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The Effect of Orally Administered Cadmium on the Ultrastructure of the Rat Prostate

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Summary. The effects of cadmium and nickel chloride, administered in drinking water at 5 and 50 ppm, on the rat prostate are described. Zinc concentrations in the two lobes of the gland were unaffected by the metals and no consistent changes were observed at the subcellular level by X-ray microanalysis. The ultrastructural appearance of the prostate gland in rats of varying ages was unaltered following cadmium administration at those levels, while plasma testosterone concentrations did not differ significantly in cadmium treated animals. Low levels of cadmium (<5 ppm) were accumulated by the ventral lobe of the prostate, although the metal was not detectable subcellularly. The results are discussed in relation to human prostatic carcinoma.

Key words: Prostate, Cadmium, Ultrastructure, Testosterone.

Introduction

The high incidence of prostatic carcinoma in the human male (Registrar General's Statistical Review, 1970–1972) has promoted interest in possible aetiological factors involved in its occurrence. A number of epidemiological studies have indicated an increased incidence of the disease amongst workers in the cadmium industry [35, 20, 23], but this remains controversial [22, 21]. The toxic effects of cadmium to the male reproductive system have long been known [30, 31], high levels of the metal causing testicular necrosis, which is accompanied by atrophic and inflammatory changes in the prostate [39]. It has, however, proved difficult to divorce the direct effects of the metal on the prostate from indirect changes induced by reduced testosterone synthesis.

Studies employing low levels of the element administered via water have shown neither an increase in tumour incidence nor significant light microscopic changes in the prostate [25]. These investigations were, however, mainly histological in nature and in the present study were extended to an ultrastructural investigation of the effects of low concentration of the metal in the lateral and ventral lobes of the rat prostate at different ages. Age was considered to be an important factor, since the incidence of prostatic neoplasia in rats, as in man, is found to increase dramatically with age [33, 40]. The morphological studies were accompanied by measurement of total cadmium uptake by each prostatic lobe and X-ray microanalysis of individual tissue sections. The effect of the metal on zinc content and distribution within the gland was also investigated, since zinc is known to have a protective effect against cadmium action [11, 32] and levels of the element in the prostate are reduced following cadmium treatment [41]. Nickel was also investigated because of its known carcinogenicity and natural association with cadmium.

Materials and Methods

(i) Animals

Institute bred male Sprague-Dawley rats were employed throughout the experiments. They were fed a standard laboratory diet (Pilsbury, England) and tap water *ad libitum*. In the case of experimental animals cadmium chloride at concentrations of 5 and 50 ppm and nickel chloride at 5 ppm were added to their normal drinking water. Normal levels of Ni and Cd in drinking water were <10 ppb for all control rats. Laboratory diet contained <1 ppm cadmium and nickel. The animals were selected from three age groups; 12 week old rats, 10 month animals and 18 month old rats. For the youngest rodents groups were killed at 10, 20 and 40 weeks after Cd and Ni treatment commenced, while at the other two ages prostatic tissue was removed at 20 weeks only. Ultrastructural, X-ray analytical and atomic absorption studies were performed on all prostatic tissues except at 40 weeks, when histological and plasma testosterone investigations alone were performed.

(ii) Electron Microscopy

Tissue for electron microscopy was diced into 1 mm^3 fragments and fixed in 3% glutaraledhyde (Emscope) for 3 h at 20 °C. It was

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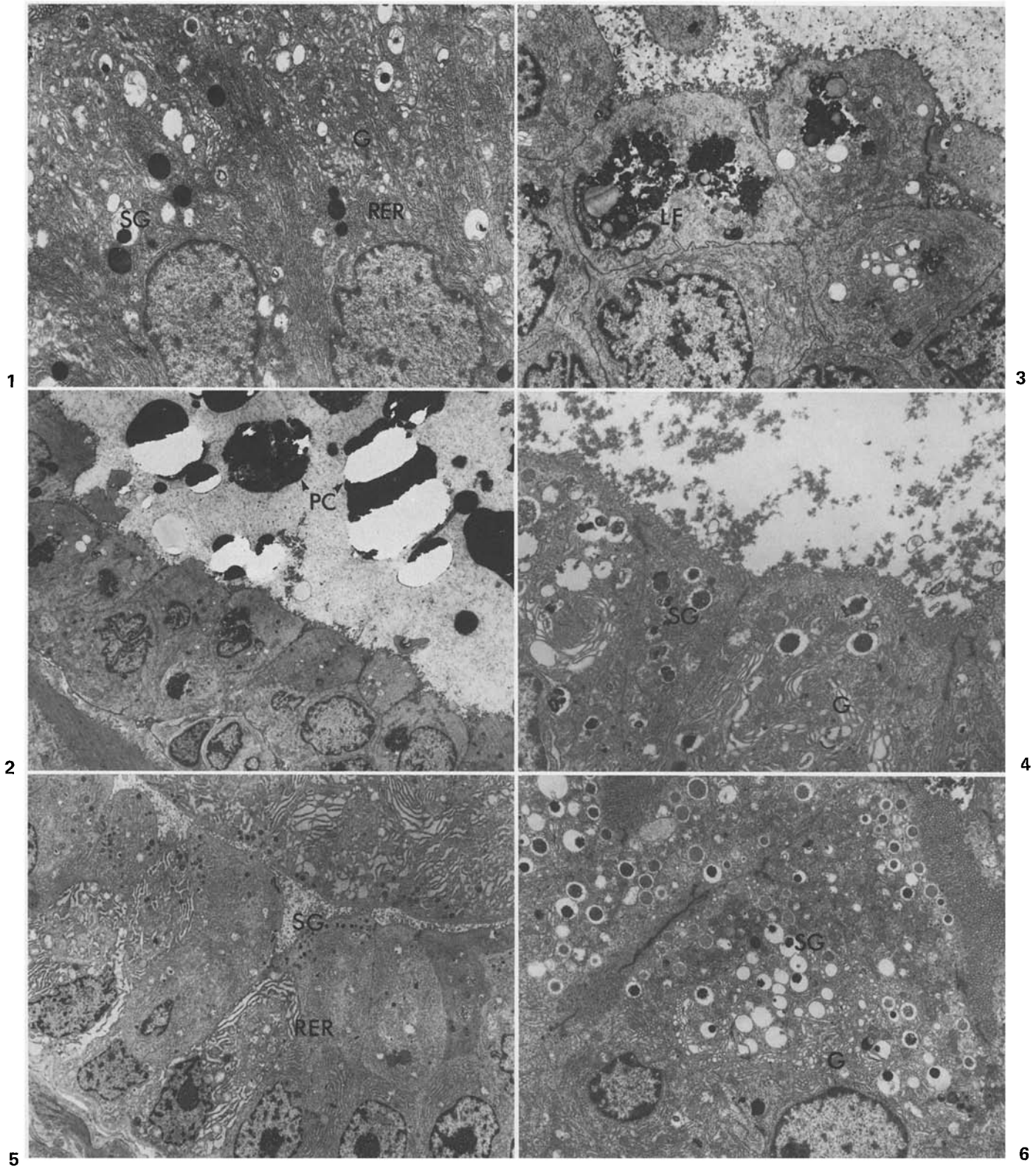

Fig. 1. Lateral prostate of normal 22 week old rats shows extensive system of granular endoplasmic reticulum (RER). *G* = Golgi Region; *SG* = Secretory Granule (x5,600)

Fig. 2. Ventral lobe of prostate in 15 month old rat exhibits short undifferentiated epithelial cells. Numerous prostatic calculi (*PC*) are evident. (x2,100)

Fig. 3. Low columnar epithelium in rat ventral prostate at 23 months. Lipofuscin bodies (*LF*) containing lipid droplets and dense granules are present in abundance. (x5,600)

postfixed in 1% osmium tetroxide [28] for 1 h and washed in 0.1 M phosphate buffer (pH 7.4). The tissue was then dehydrated in a graded series of alcohol, cleared in propylene oxide and embedded in Araldite epoxy resin.

Ultrathin section (~ 65 nm) were cut on a Cambridge-Huxley ultramicrotome and collected onto copper grids (Agar Aids, U.K.). These sections were stained with uranyl acetate and lead citrate and examined in an AEI EM6B or EMMA 4 electron microscope (Kratos, U.K.). Semithin sections (0.5 μm) were stained with toluidine blue and borax and examined with a Zeiss photomicroscope.

(iii) X-ray Microanalysis

Tissue was processed, using a modification of the method of Yarom and Chandler [44] and validated for zinc in prostatic tissue by Chandler [5]. Pieces of tissue (1 mm^3) were fixed for 1 h in 2% postassium pyroantimonate containing 1% OsO_4. They were dehydrated in 100% ethanol, infilterated and embedded in Araldite. Gold coloured sections (100 nm) were mounted on aluminium grids and carbon coated. Analysis was performed using an EMMA 4 electron microscope (Kratos, U.K.) This permitted X-ray microanalysis to be performed on subcellular regions of tissue sections, while observing the transmitted image [4].

Analysis was performed with an electron beam current of 0.1 μA at 80 kV accelerating voltage for a counting time of 40 s in each region. An energy dispersive solid state detector (Kevex, USA) was employed for analysis. Zn, Kα, and Cd Lα X-ray were collected and quantitation performed using the method of Hall [14], together with resin standards [3].

(iv) Histology

Tissue for histological examination was fixed in 10% formol saline for 7–10 days, paraffin wax embedded and 5 μm sections cut and collected onto glass slides. The sections were stained with haematoxylin and eosin, mounted and examined in the Zeiss photomicroscope.

(v) Atomic Absorption Spectrophotometry

Pieces of prostatic tissue were weighed in procelain crucibles and 3 ml of a mixture of 25 ml of nitric acid (Aristar grade) and 10 ml of perchloric acid was added. Solutions were heated almost to dryness and the contents of each crucible were made up to 5 ml with deionised water. A Varian-Techtron AA 1200 atomic absorption spectrophotometer with a standard acetylene burner was used. The instrumental parameters employed were as given in the manufacturer's analytical methods handbook. Zinc was determined at 213.9 nm and Cd at 228.8 nm.

(vi) Determination of Testosterone Concentrations

Samples of venous blood (3 ml) were obtained from the external jugular vein, under ether anaesthesia. The blood samples were immediately placed in sequestered tubes and centrifuged at 1,000 g for 15 min at 4 °C. The plasma was separated and stored at −20 °C until assayed. The concentration of testosterone in each sample was measured by radioimmunoassay in the Supraregional Assay Service Laboratory of the Tenovus Institute, using methods developed at the Institute [8].

Results

I. Ultrastructure of the Normal Untreated Rat Prostate

a) 22 Week Animals. The ultrastructure of the lateral and ventral lobes of the rat prostate has been well documented [15, 1, 16, 7] while a detailed study of age changes within the gland has recently been reported [42].

In general the lateral prostate of the rodent consists of fibroblasts, connective tissue fibres and layers of smooth muscle cells surrounding acini lined by columnar epithelial cells. These cells have basally situated indented nuclei and a well developed system of granular endoplasmic reticulum (Fig. 1). A prominent feature of the supranuclear region of the cell is a well-developed Golgi apparatus consisting of both flattened and dilated saccules, some of which contain dense secretory products. Another characteristic of the lateral lobe epithelial cells is the numerous microvilli observed apically where there are also many membrane-bound vacuoles containing dense secretory granules or flocculent material. Moderate numbers of primary and secondary lysosomes occupy the supranuclear region. Interspersed between the epithelial cells at regular intervals around the acini are small, pale basal cells, which are characterised by few cytoplasmic organelles.

Epithelial cells in the ventral lobe of the prostate differ from those in the lateral region by being taller and containing extensive long, dilated profiles of RER. The secretory granules in these cells are small and dense and confined to the apex of the cells, while microvilli are sparse.

b) 8 and 15 Month Old Animals. The prostate in 32 week old rats differed from those in the younger age group only in the presence of increased numbers of lysosomes. At 15 months, the ventral lobe of the prostate exhibited both tall columnar and shorter atrophic cells. The more normal of the epithelial cells were characterised by the presence of large lipofuscin granules in the perinuclear region. The shorter cells exhibited a reduction in the extent of the RER while numbers of secretory granules and Golgi saccules were also decreased. In these areas prostatic calculi were frequently observed in the lumina (Fig. 2).

◀ **Fig. 4.** Lateral prostate from 22 week old rat treated with cadmium chloride (50 ppm) for 10 weeks. Epithelium appears normal with large Golgi region (*G*) and abundant secretory granules (*SG*). (x3,500)

Fig. 5. Ventral lobe epithelium in 22 week old rats (+ 5 ppm $CdCl_2$ for 10 weeks) shows extensive system of endoplasmic reticulum (*RER*) and apically situated secretory granules (*SG*). (x2,250)

Fig. 6. Lateral prostate 15 month old rat reated with 50 ppm $CdCl_2$ for 20 weeks. Columnar epithelial cells show well developed Golgi region (*G*) and many secretory granules (*SG*). (x4,200)

c) 23 Month Old Animals. The lateral lobe of prostate gland in 23 month old rats contained epithelium of fairly normal appearance, although shorter epithelial cells were also encountered. The columnar cells retained their appearance although there was some reduction in the extent of the RER and increased numbers of lysosomes were observed in the supranuclear region of the cells compared with 15 month old animals. The Golgi apparatus was extensive and secretory granules were present, although not to the same extent as in younger animals. A well developed system of microvilli was again observed at the apex of these cells, while the basal cells had a normal appearance with few organelles.

Sampling of tissue proved to be a problem in the ventral lobe, where a range of epithelia from high columnar to low cuboidal was observed histologically. The low columnar or cuboidal cells showed a pale cytoplasm with few free ribosomes and fewer smaller less dilated profiles of RER than observed in younger rats. Secretory granules were greatly decreased in number, while large lipofuscin bodies containing lipid droplets and dense granules were present in abundance (Fig. 3).

II. Prostatic Ultrastructure in Cd and Ni Treated Rats

The gross appearance of all prostate glands in the experimental animals was almost unchanged compared with that seen in control animals. Histologically, changes in prostatic appearance were also related only to the age of the animals and not to cadmium treatment. Lateral lobe epithelium consisted of columnar cells, interspersed with basal cells, as described above (Fig. 4). Nuclei were irregularly shaped with a thin rim of heterochromatin and prominent nucleoli. Microvilli were abundant towards the apex of the cells and similar numbers of secretory granules were observed to control cells. The profiles of endoplasmic reticulum were observed with similar frequency to the controls, while basal cells were small and characterised by a lack of differentiation. Ventral lobe epithelium, once again, consisted of columnar cells (Fig. 5), whith irregular nuclei and a well developed Golgi region. As in controls, the RER formed an extensive network, while secretory granules were small and dense and confined to the apices of the cells.

Ultrastructural age changes in the prostatic epithelium were similar in the glands of metal treated rats to those seen in control animals. At 8 months, numbers of lysosomes, were increased in both lobes of the glands in all experimental animals, but, the size and extent of these organelles did not differ significantly from normal. The degree of atrophy observed in the lateral lobe tissue and more especially ventrally at 15 and 23 months was similar to controls (Figs. 6 and 7). The small undifferentiated cells were not seen with greater frequency, even in high cadmium treated animals, while lipofuscin bodies occurred with normal frequency (Fig. 8). Nuclei remained irregular in outline, with heterochromatin limited to their outer margins and lipofuscin, while extensive in the prostates of this age group, was not altered compared with normal old glands.

Histologically, both lateral and ventral lobes of the prostates of 52 week old rats, treated with Cd for 40 weeks appeared normal (Figs. 9 and 10).

III. Cadmium and Zinc Content of Prostatic Tissue (AAS)

(a) Zinc. The zinc content of the lateral prostatic lobes at all ages was high, but showed a large standard deviation from one animal to another (Table 1). In the ventral region of the gland, the zinc levels were much lower. Lateral lobe concentrations of the element were unaltered in either nickel or cadmium treated rats. There was, however, a significant reduction of the metal in the ventral region in 15 month old rats, following administration of 50 ppm Cd, while this concentration caused zinc uptake in the same lobe of 22 week old animals.

b) Cadmium. In the lateral prostate, cadmium was not detected in the control animals at 22 weeks, 15 or 23 months. The Cd value found for the 32 week old rats was not statistically significant and was due to one animal in the group. In the Ni treated group a significant increase of

Table 1. Zinc content of the prostate (ppm – wet weight) determined by atomic absorption spectrophotometry

		22 week old rats (+ Cd for 10 weeks)	32 week old rats (+ Cd for 20 weeks)	15 months old rats (+ Cd for 20 weeks)	23 months old rats (+ Cd for 20 weeks)
Lateral Lobe	Control	1,182 (± 1,080)	931 (± 492)	987 (± 1,053)	–
	5 ppm Cd	1,465 (± 536)	636 (± 586)	194 (± 60)	–
	50 ppm Cd	426 (± 470)	859 (± 203)	238 (± 102)	–
	5 ppm Ni	2,410 (± 481)	773 (± 645)	278 (± 207	–
Ventral Lobe	Control	17 (± 4)	16.7 (± 5)	14.5 (± 2.1)	10.8 (± 0.9)
	5 ppm Cd	40 (± 32)	13.0 (± 3.2)	–	–
	50 ppm Cd	42[a] (± 1)	10.5 (± 7.8)	8.8[a] (± 1.1)	13.2 (± 5.7)
	5 ppm Ni	35 (± 41)	–	–	–

[a] Significantly different from control ($p < 0.05$) Figures in parenthesis represent ± 1 Standard deviation

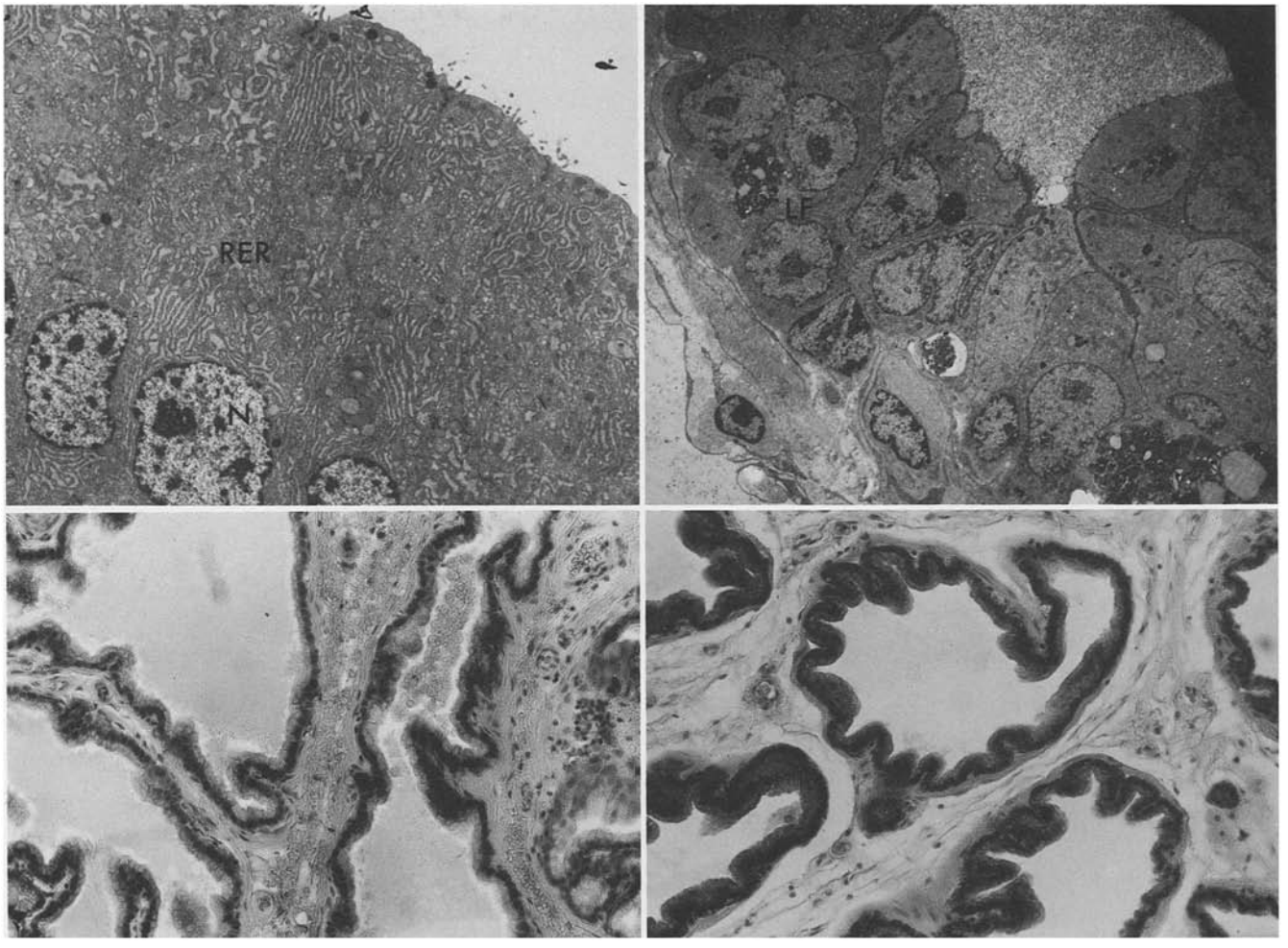

Fig. 7. 15 months ventral lobe tissue (+ 5 ppm $CdCl_2$ for 20 weeks). Tall columnar epithelial cells with basal nucleic (*N*) and an extensive system of endoplasmic reticulum (*RER*) are apparent (x3,500)

Fig. 8. Atrophic ventral lobe epithelial cells in cadmium treated 23 month old rat (5 ppm for 20 weeks) showing lipofuscin granules (*LF*) (x2,100)

Fig. 9. Lateral prostate of 52 week old rat treated with cadmium chloride (50 ppm) for 40 weeks (x182)

Fig. 10. Ventral prostate of 52 week old rat treated with cadmium chloride (50 ppm) for 40 weeks shows columnar epithelium (x182)

Table 2. Cadmium content of the prostate (ppm wet weight) determined by atomic absorption spectrophotometry

		22 week old rats (+ Cd for 10 weeks)	32 week old rats (+ Cd for 20 weeks)	15 month old rats (+ Cd for 20 weeks)	23 month old rats (+ Cd for 20 weeks)
Lateral Lobe	Control	0	0.6 (±1.0) [N.S.]	0	–
	5 ppm Cd	6.6 [N.S.] (± 11.4)	0.8 [N.S.] (± 1.4)	1.2[a] (± 1.3)	–
	50 ppm Cd	0	2.4[a] (± 1.4)	1.4[a] (± 0.7)	–
	5 ppm Ni	0	0	0	–
Ventral Lobe	Control	0.5 (± 0.1)	1.3 (± 0.7)	1.8 (± 0.1)	–
	5 ppm Cd	2.2[a] (± 1.0)	–	–	–
	50 ppm Cd	5.0[a] (± 0.1)	2.4 (± 1.2)	1.9 (± 1.1)	0.66[a] (± 0.4)
	5 ppm Ni	0.9[a] (± 0.3)	–	–	–

[a] Significant different from control ($\rho < 0.05$) Figures in parenthesis represent ± 1 standard deviation N.S. = Not significant

Table 3. Zinc levels in nuclei and secretory granules of lateral prostate of rat (% wet weight) determined by X-ray analysis

		Controls	5 ppm Cd	50 ppm Cd	5 ppm Ni
22 week old rats (+ Cd for 10 weeks)	Nucleic	0.16 (± 0.06)	0.26[a] (± 0.06)	0.10 (± 0.05)	0.29[a] (± 0.08)
	SGS	0.18 (± 0.15)	0.50[a] (± 0.14)	0.10 (± 0.07)	0.32[a] (±0.08)
32 week old rats (+ Cd for 20 weeks)	Nucleic	0.16 (± 0.02)	0.19 (± 0.05)	0.25[a] (± 0.18)	0.23[a] (± 0.12)
	SGS	0.16 (± 0.09)	0.25[a] (± 0.11)	0.28 (–)	0.45[a] (± 0.34)
15 month old rats (+ Cd for 20 weeks)	Nucleic	0.10 (± 0.04)	0.11 (± 0.05)	0.10 (± 0.04)	0.13[a] (± 0.04)
	SGS	0.16 (± 0.1)	0.16 (± 0.07)	0.19 (± 0.09)	0.14 (± 0.07)
23 month old rats (+ Cd for 20 weeks)	Nucleic	0.23 (± 0.08)	–	0.13 (± 0.05)	–
	SGS	0.23 (± 0.07)	–	0.16[a] (± 0.07)	–

[a] Significantly different from control ($p < 0.05$) Figures in parenthesis reepresent ± 1 standard deviation. Cadmium was not detected in any organelle (Minimum detectable limit = 100 ppm locally)

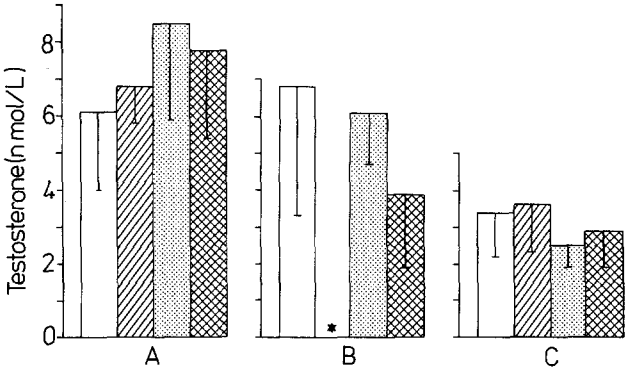

Fig. 11. Plasma testosterone concentrations in control, cadmium and nickel treated rats. Vertical bars represent one standard deviation. Key: *A* = 22 week old rats + 20 week Cd; *B* = 52 week old rats + 40 weeks Cd; *C* = 23 month old rats + 20 weeks Cd. *Asterisk* indicates values not determined. *Open columns* represent control animals; *hatched columns* represent treatment with 5 ppm $CdCl_2$; *stippled columns* represent rats treated with 50 ppm $CdCl_2$; *Cross hatched columns* represent nickel chloride treatment (5 ppm)

Cd in the ventral prostate was found at 22 weeks but less than in any Cd treated group. It was present in significantly increased amounts in the high cadmium group at 32 weeks, in both cadmium groups at 15 months.

Cadmium concentrations were generally higher in the ventral lobe than in the lateral tissue, even in control animals (Table 2) Cadmium levels, however, were significantly elevated compared with controls in all metal treated 22 week old animals and in the high cadmium treated 23 month old rats.

IV. X-ray-microanalysis

Previous studies have shown zinc to be primarily located in the nuclei and secretory granules of prostatic epithelial cells. After preliminary general analysis, therefore, these organelles were analysed in detail for zinc and cadmium. Cadmium was not detected in any organelles in sections of control or metal treated prostatic tissue (minimum detectable concentration 100 ppm locally). Standard deviations were high for all zinc analyses. In the nuclei, zinc concentrations in the rats treated with low levels of nickel and cadmium for 10 weeks were significantly higher than in control animals (Table 3). Levels were also significantly elevated in the lateral lobe nuclei of 32 week old rats, treated with high cadmium or low nickel concentrations for 20 weeks and in the nickel treated 15 month and 2 year old animals. Secretory granule zinc levels in low cadmium and nickel rats were significantly higher than controls for 22 and 32 week old rats, while in the 2 year old animals, concentrations of the element in the secretory granules of high cadmium rats were significantly depressed.

V. Plasma testosterone levels

In 8 month and 23 month old rats plasma testosterone levels were compared in control animals and those to which $CdCl_2$ and $NiCl_2$ has been administered in drinking water for 20 weeks. In both age ranges plasma testosterone concentrations, determined by RIA, were not significantly altered by cadmium or nickel treatment, although levels were consistently higher in the younger animals (Fig. 11). In a third set of rats, to which cadmium had been administered for a period of 40 weeks, again plasma testosterone levels in cadmium or nickel treated animals did not differ significantly from controls.

Discussion

Many investigations have shown the toxicity of subcutaneously administerd cadmium to the mammalian prostate [31, 11]. The link between exposure to the metal and the incidence of prostatic carcinoma in the human male, however, remains uncertain [23, 26, 35]. Several workers have

demonstrated the toxic effects of injection of high cadmium concentrations to the rodent prostate [19, 37, 41]. Few reports, however, have appeared concerning the toxic prostatic effects of low cadmium levels, which appear not to cause alterations in testicular androgen output. In the present study plasma testosterone levels and gross testicular appearance were unaffected by the concentrations of cadmium employed. This implied that any action of metal would most likely be direct, rather than indirect through testicular incompetence.

The oral route of cadmium administration employed here allows for absorption of 1%–8% of the ingested metal [43] The levels chosen (5 and 50 ppm) were considerably higher than those found in normal drinking water (Safe level = 0.01 ppm [9], but were concentrations, which although not acutely toxic to the rat, have been shown to cause ultrastructural changes in the kidney [29] and liver [27]. Nickel was also used as a comparative metal, being one to which cadmium workers are also routinely exposed [26] and a suggested carcinogen [17, 18].

Prostatic ultrastructure in the rats used in the present study was unaffected by cadmium or nickel administration. In ageing animals changes in prostatic ultrastructure were comparable with those encountered in control animals and similar to those previously described [42]. Prostatic tumours were not observed in any group. The link, albeit tenuous, between cadmium and prostatic carcinoma has previously prompted several attempts to induce prostatic tumours in experimental animals [24, 39]. As found in the present study, it has not proved possible to produce prostatic adenocarcinomas in rats or mice by the administration of cadmium either orally or by injection. Tumours induced by injection of Cd in the gland, have been sarcomas and squamous cell carcinomas [24, 39], the tumours of mesenchymal mesodermal origin, suggested by Gunn et al. [12]. These have little relation to the normal prostatic adenocarcinomas commonly found in man.

In addition to observing the effects of the metal on prostatic ultrastructure, it was of interest to investigate its effect on naturally occurring zinc in the gland. It has long been known that a 100–200 times excess of zinc can reverse the toxic action of Cd on the testis [11, 31] while cadmium also causes a decrease in ^{65}Zn uptake by the rat dorsolateral prostate [6, 11]. The possibility of interaction between zinc and cadmium in the prostate was investigated in the present study by the measurement of whole tissue zinc levels and the subcellular distribution of the metal following cadmium treatment. Cadmium, even up to the highest levels in drinking water, did not cause consistent changes in total zinc levels or depression of nuclear or cytoplasmic zinc, even though a replacement of the metal by cadmium had been thought possible. Cadmium accumulation, however, was generally lower in the lateral lobe of the gland, which supports the suggestion of a protective role for zinc against Cd action [13, 38].

As in man, the occurrence of prostatic tumours in the rat is an age related phenomenon [34]. It might, therefore, have been expected that the prostates of older animals would have a greater susceptibility to cadmium action and would exhibit an increased accumulation of the metal with time. In the experiments described here the effect of Cd on the prostate was not influenced by the age of the animals and in addition, the normal ageing effects observed in the gland were not modified by the presence of the metal.

The results, described in this study, might suggest that the differing levels of Cd administered here via an oral route compared with industrial exposure by inhalation, along with the differences in prostatic ultrastructure between man and the rat, may provide limited information with regard to cadmium as a carcinogen in man. Evironmentally, however, the metal, even at low levels is accumulated by the prostate and over a 20–40 year period may be present in sufficient concentration to enhance the latent neoplastic changes occurring in the human gland [10]. It is possible that Cd is a weak carcinogen either acting directly on the gland, as evidenced by increased Cd uptake in prostatic carcinoma [13] or indirectly via subtle modification in testicular hormone output. Indeed the contradictory results obtained on epidemiological investigation of the role of Cd in prostatic carcinoma in man [20, 22, 23] do not give a clear indication of the importance of Cd in the occurrence of the disease compared with the other factors implicated in its aetiology.

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