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Project Summary

The Effects of Cadmium on Renal Aging -- A Chronic Cadmium Feeding Study in Rats

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Cadmium (Cd), a nephrotoxin, is known to accumulate in the kidney cortex, preferentially in the renal proximal tubules. Animal and human autopsy studies have shown that damage to the renal proximal tubular cells is associated with toxicity from chronic Cd exposure. Several investigations have shown an age-associated change in the levels of renal cortical cadmium resulting from chronic exposure in laboratory animals and long-term environmental exposure in humans.

This study was undertaken to determine if Cd exposure influences the natural aging process in the kidney and the accumulation patterns of renal copper (Cu) and zinc (Zn). The concentrations of Cd, Cu, and Zn in the cortical tubules were quantitated to determine if the tubules preferentially accumulate these three metals relative to the whole cortex.

Two hundred seventy-two male Wistar rats were treated for 24 months with 0.0, 0.5, 5.0, and 50 mg/l $CdCl_2$ in the drinking water. Every three months, data were collected pertaining to renal structure, function and concentrations of Cd, Zn, and Cu.

The levels of all three metals studied were higher in the tubules than in the intact cortex, on a dry weight basis, indicating that these three metals were concentrated by the tubular cells relative to the other cortical cell types. The concentration of Cd in both the cortex and tubules increased with dose level and duration of exposure. This increase was linear up to 12 months in the two low dose groups and up to 15 months in the highest dose group. After 15 months, the levels of Cd decreased in both the tubules and the whole cortex. Zn and Cu concentrations in the cortex and tubules were significantly elevated by Cd exposure, with the greater effect on Cu. Zinc and Cu levels in the tubules also decreased significantly after 15 months.

The toxic effects from Cd exposure were mild and limited primarily to the highest dosed group. The combination of age and Cd dosing had the greatest effect on the accumulation patterns of renal Cd, Zn and Cu.

Age alone had the greatest effect on changes in the tissue structure of the kidneys and may have accounted for the decrease in the concentration of renal metals seen in older individuals. Age alone also had the greatest effect on the physiological functioning of the kidney as measured by proteinuria, decreased urine and renal leucine aminopeptidase activity, changing diurnal urine volumes and decreased urine pH and specific gravity. The combination of old age and high Cd dosing appeared to affect renal functioning as indicated by increased urine volume, increased urine glucose levels and decreased specific gravity.

Increased Cd dosing appeared to be asociated with certain structural changes in the kidney. Although not significant, the highest dosed animals had a greater prevalence of moderate to severe tubular hyperplasia and tubular dilation/cast formation. The highest dosed rats also had significantly larger kidneys as measured by their percent of the total body mass.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to

announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Cadmium, a non-essential trace element used in many industrial processes and consumer products, is now widely distributed throughout the environment. Since cadmium is a nephrotoxin, there is increased concern over the potential health effects from low-level, chronic exposures.

Previous animal and human autopsy studies have shown that chronic cadmium exposure is associated with characteristic renal morphological changes. Such studies have also demonstrated that the pattern of renal cadmium accumulation varies as a function of age

Because of the documented age-associated change in renal cadmium levels, a study was designed to examine the effects of both natural aging and cadmium exposure on the tissue structure and physiological functioning of the kidney The primary hypothesis tested was whether cadmium exposure influences the physiological, morphological, and biochemical aging of the kidney

Since cadmium is thought to accumulate preferentially in renal proximal tubules, it was desirable to separate out the tubules and relate the physiological changes in the kidney to the cadmium levels in the tubules. Traditionally, the renal cadmium burden has been evaluated as the concentration of cadmium in the intact cortex; therefore, a comparison of the levels of metals in the tubules with that of the cortex was needed to determine if the cortex levels were good indicators of tubular levels It was hoped that by separating out the tubules, it would be possible to gain a more accurate picture of the relationship between renal cadmium accumulation and renal toxicity A secondary hypothesis tested was whether cadmium concentrations in the renal cortex are sufficient to predict the cadmium dose to the renal tubules

Since tissue accumulation of cadmium is closely associated with that of zinc and copper, additional hypotheses tested were whether the renal tubules preferentially concentrate zinc and copper and if cadmium affects the accumulation of these two metals.

Every three months, eight rats from each dosage group (i.e., 0 0, 0 5, 5.0, and 50.0 mg/l cadmium) were sacrificed to obtain kidneys from which enriched cortical tubule concentrations were determined for both the intact cortex and the tubule preparation from each rat. Changes in renal function were assessed by performing urinalysis on a regular basis. Changes in renal tissue structure were assessed by morphometric analysis of fixed kidney sections.

Results and Discussion

The effect of cadmium exposure on the general health status of the rats was assessed by several parameters, including body and organ weight gain, hematocrit, and food and water consumption.

Increased cadmium exposure was associated with decreased food and water consumption, body weight and hematocrit. Reduction in the values of these variables indicated that cadmium exposure adversely affected the general health of the individuals; however, the effect was not severe at the levels of cadmium given to the rats.

The concentration of cadmium in the intact renal cortex (Figure 1) and the renal cortical tubules (Figure 2) versus time have shown that in all exposure groups, cortical and cortical tubular cadmium concentrations increase with time of exposure up to 15 months and then begin to decrease. The 15-month time point appears to be a critical time for cadmium kinetics independent of renal cadmium burden, suggesting that the effect is related more to aging than to cadmium toxicity.

Comparison with control rats showed that cadmium exposure elevated renal copper and zinc levels in a dose dependent manner. The greater effect was on copper concentrations (Figure 3).

As with cadmium accumulation, tubular and cortical copper and tubular zinc levels peaked around 15 months and declined thereafter. These results support the idea that an age component affects the renal accumulation of these three metals.

The data support the hypothesis that the renal cortical tubules preferentially accumulate cadmium, zinc and copper.

The relationship between the concentration of cadmium in the intact cortex and the concentration of cadmium in the cortical tubules for the high dose group is indicated in Figure 4. For all dosage groups up to 18 months, with few exceptions, the tubules consistently had significantly higher concentrations of cadmium than did the intact cortices ($p \leq 0.05$). The same effect was observed for both zinc and copper. Dramatic decreases of cortical tubular metals seen at 21 and 24 months may have been more artifactual than real. This was due to a significant loss of physical integrity of the tubule preparations recovered during this late phase of the study.

Further, the relative rate of accumulation of cadmium in the cortical tubules versus the intact cortex was calculated for each group for the linear portion of the cadmium accumulation curve (i.e., 0-12 months for the low dose groups and 0-15 months for the high dose group). The ratios of the rates of cortical tubule:cortical accumulation were found to be 1.2, 1.3, and 1.4 for the high, midrange and low groups, respectively. Although the differences in these ratios are very small, they do show a dose-related trend. This would suggest that the rate of accumulation of cadmium in the intact cortex may not be a good indicator of the rate of accumulation of the metal in the cortical tubules at all doses. It does suggest, however, that at higher doses, the rate of cadmium accumulation in the intact cortex would be a good estimator of the rate of accumulation in the cortical tubules. Since the preparation of tubule pellets is extremely time-consuming, it is reasonable to use the cortical data as an approximation for the cortical tubule data.

By separately analyzing the cortical tubules and the whole cortex, it was hoped that the cause of decreased cortical cadmium levels with old age could be explained. It was theorized that if the cortical levels declined with age but the cortical tubular levels plateaued or continued to increase, then the decreased cortical levels could be attributed to the age-related infiltration of renal fibrous tissue, which concentrates minimal amounts of cadmium. The data partially support this theory. First, the morphometrics showed that there was a significant decrease with age of the numerical density of glomeruli and, therefore, the number of nephrons. Second, there was a significant age-associated decrease in the volume density of intact proximal tubular walls in the outer cortex. Third, there was a significant age-associated increase in the amount of interstitial fibrotic tissue. All three effects were seen equally among the controls and the dosed animals.

These data suggest that the decrease of renal cadmium with age is due to at least two factors. First, with age, there is an actual physical loss of intact proximal tubules, the component of the renal

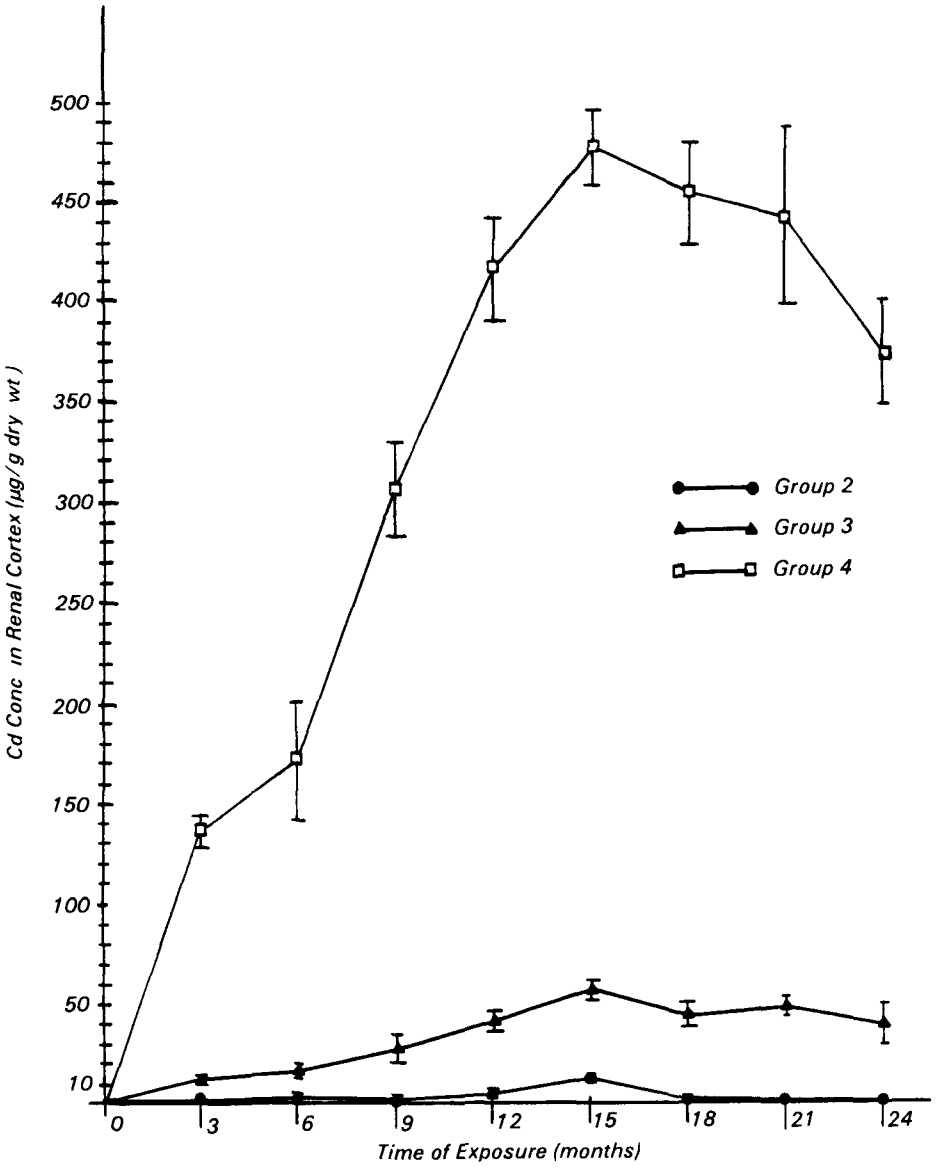

Figure 1. *Concentration of cadmium in the intact renal cortex (µg Cd/g dried cortex) as a function of exposure to cadmium.*

cortex that sequesters the highest proportion of the cadmium organ burden. Second, with increasing age, there is a change in the proportion of tissue types in the kidney. Presumably, each tissue type has a characteristic metal burden so that alterations in the tissue composition could lead to alterations in the renal metal levels. Given the present data, these factors seem to apply to the accumulation patterns of zinc and copper as well as cadmium.

All study groups showed many age-related changes in kidney structure. Consistent with the findings of others, age correlated significantly ($p \leq 0.05$) with increased inflammation, interstitial fibrosis, glomerular and tubular basement membrane thickening, tubular dilation/cast formation, and hyperplasia. The glomeruli became significantly larger ($p \leq 0.05$) with age, but their density in the outer cortex became significantly smaller. Although there were no statistically significant differences between the groups, the high dose group, in comparison to controls, tended to have a higher percentage of individuals with moderate to severe hyperplasia and tubular dilation/cast formation.

The data suggest that cadmium exposure may have had a toxic effect on the kidney that was expressed by an increase in renal mass. Although there was no statistically significant difference between the groups in the total wet weight of the kidneys, the highest dose group consistently had heavier kidneys than all other groups throughout most of the experiment. Also, when the kidney weight was recalculated as the percent of body weight, kidney values for the highest dose group were significantly higher ($p \leq 0.05$) than those of the controls.

Since the renal wet/dry weight ratios were not significantly different between the groups, increased hyperplasia in the high dosed group seemed the likely cause of the larger kidneys. Morphometric analysis supporte this idea.

At the levels tested, renal morphological changes typical of cadmium toxicity were not detected.

In general, cadmium at the doses tested had little adverse effect on the functioning of the kidney. Significant dose-response relationships were not anticipated since the maximum cortical concentrations of cadmium were approximately 90 mg/g wet weight (high dose group) and it has been suggested that 200 mg/g wet weight is the critical concentration associated with detectable renal dysfunction.

Age alone caused more significant changes in renal function than did cadmium exposure. For certain parameters, including diurnal urine volume, urine glucose and specific gravity, the effect of old age in combination with high cadmium exposure appeared to have a greater effect on renal function than did age alone. The data were suggestive of a mild toxic effect of cadmium expressed only in late life.

The rat presented a picture of gradual renal change with time that included decreased levels of renal and urine leucine aminopeptidase proteinuria, changing diurnal urine volumes, decreasing pH, and decreasing specific gravity.

Conclusions and Recommendations

Data from this study suggest many age-related renal changes which alter the kidney's physiological handling of the toxic metal, cadmium, and the essential metals, copper and zinc.

The hypothesis that chronic cadmium exposure influences the physiological, morphological and biochemical aging of the kidney has been supported partially by the findings. This hypothesis was not fully supported because of the levels of cadmium exposure tested. In general,

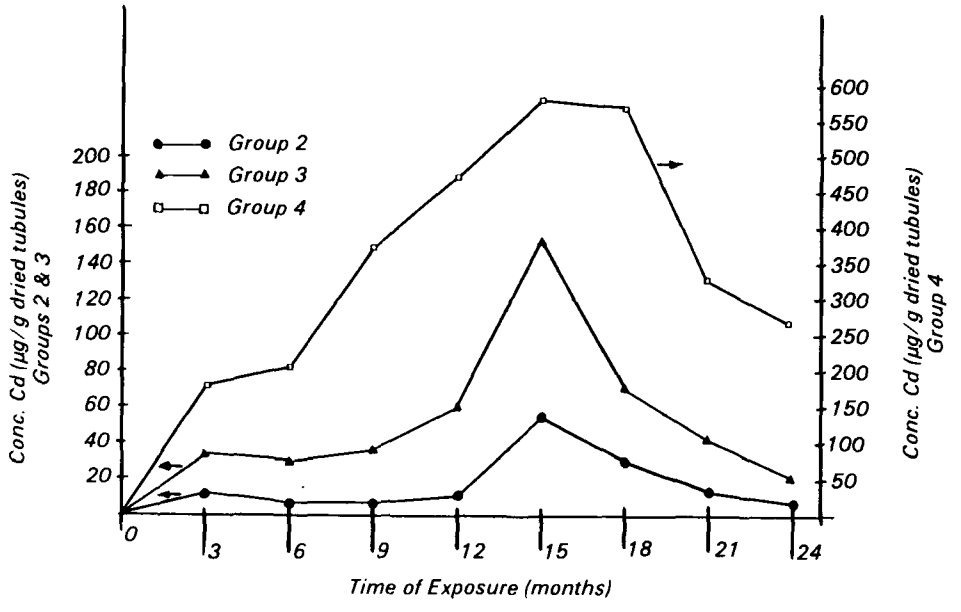

Figure 2. *Concentration of cadmium in the renal cortical tubules as a function of exposure to cadmium.*

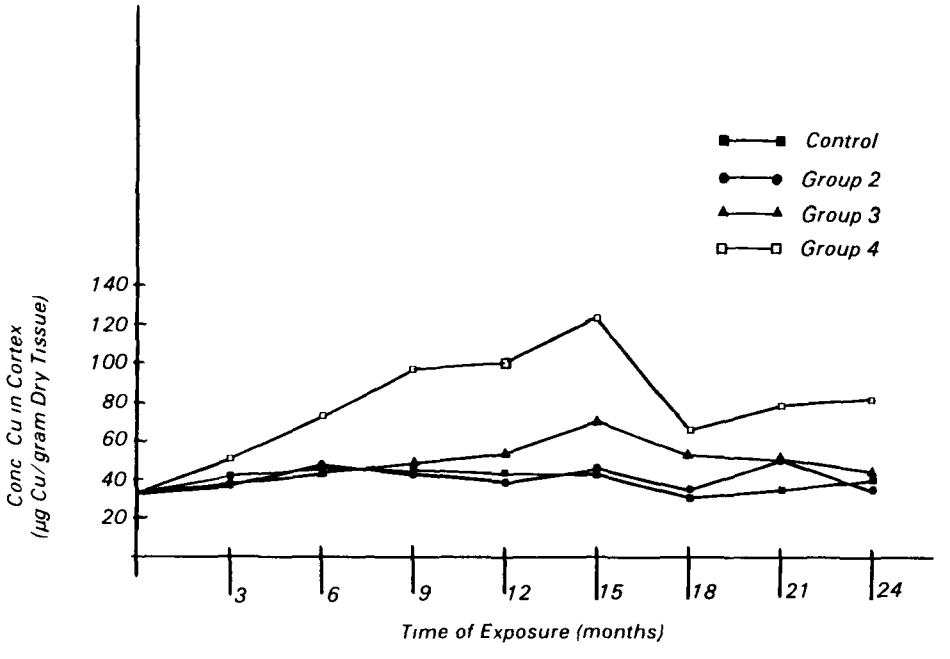

Figure 3. *Concentration of copper in the intact renal cortex as a function of age and exposure to cadmium.*

toxic effects from cadmium exposure were mild and limited to the highest dose group.

The health status of the high dosed rats, as measured by hematocrit, body weight gain, and food and water intake, was compromised. Physiological responses to both cadmium and a poor nutritional status caused by a dislike for the taste of the treated water were a likely cause of this condition.

The data have supported the hypothesis that the renal cortical tubules preferentially accumulate cadmium, zinc and coper relative to the whole cortex. In general, the cortical tubular metal concentrations were significantly higher than cortical concentrations at least through 18 months of exposure. Thereafter, tubular concentrations decreased and became equivalent to or significantly less than cortical levels. Two mechanisms were proposed to explain this change. First, susceptible tubular cells containing high concentrations of cadmium and possibly copper were lost from the tubule populations, leaving behind cells with lower, less toxic concentrations of metals. Second, cortical tubules from kidneys of old rats were more fragile, as evidenced by the poor quality of the tubule preparations. Metal could have been lost from the disintegrating tubules during the pellet preparation.

Cadmium exposure disturbs the normal distribution of copper and zinc in the kidney. The concentrations of these two essential metals are increased in both the cortical tubules and the intact cortex in a dose-response manner, with a much greater effect on the copper levels.

The relative rate of cadmium accumulation in the cortical tubules versus the intact cortex (as measured by the ratio of the slopes of the accumulation curves up to 12 or 15 months) was dose-dependent. Therefore, the cortical cadmium concentration cannot be used to predict cortical tubular cadmium concentrations at all doses.

There appeared to be definite age-related changes in the kidney which altered this organ's handling of cadmium zinc and copper. The major changes appeared in the very young (i.e., up to 3 months) and adult rats (i.e., 12-15 months). In the young rat, there was a rapid increase in the concentration of metals that corresponded to the time of most rapid organ growth. In the adult,. there was a peak, then a noticeable decrease of renal metal concentrations. This sudden decrease appeared to be linked to an ongoing aging process that slowly obliterated healthy nephrons and changed normal tubules into scar tissues and dilated, cast filled conduits.

Morphometric analysis has shown that, with age, there was a significant decrease in the amount of healthy proximal tubular tissue, and the number of glomeruli (as measured by the glomerular numerical density). Coincidentally, there were significant increases in the degree of tissue inflammation, the amount of interstitial fibrosis and hyperplasia, the extent of tubule dilation/cast formation and the thickening of glomerular and proximal tubular basement mambrane. Cadmium exposure at the levels tested showed no significant dose-response effects with the occurrence of any of these features. There was, however, a greater prevalence of moderate to severe

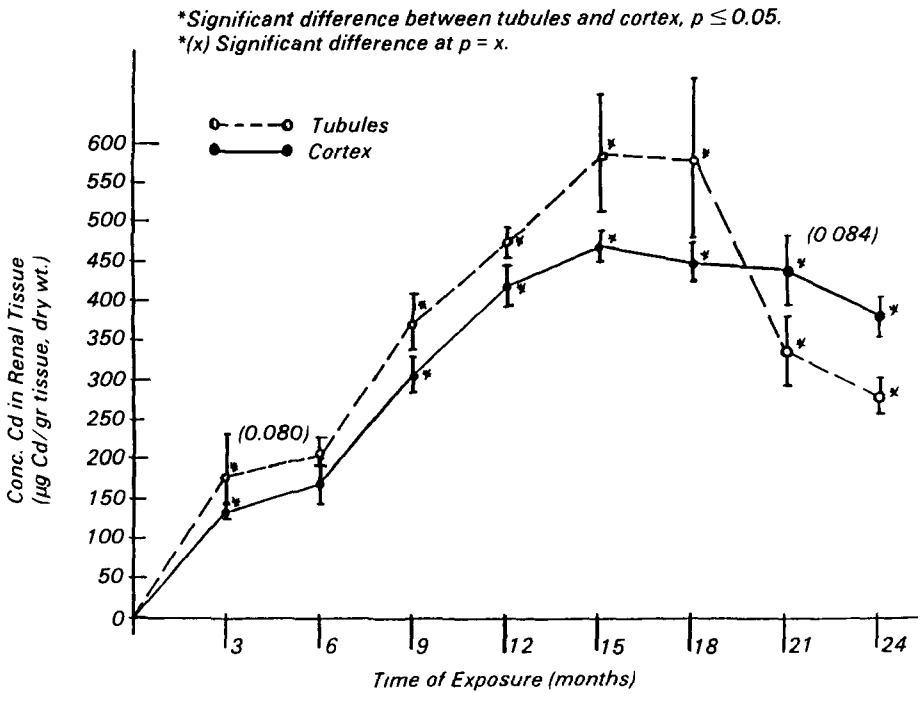

Figure 4. *Concentration of cadmium in the intact renal cortex and cortical tubules as a function of exposure in Group 4 (50 μg Cd/ml).*

hyperplasia and tubular dilation in the high dose group as compared to the controls. There appeared to be a small, but definite shift toward larger glomeruli with high cadmium exposure.

There was a significant ($p \leq 0.05$) correlation between renal cortical cadmium levels and inflammation of the renal tissue. However, this relationship is confounded by the finding that both inflammation and renal cadmium concentrations also correlate positively with age.

Of all the parameters studied, age and cadmium exposure had the most significant influence on the renal handling of cadmium, copper and, to a lesser extent, zinc.

At the levels tested, cadmium exposure had a mild effect on renal aging in terms of disturbing the tubular reabsorption of proteins, and altering both the physical integrity of the renal cortical tubules and the size of the glomeruli. Age alone did not appear to have a significant effect on diurnal urine volumes or excretion of urine glucose. However, old age, in combination with high cadmium exposure, showed a significant, positive correlation with increased urine volumes and glucose excretion. These observations suggest that cadmium exposure in the older animals compromised renal tubular functioning to a certain extent.

Cadmium exposure did not appear to significantly affect age-associated changes in the kidney structure such as increased inflammation, glomerular and tubular basement membrane thickening or interstitial fibrosis. On the other hand, cadmium exposure did appear to have a mild effect on renal structure as evidenced by a dose-response relationship with the degree of tubular hyperplasia.

Fifteen months of exposure appeared to be the critical time point for renal metal kinetics as evidenced by the peak in the metals accumulation curves. It also may have been a point at which the renal physiology was changing in other ways, as evidenced by changes in certain renal function tests.

In examining the relationship between the level of cadmium exposure and the concentration of cadmium in the kidney, it was found that the rate of accumulation of cadmium in the whole cortex did not increase linearly with the actual dose to the rats. These results implied that the actual dose to the rat could not be used to predict the renal cadmium concentration at a given time.

The results of this study indicate the need for further research in order to more fully answer the question of whether or not cadmium exposure influences normal renal aging.

Data from this study have demonstrated that renal concentrations of 90 μg Cd/g wet weight of cortex are associated with mild functional and structural changes in the kidney in association with old age. Since these data are suggestive of a possible effect of cadmium on renal aging, further research is needed using higher exposures. Exposures which would result in attaining the critical concentration of 200 μg Cd/g wet weight in the cortex should be used in conjunction with morphometric techniques for analyzing renal tissue pathology. Also, appropriate measurements of the total amount of cortical tissue in the fresh and fixed kidney should be made so that an estimate of the total number of nephrons and total mass of proximal tubular tissue per kidney can be calculated. Estimates of the total amount of a specific tissue type rather than the proportion of that tissue type in the kidney may result in a more sensitive measure of the effects of cadmium exposure on renal structure and aging.

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The complete report, entitled "The Effects of Cadmium on Renal Aging: A Chronic Cadmium Feeding Study in Rats," (Order No. PB 84-191 022; Cost: $22.00, subject to change) will be available only from:
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