32 calves of both sexes. The experiment lasted 94 days from 2 to 14 weeks of age of the animals. Cadmium was given to all groups as $CdCl_2$ in their diet, zinc (Zindep) at a subcutaneous dose of 0.8 ml/10 kg live body mass and selenium (Selevit) at a subcutaneous dose of 20ml/100 kg live body mass . The experimental animals were allocated to 7 groups:

Group 1 (7 animals) - received 1 mg Cd/animal/day and 2 doses of Zindep
Group 2 (3 animals) - received 2 doses of Zindep
Group 3 (2 animals) - received 1 mg Cd/animal/day and 1 Selevit dose
Group 4 (6 animals) - received 1 mg Cd/animal/day.
Group 5 (3 animals) - received 2 mg Cd and 1 Selevit dose
Group 6 (3 animals) - received 2 mg Cd/animal/day
Group 7 (8 animals) - controls. The calves were fed the same diet as the experimental animald but without heavy metal addition.

The average body mass of animals at the end of the experiment: control group, 100.3 kg; groups receiving Zn, 101 kg; groups given Cd and Zn, 94 kg.

II. In the second experiment, 21 bulls were used. It lasted 92 days, i.e. from 3 to15 weeks of age. Cadmium, as $CdCl_2$, was given at a dose of 5 mg Cd /kg feed dry weight, copper (Cu Bioplex) at a dose of 10 mg/kg feed dry weight and zinc (Zn Bioplex) at a dose of 100 mg/kg feed dry weight. Heavy metal doses increased as the growing bulls consumed more feed. The animals were allocated to 4 groups:

Group 1 (6 animals) - received 5 mg Cd/kg feed dry weight. The total Cd amount was 900 mg.

Group 2 (6 animals) - received 5 mg Cd/kg feed dry weight and Cu Bioplex. Total amounts of Cd and Cu were 900 mg and 1.8 g, respectively.

Group 3 (6 animals) - received 5 mg Cd/kg feed dry weight and Zn Bioplex. Total amounts of Cd and Zn were 900 mg and 18 g, respectively.

Group 4 (3 animals) - received equal feed rations but without heavy metal addition.

The average body mass of animals at the end of the experiment: control group, 146 kg; Cd-Cu group, 133,6 kg; Cd-Zn group, 140 kg; Cd group, 139 kg.

For ultrahistochemical examination in experiment I, liver, kidney, pancreas, testis or ovary and diaphragm samples were collected. After preliminary evaluation of the results from experiment I, samples of liver, kidney, pancreas and testis were collected in experiment II. The selection of organs was based on literature data and the authors' experience

concerning the frequency of histological findings of heavy metals in organs and tissues of experimental animals. In each group, samples of all collected organs from at least 3 animals were taken for examination.

Immediately after collection, the samples were fixed for 2 h in 300 mmol/l glutaraldehyde in 100 mmol.l^{-1} cacodylate buffer (pH 7.2) saturated with hydrogen sulfide and for additional 2 h in the glutaraldehyde solution without hydrogen sulfide. They were subsequently washed (3 washes, 2 hours each) in open vessels with 300 mmol.l^{-1} saccharose and maintained overnight in a saccharose solution in a desiccator over a saturated $CuSO_4$ solution. On the following day, the samples were washed with 300 mmol.l^{-1} saccharose for 30 min, dehydrated, infiltrated and embedded (in Durcupan-ACM) in the routine manner. Sections were cut with an LKB Nova ultramicrotome.

The ultrathin sections were developed in a mixture of acacia gum (200g.l^{-1}), hydroquinone (20 g.l^{-1}), citric acid (50 g.l^{-1}) and $AgNO_3$ (100 g. l^{-1}) at 20 ćC in the dark for 60 to 90 min. Neither incubation in 5% sodium thiosulphate nor additional staining was performed. The reduced silver appeared as dense granules of varying sizes which were clearly discernible in ultrathin sections viewed and photographed in a Tesla 500 electron microscope.

Results

Experiment I

Granules of reduced silver were seen, in varying amounts, in hepatocytes in all the groups. In the groups receiving Cd and Zn, they appeared in great numbers in lysosomes (Plate XIII., Fig. 1). In some of them, they were aggregated in clusters and filled the entire lysosome, giving it a dark, homogeneous appearance. In other lysosomes, a lower number of granules among light areas produced a net-like appearance. Ingestion of these two metals was rarely associated with the presence of dark granules in the nucleus and nucleolus or other organelles of hepatocytes.

In the kidney, dark granules were observed predominantly in the cytoplasm of cells of the proximal and distal tubules (Fig. 2). They were occasionally present also in the nucleus and nucleolus. They were found neither in the cells of Henle's loop or the renal corpuscle.

In the testis, granules of reduced silver seen in germinal epithelium were located only in the nucleoli, nuclei and cytoplasm of spermatogonies (Plate XIV., Fig. 3). The granules were very small and diffusely scattered in the whole cytoplasm. A small number of them also appeared in the basement membrane and tunica propria. The cytoplasm of neither Sertoli's cells nor myoid cells showed any presence of the granules.

Pancreatic tissue contained the lowest amounts of reduced silver granules. These were seen as fine, dark grains on the surfaces of zymogenic granules and in lysosomes where they were present as granule clusters (Fig. 4).

In diaphragm samples, granules of reduced silver were occasionally observed on the fibers of striated muscle; the same rare occurrence was noted in the connective tissue of the cortical region and in ovarian follicles.

The intake of Cd and Se resulted, similarly to the previous metal combination, in the most marked response to be seen in hepatocytes. The heavy metals were largely accumulated in lysosomes (Plate XV., Fig. 5) in which the presence of fusing granules produced a homogeneous appearance. Individual grains of reduced silver could be distinguished only in some of the lysosomes. In addition, individual granules were also seen in the cytoplasm of hepatocytes where they were located between mitochondria and the granular endoplasmic reticulum (GER).

In contrast to the Cd and Zn effects, Cd and Se produced large numbers of fine granules in the testis. Reduced silver grains were detected in all parts of the tubuli seminiferi contorti, particularly in germinal epithelium and interstitial connective tissue (Fig. 6). The important finding was the presence of these granules in the nucleus and nucleolus of spermatogonies, in the cytoplasm of Sertoli's cells and gametes. In several cases, the presence of these heavy

metals was recorded in the cytoplasm of interstitial connective tissue epithelioid cells. However, because the ultrastructure of mitochondria was not sufficiently preserved (moreover, the sections were not stained) it was not possible to determine for certain whether these cells were Leydig's cells. In the remaining organ samples from the animals receiving Cd and Se, the heavy metal concentration was so low that these xenobiotics could not be detected by the method used.

Cadmium alone was administered at a dose of 1 mg and 2 mg per day to each animal of groups 4 and 6, respectively. After both doses, the increased accumulation of the metal was observed in their liver. The appearance and distribution of reduced silver granules were similar to those in the hepatic tissues of the animals receiving Cd in combination with either Zn or Se. The Cd levels in the other organs examined were very low and approached detectability and thus failed to provide constant results of observation. The same applies to observations made in the group which received two doses of Zn alone; the metal was demonstrated only in hepatocytes.

Experiment II

Increased doses of heavy metals, as compared to experiment I, given singly or in combination (Cd and Zn; Cd and Cu) resulted in their accumulation, as numerous granules of reduced silver, in organs of the experimental animals. In this experiment, samples of liver, kidney, testis and pancreas were taken for examination.

In the liver, combined Cd and Zn at increased doses led to the accumulation of heavy metals in nearly all hepatocyte compartments. The nucleus showed large granules of reduced silver in the nucleolus, karyosomes and in euchromatin areas (Plate XVI, Fig. 7). The nuclear structure was well preserved. In the cytoplasm, the highest numbers of granules were seen in lysosomes which were completely filled with them. In addition, numerous large and small granules were diffusely distributed among organelles and frequently located in the ground cytoplasm close to mitochondria or inside them.

In the testis, the Cd-Zn combination resulted in the presence of large amounts of small dense granules of reduced silver in the nucleus and nucleolus of spermatogonies and in their cytoplasm, and in the cytoplasm of Sertoli's cells (Fig. 8). No granules were found in either the basement membrane or interstitial cells.

In the kidney after ingestion of Cd and Zn, reduced silver granules were present predominantly in the nucleoli and nuclei of the proximal tubule cells (Plate XVII, Fig. 9). The nucleoli contained large amounts of small, dense granules while perinuclear chromatin showed granules lying in the close vicinity. The cytoplasm showed, apart from large granules, lysosomes filled with reduced silver (Fig. 9) and located close to the tubule basement membrane. The distal tubules contained only a small amount of these granules and the cells of the thin segment of Henle's loop showed no granules at all.

In comparison with experiment I, Cd and Zn combined intake resulted in a high accumulation of dense granules of reduced silver in the glandular cells of the pancreas (Fig. 10). Large numbers of small, dark granules were located on nucleoli. Granules larger in size were seen on karyosomes and in the euchromatin area and close to the nuclear envelope. The cytoplasm showed numerous large grains located on zymogenic granules, mitochondria and GER cisternae.

The ingestion of increased doses of Cd appeared to result in numerous small granules in hepatocytes. The highest amounts of granules were seen in nucleoli within which they were aggregated into groups or chains (Plate XVIII, Fig.11). In addition, granules were situated close to perinucleolar chromatin, near karyosomes at the nuclear envelope and were also seen irregularly distributed in a cross-section through the nucleus. The cytoplasm showed,

apart from small granules, larger-sized granules located in mitochondria and adjacent to GER cisternae. The highest concentration of granules was observed in lysosomes, which was in agreement with the findings made in the other experimental groups.

Cadmium given alone at increased doses was detected in the proximal and distal tubules of the kidney. The highest amount of small granules of reduced silver was located in the nucleolus, karyosomes and in the euchromatin area (Fig. 12). Similar fine granules were attached to the outer membrane of the nuclear envelope and were also scattered in the cytoplasm.

The lowest number of granules after Cd intake was demonstrated in the testis. Spermatogonies showed only small amounts of gross granules of reduced silver in the nucleus (Plate XIX, Fig.13) in areas other than heterochromatin ones. The cytoplasm contained granules of varying sizes distributed irregularly among cell organelles.

Administration of Cd with Cu had the greatest effect on the liver of all organs. Hepatocytes showed highest amounts of granules in lysosomes (Fig. 14); these were full to such an extent that some of them acquired a homogeneous appearance. Nuclei and nucleoli, on the other hand, showed only small numbers of granules.

In the kidney, the combined Cd and Cu intake led to the presence of reduced silver granules in nearly all of the proximal tubule cells and in some of the distal tubule cells (Plate XX, Fig. 15). Large amounts of small granules were seen in the nucleolus and occasional granules appeared in heterochromatin areas. Some fine granules of reduced silver were attached to the outer membrane of the nuclear envelope. The cytoplasm showed clusters of fine granules among mitochondria of the basal labyrinth and in the lamina basalis; occasional gross granules were located in the apical cell parts.

Intake of Cd and Cu resulted in the appearance of large amounts of reduced silver granules in the germinal epithelium of tubuli seminiferi contorti (Fig.16). The granules varying in size were located in the nucleoli, nuclei and cytoplasm of spermatogonies. Their presence was also demonstrated in Sertoli's cells and in the cells of interstitial connective tissue. A small amount of granules were also found in the tunica propria tubuli seminiferi.

No reduced silver granules were found in any of the organs collected from the control animals and thus the results of electron microscopic examination are not presented.

Discussion

A method for histochemical assessment of heavy metals in animal tissues and organs was developed by Timm in 1958. It was later modified by its author (Timm 1963) and by other researchers (Danscher 1981; Danscher et al. 1994; Jin et al. 1995) to be used for a determination of heavy metals in any organ of laboratory mammals as well as in human tissue. Kodama et al. (1993) later adapted this method for electron microscopic studies. This approach, however, has been used less frequently than histochemical methods for light microscopy, perhaps because of higher demands for costs and labor. When it was used, it was applied to investigations in laboratory mammals, birds and, exceptionally, dogs (Cigánková et al. 1994; Chishti and Rotkiewics 1993; Hamada 1991, Patra and Bose 1990). These authors were mostly interested in the submicroscopic changes in organs resulting from heavy metal intake rather than in the localization of these metals. This is also true for the studies published by Abdelrahman and Kincaid (1993) who studied accumulation of Cu, Mn, Zn and Se in the livers of calves during their intrauterine development and reported intracellular distribution of these heavy metals. These authors, however, used a mere biochemical determination of changes in metal concentrations at different stages of pregnancy.

Interesting findings concerning the effects of Cd, Zn, Cu and Ni administered intratracheally have been reported by Murthy and Holovack (1991). These authors found elevated numbers of macrophages, multivesicular and lamellar bodies after exposure to CdO and ZnO and increased numbers of phagolysosomes after exposure to CdO and CuO. These observations were in agreement with the findings made by Farina et al. (1996) and Loose et al. (1978). In addition, exposure to Ni and Cd produced changes on mitochondria. The data on the effects of cadmium on the renal parenchyma of rats was objectivized by Condron et al. (1994) who performed a morphometric analysis of renal proximal tubules. They administered cadmium either subcutaneously or in drinking water and, subsequently, demonstrated distention of the proximal tubules, shortening of the brush border associated with its local impairment and a decrease in the number of microvilli by up to 25%. They, however, did not use a histochemical assessment of cadmium and thus, on the basis of our results, we can confirm their findings only in part. The morphometry of hepatocytes in relation to lysosome distribution is reported in another study (Horký et al. 1998).

Cadmium toxicity and its accumulation in different tissues of the organism has been studied in detail in model experiments in rats. It has been reported that low doses of cadmium (1 to 30 $mg.kg^{-1}$ of the diet fed for 3 months) did not have any adverse effects on the growth of animals and did not produce any histopathological changes in livers, kidneys and blood (Loesser and Lorke 1977). This information, particularly in relation to the liver, seems to be highly improbable (Horký et al. 1998a). On the other hand, a dose of 300 mg. kg^{-1} diet resulted in the accumulation of cadmium in hepatic and renal tissues (Abdelrahman and Kincaid 1993; Chishti and Rotkiewicz 1993; Exon et al. 1977; Condron et al. 1994; Hamada et al. 1991; Sabbioni et al. 1978), which is in accordance with our observations (Horký et al. 1998a). As early as in 1957, Pařízek described irreversible destruction of testicular tissue in male rats following subcutaneous administration of cadmium compounds. These effects are corroborated by the findings reported by other authors (Fowler et al. 1982; Hew et al. 1993 and others) who demonstrated the harmful influence of cadmium on meiosis during spermatogenesis in experimental rats due to early separation of sex chromosomes. Our results were similar showing large numbers of reduced silver granules in the nuclei, nucleoli and cytoplasm of spermatogonies after heavy metal ingestion. Different results were reported by Sacerdote and Cavicchia (1995) who found refraction of the epididymis in rats exposed to cadmium. It is necessary to add that they used 15-day-old, i.e., immature, males. This is similar to some observations in our experiments which also involved sexually immature bulls and heifers; reduced silver granules were seen in greater numbers only in the testicular tissue of the group which received both Cd and Cu.

The fact that, in all the groups of experimental animals, the granules were found in the liver is related to the mechanism of detoxication of this xenobiotic in the organism. During detoxication, macrophages first accumulate insoluble Cd or Cd bound to colloidal particles. With an increasing Cd level in the organism, metallothionein is produced in the liver and kidneys. Cadmium is bound to thionein by forces 3000-times stronger than are those binding zinc (Kaegi and Valce 1961). Metallothionein biosynthesis attains its maximum at 4 to 6 hours after Cd administration. It has been found in mice that metallothionein concentration in the liver increases proportionally to cadmium intake (Probst et al. 1977). It has been demonstrated that binding to this protein makes cadmium harmless because it can no longer adversely affect cell organelles or enter biochemical processes. The toxicity of cadmium depends essentially on the action of free, unbound Cd (Norberg 1977; Cibulka et al. 1991). A strong link between Cd and canine hepatocytes has been demonstrated, using

histochemical and electron microscopic methods, by Hamada et al. (1991) whose observations are in agreement with our findings made in calf hepatocytes. In another study (Horký et al. 1998a) the results of our observations on heavy metal localization are analyzed and compared to the observations of other authors.

Only little information can be found in the literature concerning the ultrahistochemical determination of heavy metals in the organs and tissues of farm animals. All the available data deal only with histochemical methods of detection of these xenobiotics in organs and with histopathology and changes in cell ultrastructure following administration of one or more metals to experimental animals. In view of this, the results of the present study are an important contribution to the understanding of heavy metal effects on farm animals. Our results concerning the biochemistry of heavy metals and their interactions which will be published elsewhere (Illek et al. in press).

Ultrahistochemický průkaz a distribuce těžkých kovů v orgánech telat

Na submikroskopické úrovni byla sledována distribuce různě vysokých dávek Cd, Zn, Se a Cu, podávaných různě dlouhou dobu. Těžké kovy byly podávány v I. experimentu 32 telatům obojího pohlaví stáří 3měs. buď samostatně (Cd a Zn), nebo v kombinaci (Cd + Zn, Cd + Se). Jedna skupina byla kontrolní. Ve II. experimentu bylo 21 býčkům stáří 3,5 měs. podáváno Cd, Cd + Cu a Cd + Zn. V experimentu I. byly pro ultrahistochemický průkaz odebírány vzorky jater, ledvin, pankreatu, varlete ev. ovaria a bránice, ve II. experimentu byly odebírány vzorky jater, ledviny, pankreatu a varlat.

Pro průkaz a zjišťování distribuce těžkých kovů na submikroskopické úrovni bylo použito modifikované metody průkazu sulfidů těžkých kovů hydrochinonovou vývojkou s přidáním dusičnanu stříbrného. Ložiska redukovaného stříbra na molekulách sulfidů lze identifikovat jako tmavá granula na řezu buňkami.

V experimentu I. se po podání Cd + Zn objevila granula redukovaného stříbra v největším množství v cytoplasmě hepatocytů a buněk proximálních a distálních kanálků ledvin, nejmenší množství jich obsahovaly žlázové buňky pankreatu. Po aplikaci Cd + Se se větší množství granul objevilo v jádrech a jadérkách spermatogonií. Ve II. experimentu po podání Cd + Zn se u všech skupin projevilo zvýšení množství granul v jadérkách, jádře a cytoplasmě buněk, zejména v testes, ledvinách a pankreatu. Po podání Cd se zvýšilo množství granul v jadérkách hepatocytů, buněk proximálních a distálních tubulů nefronu. Po aplikaci Cd + Cu lze nejvíce granul pozorovat v buňkách proximálních a distálních tubulů ledvin a v jadérkách a jádrech germinativního epitelu.

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References

ABDELRAHMAN, M. M., KINCAID, R. L. 1993: Deposition of copper, manganese, zinc, and selenium in bovine fetal tissue at different stages of gestation. J. Dairy Sci. **76**: 3588-3593

CIBULKA, J. (ed) 1991: Pohyb olova, kadmia a rtuti v biosféře. Akademia, Praha, J. Cibulka (ed.). 432 p.

CHISHTI, M. A., ROTKIEWICZ, T. 1993: Hepatic and renal ultrastructural changes in cockerels exposed to cadmium chloride and subsequent interaction with organophosphate insecticide. J. Environ. Patol. Toxicol. Oncol. **12**: 35-45

CIGÁNKOVÁ, V., MESÁROŠ, P., BÍREŠ, J., TOMAJKOVÁ, E., ČERNOTA, S. 1994: Morphological structure of the testes of bulls with zinc deficiency and the effect of administering Zindep inj. on the recovery of spermatogenesis. Slov. Vet. čas. **19**: 134-138

CONDRON, R. J., SCHROEN, C. J., MARSHALL, A. T. 1994: Morphometric analysis of renal proximal tubules in cadmium-treated rats. J. Submicrosc. Cytol. Pathol. **26**: 51-58

DANSCHER, G. 1981: Histochemical demonstration of heavy metals. Histochemistry **71**: 1-16

DANSCHER, G., STOLTENBERG, M., JUHL, S. 1994: How to detect gold, silver and mercury in human brain and other tissues by autometallographic silver amplification. Neuropathol. Appl. Neurobiol. **20**: 454-467

EXON, J. H., LAMBERTON, J. G., KOLLER, L. D. 1977: Effect of chronic oral cadmium exposure and withdrawal on cadmium residues in organs of mice. Bull. Environ. Contam. Toxicol. **18**: 74-76

FARINA, J., RIBAS, B., FERNANDEZ-ACENERO, M. J., GASCON, C. 1996: Pulmonary toxicity of cadmium in rats: a histologic and ultrasound study. Gen. Diagn. Pathol. **141**: 365-369

FOWLER, A. J., SINGH, D. N., DUIVEDI, CH. 1982: Effect of cadmium on meiosis. Bull. Environ. Contam. Toxicol. **29**: 412-415

HAMADA, T., NAKANO, S., IWAI, S., TANIMOTO, A., ARIYOSHI, K., KOIDE,O. 1991: Pathological study on beagles after long-term oral administration of cadmium. Toxicol. Pathol. **19**: 138-147

HEW, K. W., ERICSON, W. A., WELSH, M. J. 1993: A single low cadmium dose causes failure of spermiation in the rat. Toxicol. Appl. Pharmacol. **121**: 15-21

HORKÝ, D., LAUSCHOVÁ, I., ILLEK, J., PECHOVÁ, A., ŠINDELÁŘ, M. 1998: Distribution of exogenous heavy metals in hepatocytes of calf: a morphometric study. In press.

HORKÝ, D., ILLEK, J., PECHOVÁ, A. 1998a: Distribution of heavy metals in calf organs. In press.

JAGETIA, G. C., ADIGA, S. K. 1994: Cadmium chloride induces dose-dependent increases in the frequency of micronuclei in mouse bone marrow. Mutat. Res. **306**: 85-90

JIN, LI., MURAKAMI, H. T., JANJUA, N. A., ITANO, T. 1995: Histochemical demonstration of heavy metals in mouse skin. Acta histochem. (Jena) **97**: 383-388

KAEGI, J. H. R., VALEE, B. L. 1961: Metallothionein: a cadmium and zinc containing protein from equine renal cortex. II. Physicochemical properties. J. Biol. Chem. **236**: 2435-2442

KODAMA, H., ABE, T., TAKAMA, M., TAKAHASHI, I., KODAMA, M., NISHIMURA, M. 1993: Histochemical localization of copper in the intestine and kidney of macular mice: light and electron microscopic study. J. Histochem. Cytochem. **41**: 1529-1535

LOESER, E., LORKE, D. 1977: Semichronic oral toxicity of cadmium. Studies on rats. Toxicology **7**: 215-224

LOOSE, L. D., SILKWORTH, J. B., WARRINGTON, D. 1978. Cadmium-induced phagocyte cytotoxicity. Environ. Contam. Toxicol. Bull. **20**: 582-588

MARETTA, M., MARETTOVÁ, E., ŠKROBÁNEK, P., LEDEČ, M. 1995: Effect of mercury on the seminiferous epithelium of the fowl testis. Acta Vet. Hung. **43:** 153-161

MURTHY, R. C., HOLOVACK, M. J. 1991: Ultrastructural changes in rat lungs exposed to combinations of cadmium, zinc, copper, and nickel. J. Submicrosc. Cytol. Pathol. **23**: 289-293

NORBERG, M. 1997: Studies on metallothionein and cadmium. Environ. Res. **15**: 381-404

PAŘÍZEK, J. 1957: Kastrace kadmiem. SZN Praha, pp. 24-42

PATRA, S. P., BOSE, P. K. 1990: A new approach for intraovarian injection of cadmium chloride to perform mass sterilization in adult bitches. Indian J. Anim. Health. **29**: 115-117

PROBST, G. S., BOUSQUET, W. F., MIYA, T. S. 1977: Kinetics of cadmium induced hepatic and adrenal metallothionein synthesis in the mouse. Toxicol. Appl. Pharmacol. **29**: 61-69

SABBIONI, E., MARAFANTE, E., AMANTINY, L., UBERTALLI, L. 1978: Cadmium toxicity studies under long-term low level exposure (ILE) conditions. I. Metabolic patterns in rats exposed to present environmental dietary levels of Cd for two years. Sci. Total Environ. **10**: 135-161

SACERDOTE, F. L., CAVICCHIA, J. C. 1995: Refractoriness of the immature rat epididymis to the early cadmium lesion. Ultrastruct. Pathol. **19**: 187- 191

TIMM, F. 1958: Zur Histochemie der Schwermetalle mit dem Sulfid-Silber-Verfahren. Dtsch. Z. Gerichtl. Med. **46**: 706-711

TIMM, F. 1963: Histochemische Lokalisation und Nachweis der Schwermetalle. Acta Histochem. Suppl. **3:** 142-158

WATANABE, T., ENDO, A. 1991: Effect of selenium deficiency on sperm morphology and spermatocyte chromosomes in mice. Mutat. Res. **262**: 93-99

WILDMAN, R. E., MEDEIROS, D. M., JENKINS, J. 1994: Comparative aspects of cardiac ultrastructure, morphometry and electrocardiography of hearts from rats fed restricted dietary copper and selenium. Biol. Trace. Elem. Res. **46**: 51-66

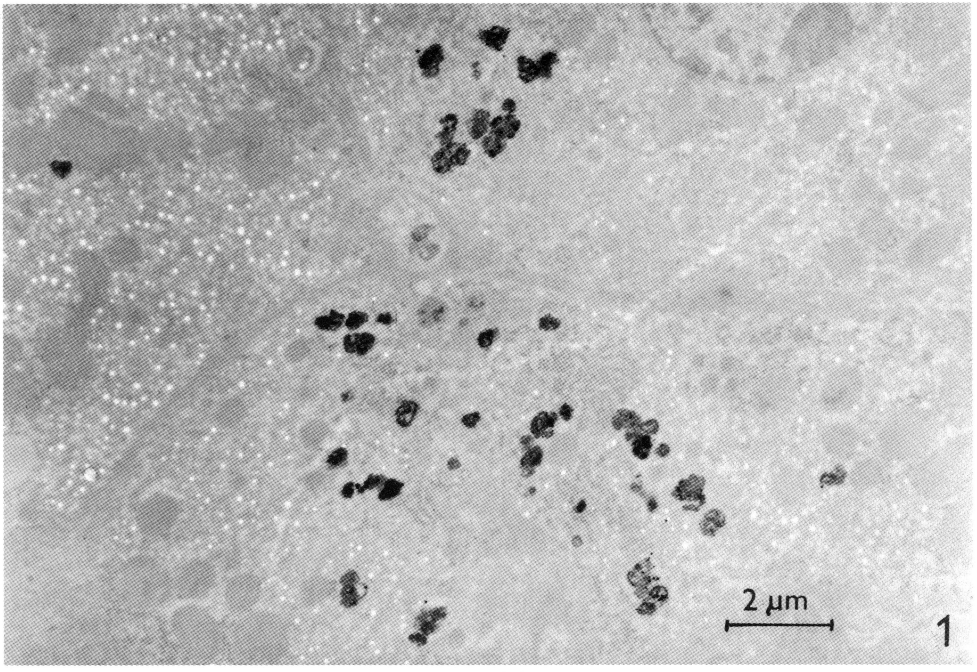

Fig. 1. Lysosomes in hepatocytes are filled with dense granules. Calves received Cd and Zn. × 7000.

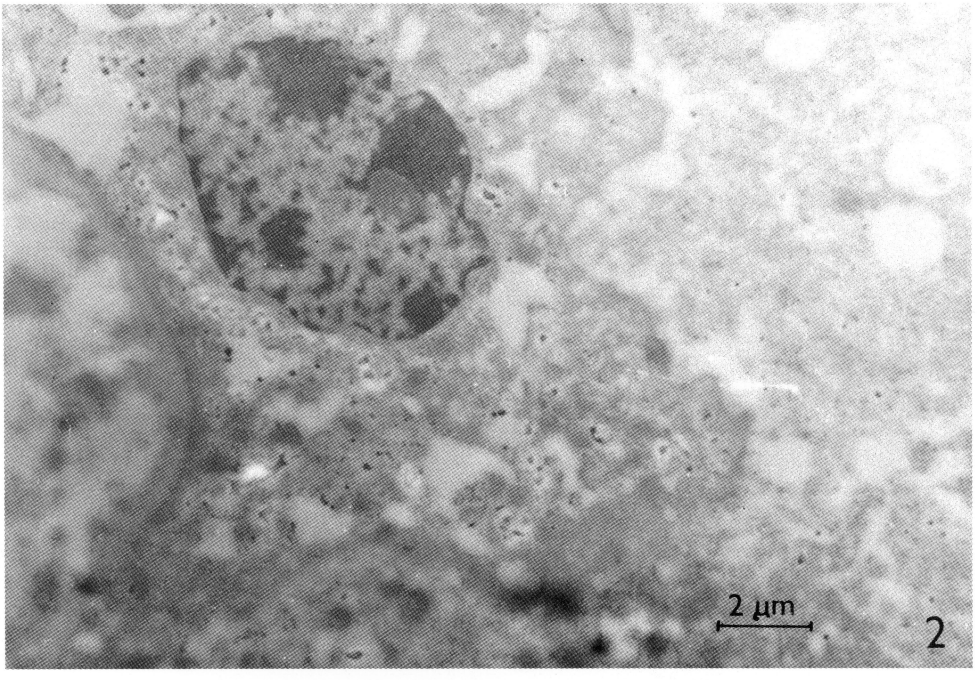

Fig. 2. A cell of the proximal renal tubule with numerous dark granules in the cytoplasm. Calves received Cd and Zn. × 9000.

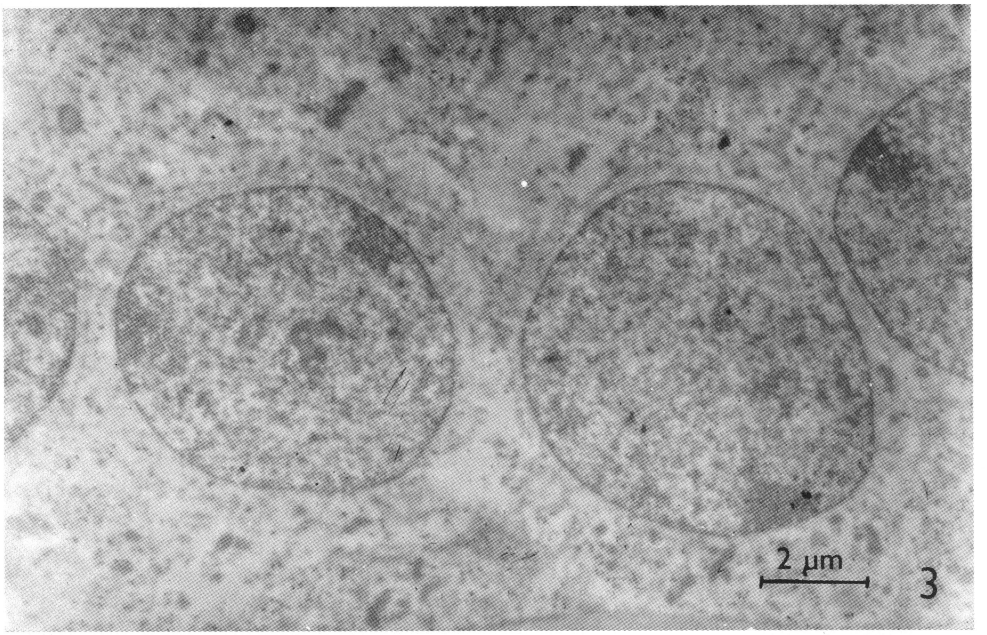

Fig. 3. Nuclei and nucleoli of spermatogonies containing occasional dark granules; numerous small granules in the cytoplasm. Calves received Cd and Zn. × 7000.

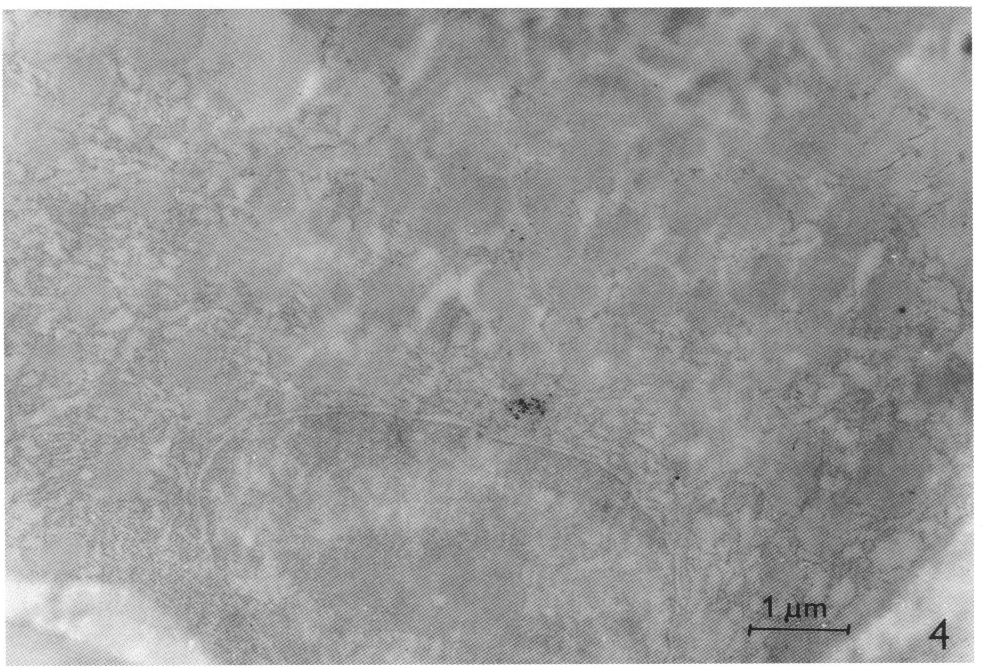

Fig. 4. Reduced silver granules in a lysosome and among organelles of a pancreatic glandular cell. Calves received Cd and Zn. × 13000.

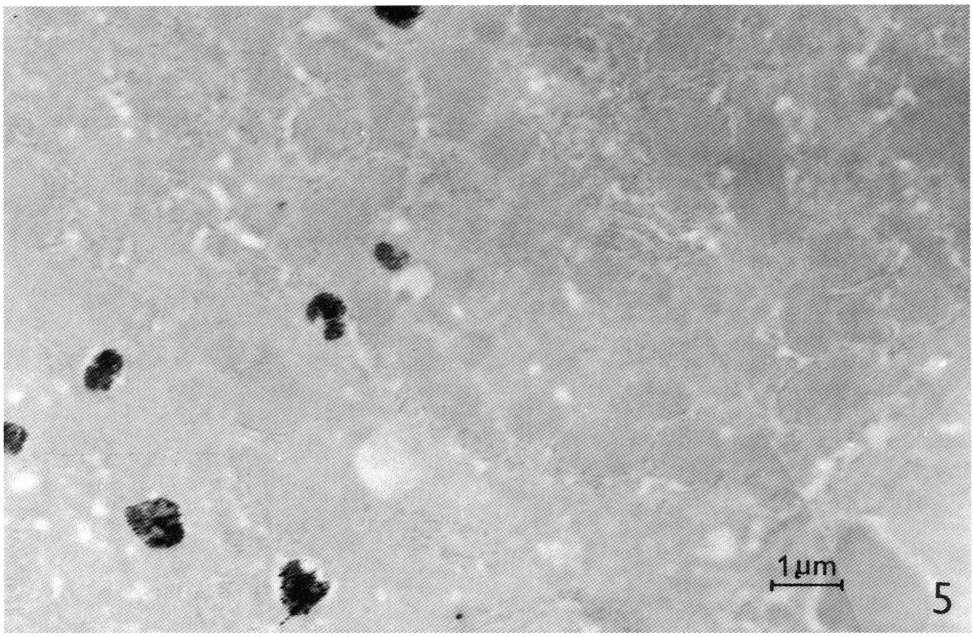

Fig. 5. Cytoplasm of a hepatocyte with lysosomes completely filled with dark granules. Calves received Cd and Se. × 9000.

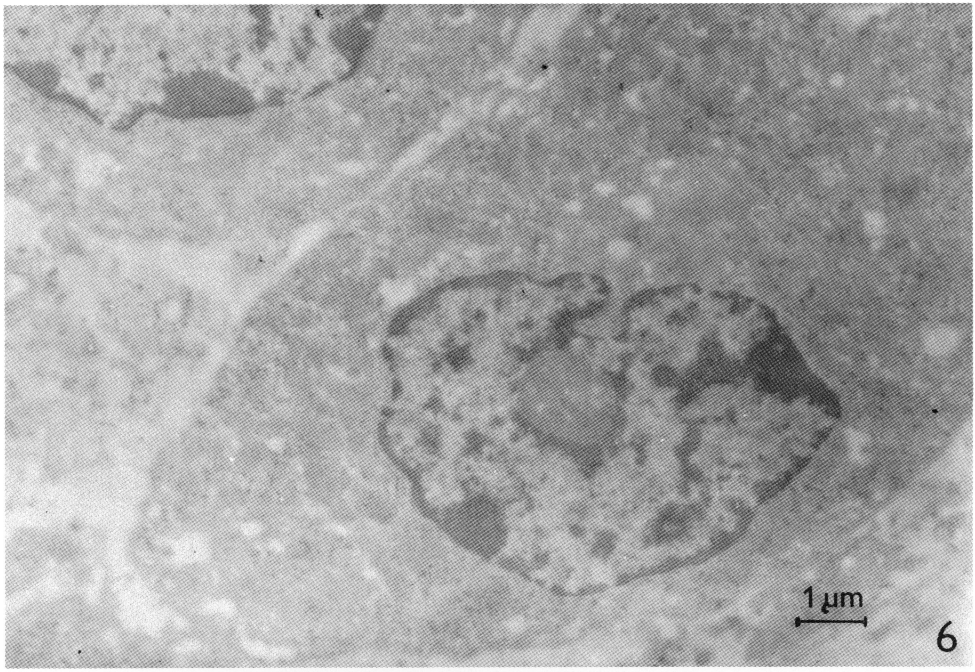

Fig. 6. Nuclei and cytoplasm of the cells of testicular interstitial connective tissue with small, dispersed dense granules. Calves received Cd and Se. × 9000.

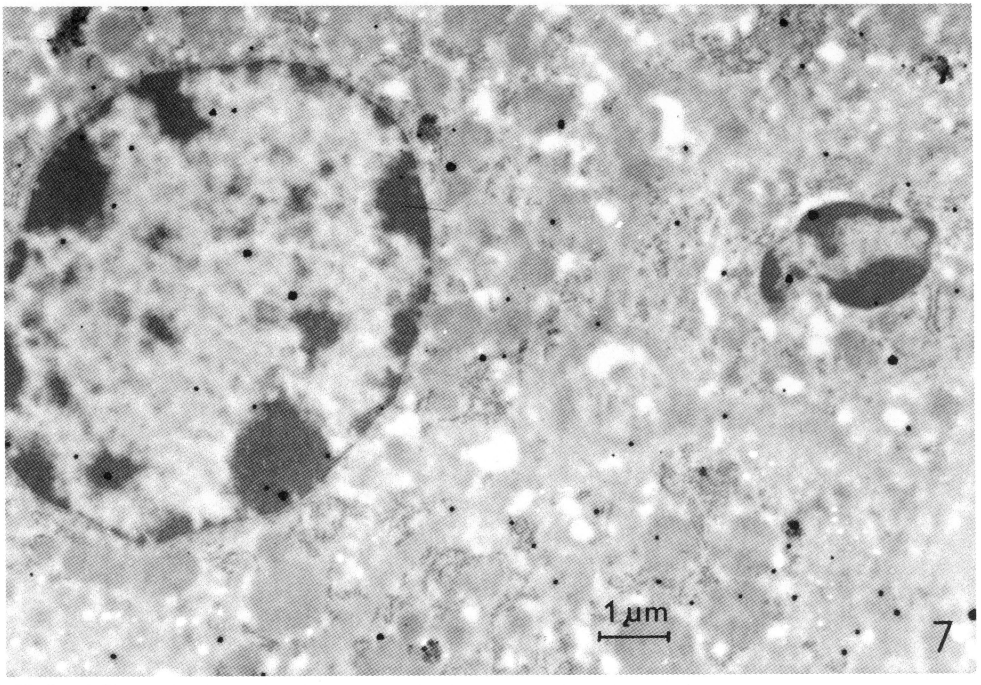

Fig. 7. Nucleus and a part of cytoplasm of a hepatocyte; numerous large granules in the nucleolus, nucleus, lysosomes and among organelles. Calves received Cd and Zn. × 9000.

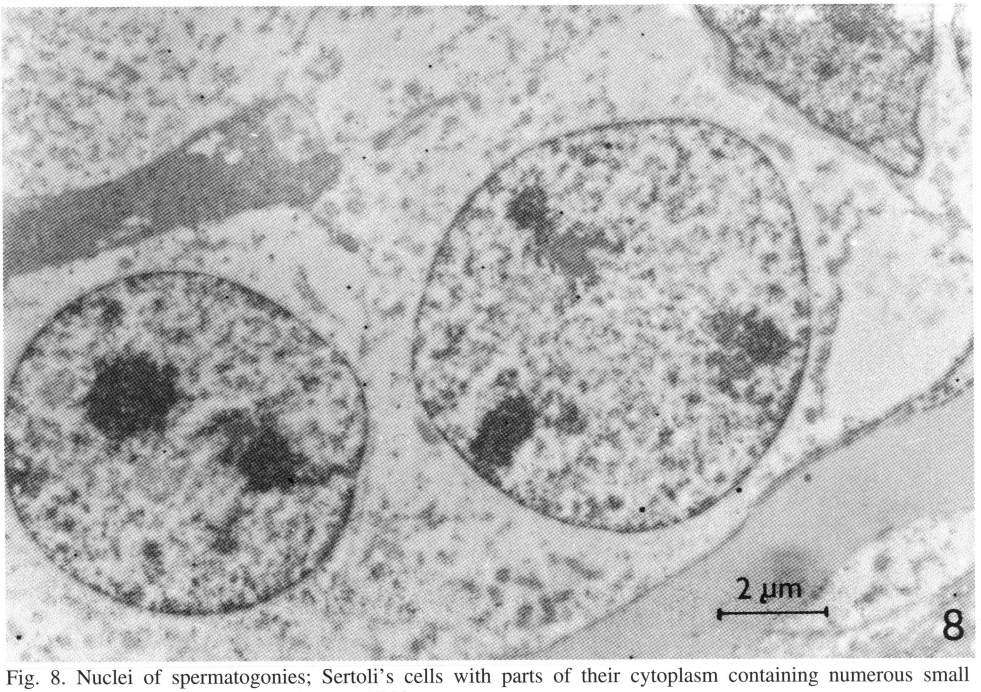

Fig. 8. Nuclei of spermatogonies; Sertoli's cells with parts of their cytoplasm containing numerous small granules. Calves received Cd and Zn. × 7000.

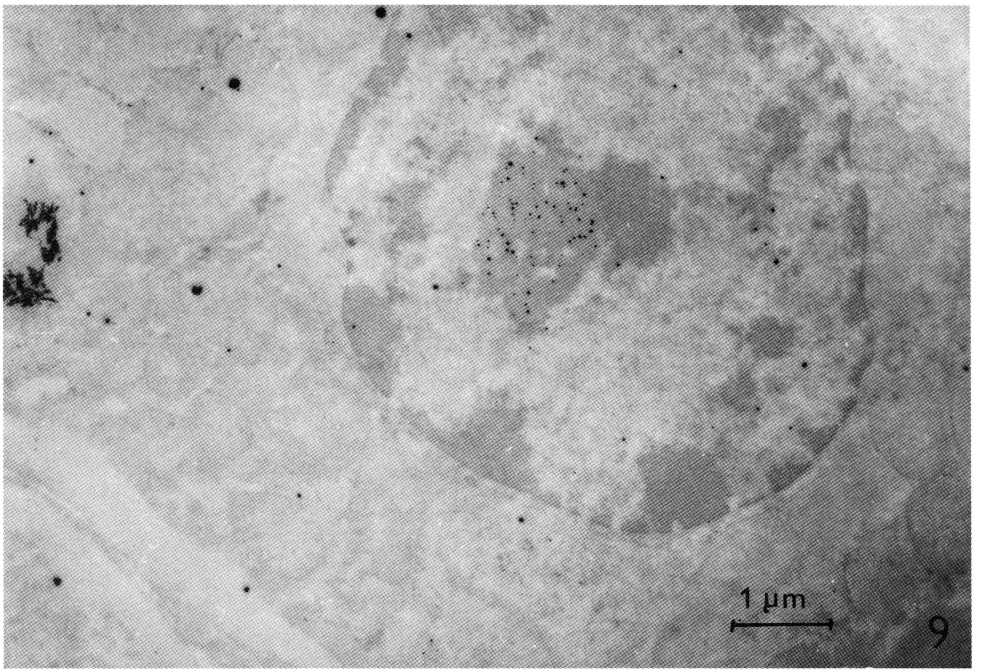

Fig. 9. Part of the nucleus and cytoplasm of an epithelial cell of the proximal renal tubule; numerous granules in the nucleolus, nucleus and cytoplasm. Calves received Cd and Zn. × 13000.

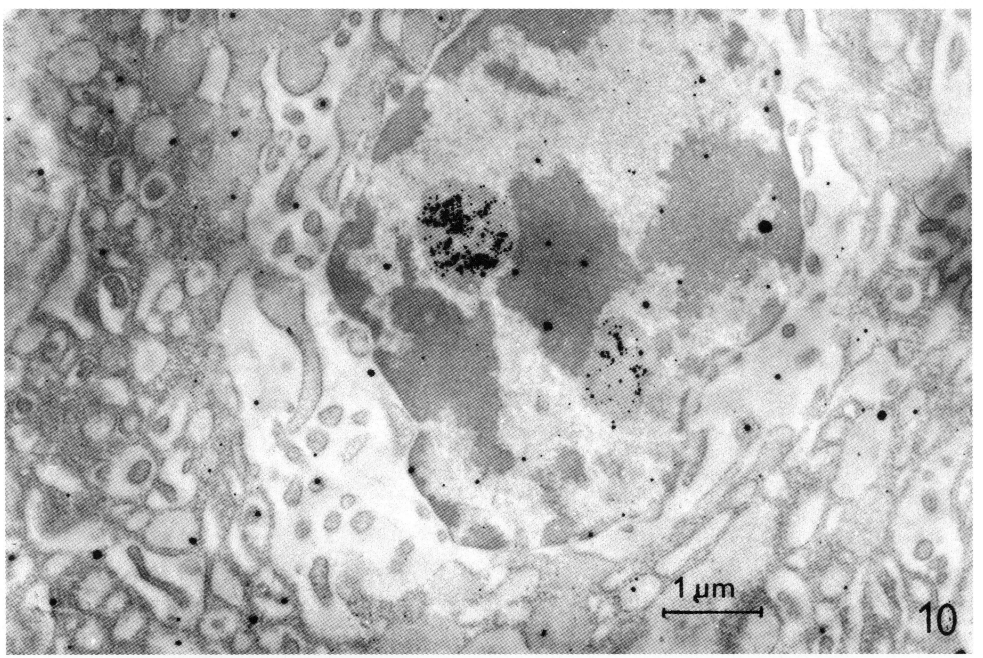

Fig. 10. Nucleus and part of cytoplasm of a pancreatic glandular cell with many small granules in both nucleoli and karyosomes and in GER cisternae in the cytoplasm. Calves received Cd and Zn. × 13000.

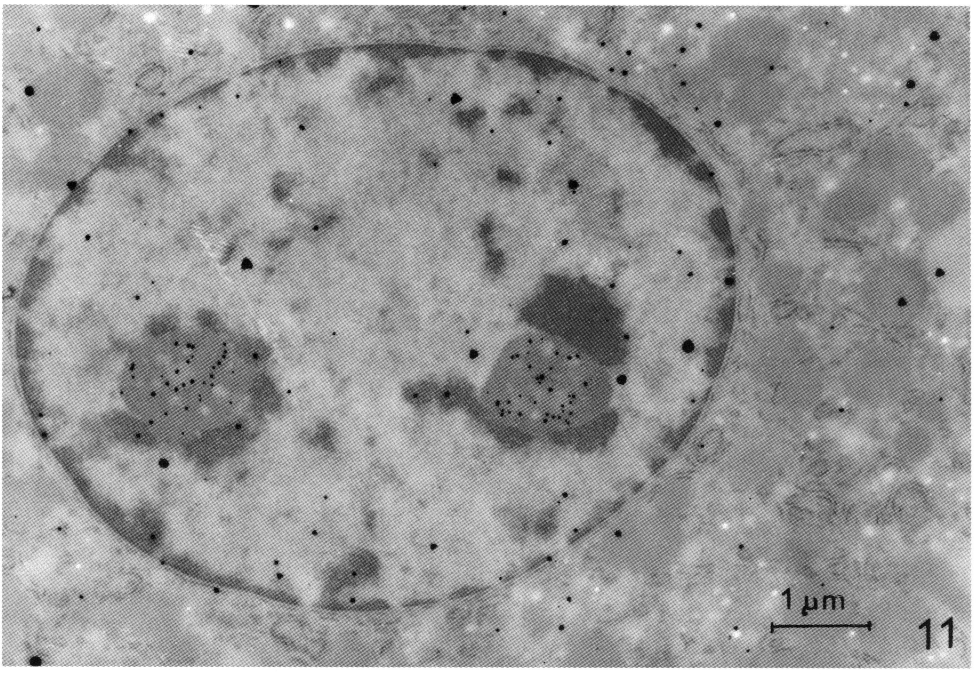

Fig. 11. Nucleus and a part of cytoplasm of a hepatocyte with numerous granules in both nucleoli, karyosomes and among organelles in the cytoplasm. Calves received Cd. × 13000.

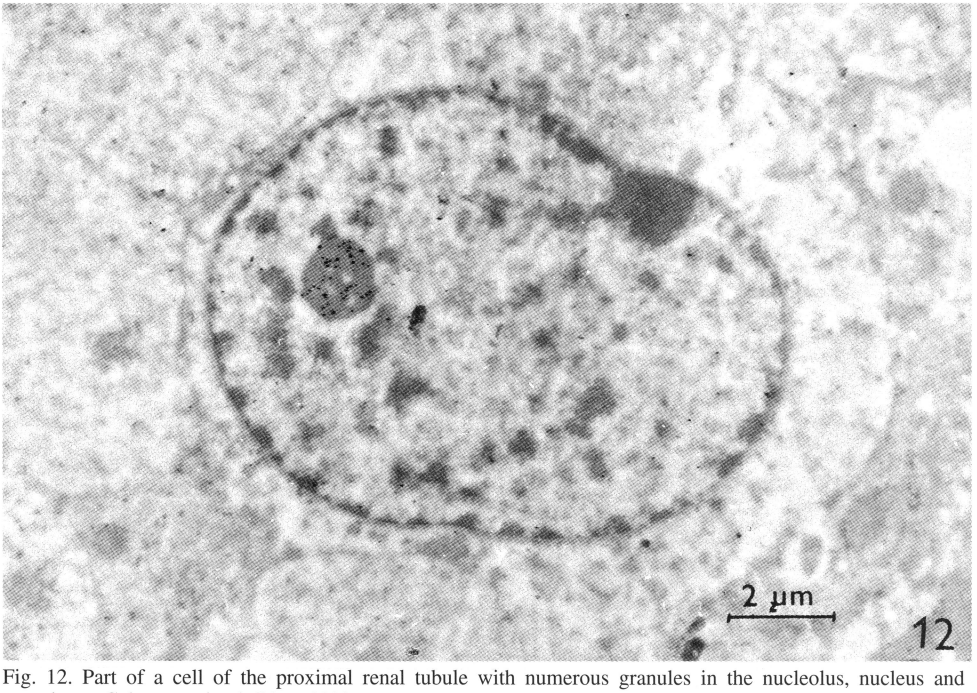

Fig. 12. Part of a cell of the proximal renal tubule with numerous granules in the nucleolus, nucleus and cytoplasm. Calves received Cd. × 8000.

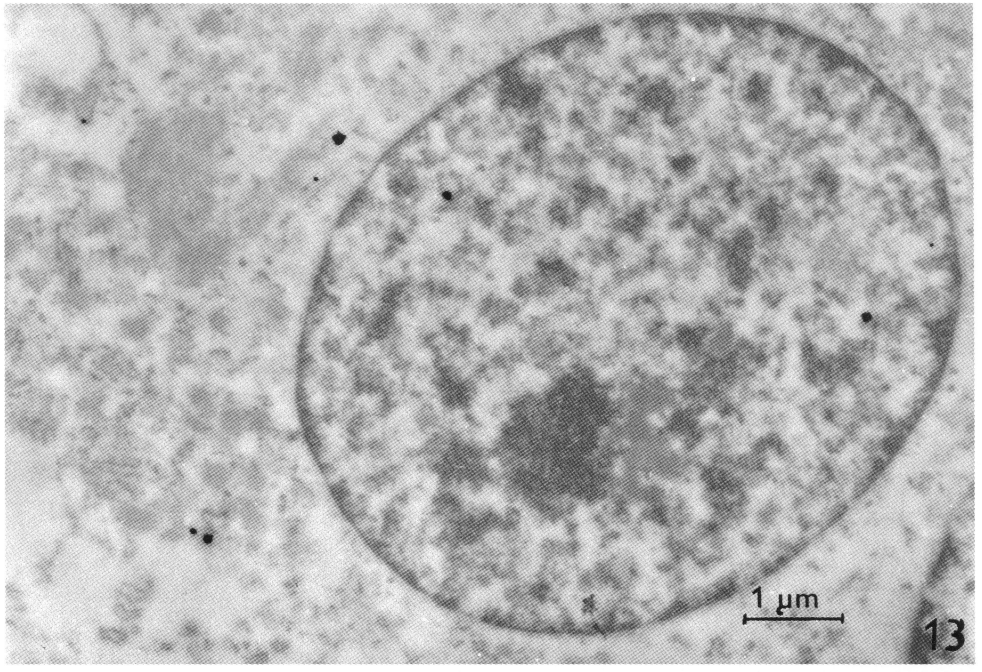

Fig. 13. Nucleus and part of the cytoplasm of a spermatogonium with few large granules. Calves received Cd. × 14000.

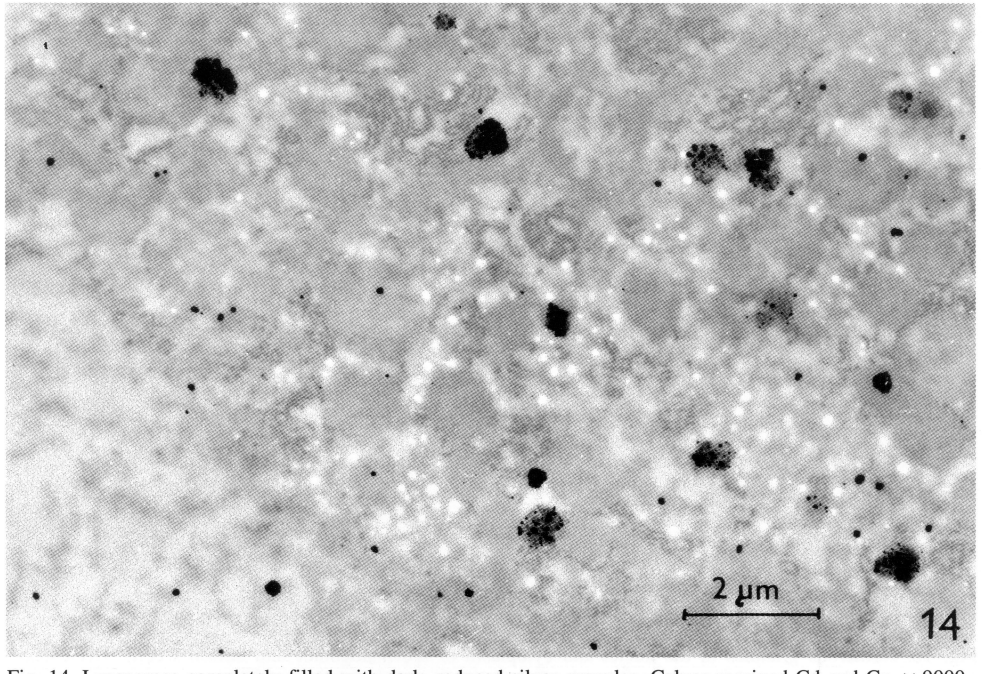

Fig. 14. Lysosomes completely filled with dark, reduced silver granules. Calves received Cd and Cu. × 9000.

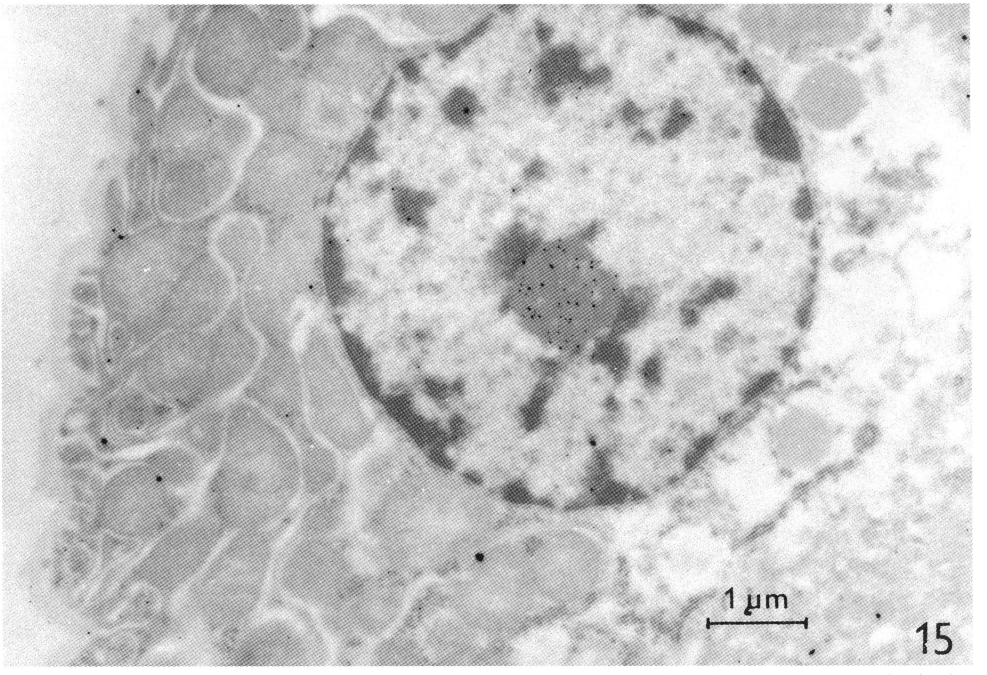

Fig. 15. Nucleus and part of the cytoplasm of a cell of the proximal renal tubule; numerous granules in the nucleolus and heterochromatin and occasional granules among organelles. Calves received Cd and Cu. × 13000.

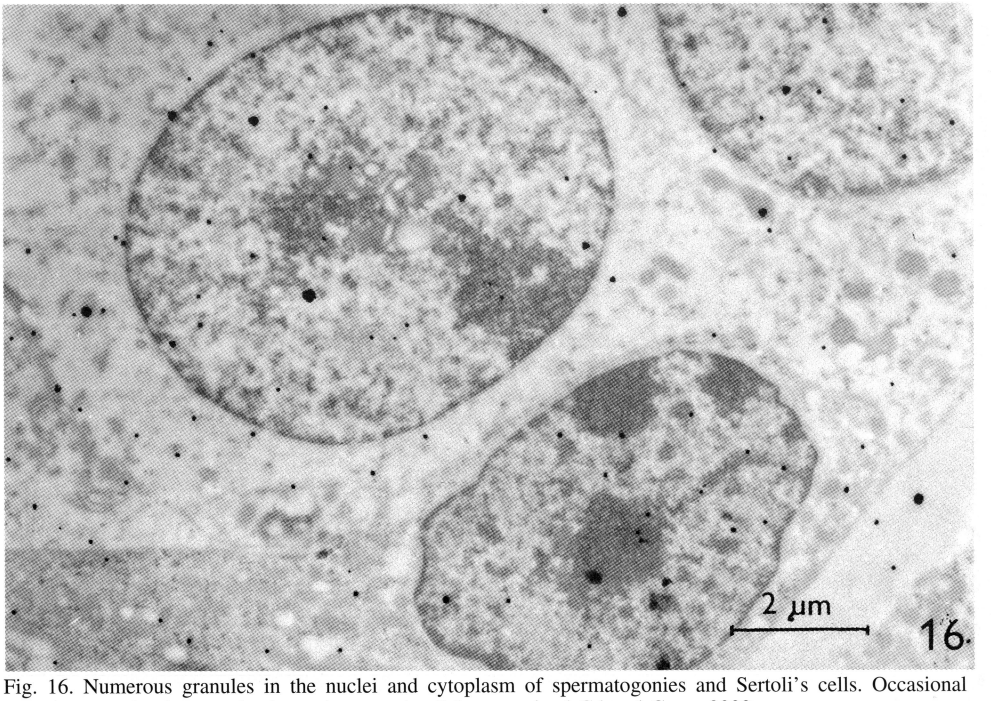

Fig. 16. Numerous granules in the nuclei and cytoplasm of spermatogonies and Sertoli's cells. Occasional granules can also be seen in the tunica propria. Calves received Cd and Cu. × 9000.