CHAPTER 13

The Aging Kidney and Exposure to the Nephrotoxic Metals Cadmium and Mercury

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13.1 Introduction

Aging is an inevitable fact of life. Owing to advances in modern medicine over the past century, the life-expectancy of humans in developed and developing countries has lengthened significantly. According to the World Health Organization, the global life expectancy has risen from 64 years in 1990 to 68 years in 2011.¹ In developed countries such as the United States, the average life expectancy has increased from 47.3 years in 1900 to 77.9 years in 2007.² Similar trends exist for other developed and developing countries, such as the United Kingdom, Japan, Chile and Sri Lanka. Increases in life expectancy have led to an increase in the aged and elderly population. World-wide, it is currently estimated that approximately 650 million individuals are over the age of 60. Because of lengthening life expectancies, this figure is expected to increase to 2 billion by 2050.¹ In the United States alone, the percentage of individuals over the age of 65 has increased approximately tenfold in the past century. In 1900,
approximately 3.1 million individuals, or 4.1% of the population, were over the age of 65. This number increased to approximately 35 million (12.4% of the population) in 2000. A thorough understanding of the impact of aging on organ systems, such as the kidney, will be critical when managing the healthcare of elderly and aged individuals. Furthermore, aging may enhance individual susceptibility to disease as well as increase one’s risk of being negatively affected by exposure to environmental and/or occupational toxicants. Because of increased life expectancy and increased pollution in the environment, it is likely that elderly and aged individuals will be exposed more often and possibly to higher levels of toxic pollutants than individuals were decades ago. Given this trend, it is important that we understand not only the normal aging process, but also the effects of exposure of aging individuals to potential nephrotoxicants, such as the toxic metals cadmium (Cd) and mercury (Hg).

13.2 Aging and the Normal Kidneys
Aging results in many deleterious structural and physiological changes in the kidneys. These changes may be related entirely to renal cell senescence or, alternatively, they may be consequences of multiple factors, such as age-related hemodynamic changes, renal or non-renal disease, and/or life-long exposure to environmental and/or occupational toxicants. The aging kidneys are capable of maintaining normal renal function and systemic homeostasis in healthy individuals, despite the fact that the normal aging process has been shown to have significant deleterious structural and physiological consequences. These changes are thought to significantly affect the functional reserve of the kidneys in that the kidneys have a reduced capacity to respond to challenges such as changes in hemodynamics and exposure to toxicants. Because of this reduction in functional reserve, the kidneys, and possibly other organs, may be more susceptible to physiologic, pathologic, and toxicologic challenges.

13.2.1 Structural Changes within the Glomerulus
One of the primary targets of the aging process is the renal glomerulus. Significant ultrastructural changes occur within numerous glomeruli as a result of the normal aging process. Indeed it has been reported that approximately 30%–40% of all glomeruli become sclerotic by the eighth decade of life. Structural characteristics of a typical sclerotic glomerulus include a thickened glomerular basement membrane (GBM), expanded mesangial matrix, and shrinkage and occlusion of the glomerular capillaries (Figure 13.1).

Although the pathogenesis of this process is not completely understood, it is thought that age-related glomerulosclerosis and the resulting drop in glomerular filtration rate (GFR) are the results of multiple factors, including increased susceptibility to inflammatory cytokines, alterations in blood flow, and damage to the glomerular filtration barrier (Figure 13.2). A reduction in the total number of functioning nephrons appears to be an additional
Contributing factor in the development of glomerulosclerosis. As nephrons are lost, vascular and glomerular changes occur in remaining functional nephrons in an attempt to compensate for the reduction in GFR."11,15 These changes lead to glomerular hypertrophy, hyperperfusion, and hyperfiltration, which increase the single nephron GFR (SNGFR) and thus predispose affected glomeruli to sclerotic changes.11,16–18

Indeed a positive correlation between glomerular hypertrophy and the development of glomerulosclerosis has been demonstrated in aging mice.19 In addition, proliferation of mesangial cells and expansion of mesangial matrix, both of which are often associated with glomerular hypertrophy, appear to precede and contribute to the development of glomerulosclerosis.20 Alternatively, age-related glomerulosclerosis may occur via immunologic mechanisms whereby formation of circulating or in situ immune complexes leads to glomerulonephritis.13

Interestingly, glomeruli in the outer cortex appear to be affected earlier and more severely by sclerotic changes than those in the juxtamedullary region.6,21,22 As cortical glomeruli degenerate, glomerular capillaries atrophy, which leads to sclerosis. Interestingly, in juxtamedullary glomeruli, a direct channel is formed between the afferent and efferent arterioles, resulting in

**Figure 13.1** Glomeruli of young rats display normal glomerular morphology (a) [periodic-acid Schiff (PAS) stain]; At 24 months of age rat kidneys contained glomeruli that were characteristic of both focal segmental glomerulosclerosis (b) and global glomerulosclerosis (c). Adhesions (arrow) between Bowman’s capsule and the glomerular tuft were also observed in some glomeruli (d) [Silver stain]. (400×).

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arterioles that do not communicate hemodynamically with a glomerulus. In general, these aberrant glomeruli tend to be larger than other cortical glomeruli; therefore, a significant reduction in the functionality of this population of glomeruli likely leads to significant reductions in whole-body GFR. An important marker of glomerular damage is proteinuria, which results from a disruption of the glomerular filtration barrier. Indeed recent studies have suggested that injury to and dysfunction of podocytes, which are key components of the glomerular filtration barrier, may play a role in the pathogenesis of age-related glomerulosclerosis.

Figure 13.2 Possible pathogenesis of age-related glomerulosclerosis. Many factors have been proposed to contribute to age-related glomerulosclerosis; these include hemodynamic and structural alterations at the site of the glomerulus. These changes may lead to glomerular hypertrophy followed by hyperperfusion and hyperfiltration of affected glomeruli. Owing to these hypertrophic changes, single nephron glomerular filtration rate increases and may lead to glomerular injury, and ultimately glomerulosclerosis and renal insufficiency.
13.2.2 Structural Changes in Renal Tubules and Interstitium

Age-related changes have also been observed within renal tubules. These changes include atrophy and degeneration, formation of diverticula, irregular thickening of the tubular basement membrane, and tubulointerstitial fibrosis, which is associated with interstitial inflammation, fibroblast activation, and increased deposition of collagen. 10,26–28 Ding and colleagues 26 examined senescent tubular epithelial cells and found increased expression of transforming growth factor β-1 (TGF-β1), which plays a role in cell cycle regulation, and p21WAF1/CIP1, a cyclin-dependent kinase inhibitor thought to be involved in regulating cell growth. Although the reason for the upregulation of TGF-β1 and p21WAF1/CIP1 is unclear, it is possible that this increased expression leads to a dysregulation of the cell cycle and consequently, cell growth. Similarly, increased expression of genes such as the hypoxia-inducible factor 1 (HIF-1), which mediates cell and tissue responses to hypoxia, 29 appears to play a role in the development of interstitial fibrosis. 30 In addition, accumulations of macrophages and myofibroblasts, which are involved in collagen deposition, have been shown to be present in interstitial areas of fibrosis. 31 Given the presence of myofibroblasts, it is not surprising that increased deposition of collagens (types I and III), 31 as well as noncollagenous proteins, 27 has been detected in the interstitial space of fibrotic nephrons. This deposition is thought to contribute to fibrosis and expansion of the interstitium. An increase in apoptosis of tubular and interstitial cells has also been observed in areas of fibrosis. 10 This apoptosis may be one reason for the documented decreases in volume, length, and number of tubular segments from fibrotic nephrons. 13,28,32 As a consequence of these structural changes, alterations in tubular function are also likely to occur. These alterations include a reduced ability to concentrate/dilute urine, 33 maintain acid/base balance, 34 and filter solutes, especially sodium. 28,35

13.2.3 Physiologic Changes in the Kidney

Since the aging kidney is subjected to considerable changes in structure, it is not surprising to find that GFR also changes significantly as the kidney ages. 6,9,22,28,36,37 Estimates indicate that beginning at an age of 30–40 years, total GFR decreases by approximately 10% per decade of life. 9,13,17,37,38 The rate of decline has been shown to increase after the age of 65. 35,37,39 The Baltimore Longitudinal Study of Aging, which collected data from patients aged 17 to 96, over a 23-year period, found that creatinine clearance (i.e., GFR) declined by 0.75 mL min⁻¹ per year. 40,41 This decrease is most likely a consequence of multiple factors, including damage to and inadequate regeneration of podocytes, glomerulosclerosis, and an eventual reduction in the total number of functioning nephrons. 25,42,43 Interestingly, the findings from the Baltimore Longitudinal Study suggested that GFR may not decrease in every patient. Although the overall findings of this study suggested that GFR is reduced steadily over time, of the 254 patients evaluated, 92 (36%) showed no
significant reduction in GFR. Since the publication of this study, weakness in the design and analysis of data have been noted by others. Interestingly, several recent studies have proposed a link between one’s genetic background and the tendency to develop age-related glomerular changes that lead to reductions in GFR. Thus individual genetic differences may account for the lack of change in GFR observed in some patients. Despite the mixed findings of the Baltimore Longitudinal Study, there is overwhelming evidence to suggest that a decline in GFR is a normal consequence of aging.

In addition to changes in GFR, aging also appears to affect renal blood flow (RBF) to the kidney. Maximum RBF is reached around the third decade, following which RBF has been shown to decrease by approximately 10% per decade of life. This decrease appears to be related to changes in the vascular resistance in afferent and efferent arterioles rather than to changes in cardiac output or renal perfusion. Because the decrease in total GFR is generally less than the decrease in RBF, filtration fraction (FF) increases in most patients. It should be pointed out that although overall RBF decreases, variable changes in blood flow occur at the level of the individual nephron. Within a single, hypertrophied nephron, RBF increases as a result of the hypertrophic changes that occur within that nephron. Increases in RBF may lead to increased intraglomerular pressure, which may then lead to glomerular injury.

Renal functional reserve may also be altered in elderly and aged individuals. Renal functional reserve is the ability of the kidney to increase its basal RBF and GFR by 20% or more after a stimulus, such as a protein load. Studies of renal functional reserve in elderly individuals have yielded mixed results. While some studies found that the functional reserve of the kidneys is preserved to some extent in healthy elderly individuals, a separate study reported that renal functional reserve is reduced or depleted in the elderly and aged, in order to accommodate for the age-related decline in renal function and as an attempt to preserve normal renal function. Another study found that the renal functional reserve of the elderly remained intact, but its magnitude was lower in older individuals than in younger individuals. Similarly, in vivo studies using aged Sprague-Dawley rats provided evidence for an age-related decline in renal functional reserve. Moreover, it has been shown that functional reserve of the kidneys is decreased or absent in patients with diseases such as diabetes and hypertension.

Aging and cell senescence also leads to changes at the cellular level. Under normal conditions, the mitotic index for renal epithelial cells is low, with proliferation occurring in approximately 1% of renal tubular cells. This percentage declines with age and may impair the ability of renal tubules to repair themselves. Similarly, studies in mouse kidneys have shown that the normal burst of proliferation observed following an acute insult to renal tubules is reduced in aged kidneys. These data are supported by the findings of Shurin and colleagues, who assessed plasma levels of various cytokines in individuals ranging from 45 to 78 years old and found that levels of insulin-like growth factor-1 (IGF-1) were lower in aged individuals. Others have
reported decreases in epidermal growth factor (EGF). Furthermore, results from cDNA microarray profiling of genes from human kidneys indicate that many genes are down-regulated in aged kidneys. Most of these genes appear to be related to energy metabolism, as well as nucleotide, amino acid, and protein turnover. An increase in gene expression was noted in a selected set of genes responsible for immune and inflammatory responses. Indeed, in separate studies, increases in the expression of tumor necrosis factor (TNF)-\(\alpha\) and interleukin (IL)-6, which are both involved in inflammatory processes, were found to be associated with aging. In addition to changes in cytokines responsible for growth and inflammation, it appears that aging also negatively affects the regulatory control of apoptosis, leading to an increase in the basal levels of apoptosis in the kidney.

13.3 Aging and Kidney Disease

There is a clear association between aging and the development of kidney disease. One can speculate that as the kidneys age, they lose their capacity to cope with certain challenges. Therefore, when an aged kidney is challenged, physiologically, pathologically, or toxicologically, renal function will likely be affected. Indeed an epidemiological study analyzing 437 cases of acute renal failure in Spain demonstrated that acute renal failure was 3.5 times more prevalent in adults over the age of 70 than in younger adults. Similarly, an analysis of data obtained from the Third National Health and Nutrition Examination Study (NHANES) in the United States identified an association between age and the incidence of chronic renal failure. It was noted that, even in the absence of other diseases such as hypertension and diabetes, approximately 11% of individuals over the age of 65 have been diagnosed with moderate to severe renal failure. The progression of renal failure also appeared to occur more rapidly in aged patients than in younger patients. Another study of elderly patients with acute or chronic renal failure suggested that older individuals are at greater risk of morbidity associated with their disease.

Comorbidities such as hypertension and diabetes are common in the elderly population and are likely to increase the susceptibility of individuals to renal disease. In the United States, approximately 30% of the adult population is affected by hypertension, the incidence of which has been shown to increase with age. Approximately 70% of adults over the age of 65 have been diagnosed with the disease. A similar trend exists for diabetes, which affects approximately 8% of the population in the United States. Of individuals aged 65 and older, nearly 27% have been diagnosed with diabetes. The presence of diseases such as hypertension and/or diabetes in aging patients likely enhances the normal age-related decline in renal function compared with healthy individuals. Therefore, it is reasonable to suggest that aged and elderly patients with superimposed diseases are more susceptible to the development of acute or chronic renal failure.
It is also important to consider the increase in prescription drug use in the elderly and aged population. Aging not only leads to a reduction in renal function, but can also lead to a reduced ability to metabolize drugs and eliminate drug metabolites.\(^7\) For example, non-steroidal anti-inflammatory drugs (NSAIDs) such as salicylate may be used frequently by aged and elderly individuals. In fact, the use of these drugs by the aged and elderly population is estimated to be three times that of the younger population.\(^7\) In addition, time required to eliminate drugs such as salicylate has been shown to be greater in the elderly population than in younger individuals.\(^7\) This reduction may be due, in part, to age-related loss of renal function. Considering the increased use and reduced elimination of certain drugs in the elderly and aged population, it is possible that the use of these drugs may predispose individuals to kidney diseases, such as analgesic nephropathy (which often leads to renal insufficiency or failure).

13.4 Aging and Exposure to Toxic Metals

Owing to naturally occurring and chemically manufactured toxicants in the environment, individuals are exposed frequently over their lifetime to toxicants that have the capacity to negatively affect various organ systems. Just as aging in the presence of diseases can have a negative impact on renal function and may enhance the development of renal disease, exposure of elderly and aged individuals to nephrotoxicants may promote or enhance the progression of renal disease. Since not all relevant nephrotoxicants can be addressed here, we will focus on two prevalent environmental metal toxicants, cadmium and mercury. Because of their abundance in the environment and in numerous occupational settings, exposure of humans to each of these metal toxicants is nearly unavoidable. The toxic effects of cadmium and mercury have been characterized extensively and a great deal is known about the mechanisms by which they gain access to target cells. Based on that knowledge and the frequency of human exposure, we have chosen to use cadmium and mercury as example nephrotoxicants in our discussion of aging and exposure to toxic metals.

As discussed in previous sections, the major age-related change within the nephron is glomerulosclerosis. As the sclerotic process progresses and glomeruli become non-functional, this decrease in functional renal mass leads to hypertrophy of remaining functional glomeruli. Glomerular and tubular hypertrophy is associated with hyperperfusion and an increase in SNGFR.\(^8\) Consequently, the luminal and basolateral surfaces of renal tubular epithelial cells are potentially exposed to higher levels of xenobiotics, metabolic wastes, and nephrotoxicants. In addition, these substances may be taken up more readily by hypertrophied tubular cells because of increases in the expression of certain cellular transport mechanisms. The increased exposure to, and probable uptake of, available xenobiotics, metabolic wastes, and nephrotoxicants likely enhances the risk of hypertrophied tubular cells being affected adversely by these substances.\(^11\) These adverse effects could conceivably lead to additional
cell death and glomerulosclerosis, which would further reduce the functional renal mass of the patient.

13.4.1 Cadmium

Cadmium (Cd) is a prevalent environmental pollutant and nephrotoxicant. Industrial uses of cadmium include manufacture of batteries, pigments, coatings, and plastics. Current regulations regarding cadmium emissions and disposal have reduced occupational exposure to cadmium, yet the environments and surrounding areas where cadmium is/was used industrially remain heavily contaminated. Additionally, the use of this metal in phosphate fertilizers can leave soil and water heavily contaminated with cadmium residue. Cadmium concentrates in soils and subsequently accumulates in plants, particularly root vegetables, as well as grains and tobacco. Cadmium is also present in high concentrations in aquatic animals such as seals and mollusks, and in crustaceans such as oysters and crabs.

Diet is the primary means by which the general, non-smoking population is exposed to cadmium. In contrast, individuals who smoke are exposed to this metal primarily via the inhalation of cigarette smoke due to the high concentration of cadmium in tobacco. Each cigarette contains approximately 1–2 μg of cadmium. About 10% of the cadmium contained in a cigarette is inhaled with approximately 50% of that being absorbed in the lungs. Therefore, it is estimated that individuals who smoke one pack of 20 cigarettes each day will absorb approximately 1–2 μg of cadmium daily. Cadmium is also present in air and drinking water in various regions of the world, although the concentration of cadmium in air is relatively low and drinking water is generally not a major source of exposure for the general population.

In a recent assessment by the US Centers for Disease Control and Prevention, as part of the NHANES, blood and urine content of cadmium was analyzed in over 5000 individuals. In individuals over the age of 20, the average blood level was 0.376 μg L⁻¹ while the average urinary concentration of cadmium was 0.232 μg L⁻¹. Both urinary and blood levels of cadmium have remained fairly steady over the past decade. These data suggest that individuals continue to be exposed chronically to cadmium. Thus, a thorough understanding of the effects of exposure to cadmium on an organ system is important to overall human health.

13.4.1.1 Renal Handling of Cadmium

Cadmium appears to gain access to renal epithelial cells by several different mechanisms. When bound to the metal-binding protein, metallothionein (MT), it is thought to be taken up via receptor-mediated endocytosis. Cadmium is also a strong electrophilic cation, which enables it to compete for and interact with membrane transporters that are involved in the uptake of nutritive metals, such as calcium, iron, and zinc. Alternatively, cadmium(II) ions may form linear complexes with select sulphydryl (thiol)-containing biomolecules, such as...
glutathione (GSH), cysteine (Cys), or homocysteine (Hcy). These cadmium-thiol complexes may gain access to cells at the site of membrane transporters normally involved in the transport of endogenous amino acids, oligopeptides, organic anions, or organic cations.

Following exposure and absorption, a significant amount of cadmium is transported to the liver, where it becomes associated with MT to form CdMT. It is believed by numerous investigators that when cadmium induces hepatocellular necrosis, CdMT is released into sinusoidal blood. This is especially true following acute exposure to nephrotoxic doses. CdMT is filtered freely at the glomerulus and is then thought to be taken up at the luminal plasma membrane of proximal tubular epithelial cells via receptor-mediated endocytosis. This route of uptake appears to be a major route of proximal tubular entry for cadmium. Indeed, the cells of the proximal convoluted tubule are the primary sites adversely affected by CdMT. Following uptake by proximal tubular cells, CdMT is delivered to endosomes and lysosomes, where Cd\(^{2+}\) is dissociated from MT and transported into the cytoplasm via the divalent metal transporter 1 (DMT1). DMT1 is also localized in the luminal plasma membrane of the epithelial cells lining the ascending thick limb of the loop of Henle, the distal convoluted tubule, and the principal cells of the cortical collecting duct, where it may play a role in the uptake of cadmium ions and lead to adverse effects within these cells.

Cadmium ions may also be taken up from the proximal tubular lumen by a mechanism involving ligand exchange. Cadmium may dissociate from MT or other ligands under certain conditions, and the process of ligand exchange may allow the cadmium ion to exchange from a protein or non-protein thiol to the binding site of a cation transporter. Certain zinc transporters appear to be capable of utilizing cadmium ions as substrates. ZIP8 (SLC39A8) and ZIP14 (SLC39A14) have both been identified in the kidney and have been shown in vitro to mediate the uptake of cadmium ions into cultured renal epithelial cells stably transfected with either of these carriers. Cadmium ions and complexes appear to utilize additional mechanisms to gain access to renal epithelial cells; however, those mechanisms and processes remain unclear at present.

13.4.1.2 Renal Effects of Cadmium Exposure

Exposure to cadmium can be assessed by measuring cadmium concentrations in urine or blood. Urinary excretion of cadmium is considered to be a reliable indicator of renal and body burden and typically represents chronic levels of exposure. In contrast, plasma levels of cadmium usually reflect a more recent exposure, such as one occurring within the previous month. Cadmium has a long half-life within the body, partly due to its incorporation into bone. Therefore, following exposure, decades may be required for complete elimination of this toxic metal.
Following chronic exposure to cadmium, approximately 50% of the total body stores accumulate in the kidney. Thus, it is not surprising to find that this organ is a primary target of cadmium toxicity, which leads to reductions in GFR and generalized tubular dysfunction (i.e., Fanconi’s syndrome). One of the earliest signs of renal damage is the presence of urinary biomarkers, such as β2-microglobulin, N-acetyl-β-D-glucosamidase (NAG), and kidney injury molecule-1 (Kim-1). β2-microglobulin is a low molecular weight protein that is filtered freely at the glomerulus and is normally absorbed by proximal tubules. Following tubular damage, a small fraction of β2-microglobulin escapes absorption and is excreted in urine. Alternatively, NAG is derived from mitochondria within proximal tubular epithelial cells and thus, following cellular damage, this enzyme is released into tubular fluid for eventual elimination in urine. More recently, Kim-1, which is a transmembrane protein not normally detectable in urine, has been shown to be a useful marker of renal tubular cell injury and/or death.

Recently, studies have shown that chronic exposure to even low levels of cadmium can result in early signs of renal toxicity. The earliest sign of cadmium-induced renal damage is microproteinuria, which is usually identified by the presence of β2-microglobulin in urine. After exposure to greater doses of cadmium, tubular damage is evidenced by the presence of a Fanconi’s syndrome, which is typically characterized by glucosuria, aminoaciduria, hyperphosphaturia, and hypercalciuria. In addition, the glomerulus is injured and consequently, GFR declines. This decline results in increased detectable levels of urinary protein. The incidence of kidney stones also increases in individuals exposed chronically to (or to larger doses of) cadmium, possibly due to the increased concentration of calcium in tubular fluid and urine. Owing to the fact that the active form of vitamin D (1,25 dihydroxy-cholecalciferol) is formed in the kidneys, it is possible that renal damage would impede the conversion of the inactive form of this vitamin to the active form. Indeed, studies from cadmium-polluted areas report an association between cadmium-induced renal damage and lowered plasma levels of active vitamin D. Interestingly, the renal changes that are observed following chronic exposure to a low dose of cadmium are similar to those observed with normal aging of the kidneys.

### 13.4.1.3 Aging and the Effects of Cadmium Exposure on the Kidneys

Epidemiological studies have demonstrated a positive correlation between age and the renal accumulation of cadmium in individuals exposed chronically to this metal. Since the normal filtration capacity of the kidney appears to decline with aging, exposure of elderly individuals to cadmium appears to potentiate the negative effects of age-related renal dysfunction. Consequently, it is reasonable to postulate that exposure of elderly and aged individuals to cadmium may be especially detrimental to target organs. In aged kidneys, the
threshold at which nephrotoxic effects are observed may be lower than in the kidneys of a younger individual. Indeed, it has been suggested that long-term exposure to cadmium exacerbates the age-related decline in GFR. Moreover, exposure to cadmium or other nephrotoxicants may further reduce or completely eliminate the renal functional reserve and the ability of the remaining functional renal mass to maintain normal homeostasis when challenged. Sprague-Dawley rats exposed chronically to oral cadmium have been shown to have less renal functional reserve than unexposed rats. Similarly, in an epidemiological study assessing renal function in cadmium/zinc smelter workers with detectable amounts of cadmium in their urine, it was found that age-related reduction in renal functional reserve was enhanced following exposures to cadmium that resulted in microproteinuria. Collectively, these studies suggest that exposure to cadmium, and perhaps other nephrotoxicants, can abolish renal functional reserve, which may increase the susceptibility of these individuals to renal failure resulting from other risk factors, such as hypertension and diabetes.

Given that smoking is a common route of exposure to cadmium, it is not surprising to find that urinary and blood levels of cadmium increase with age in current smokers. Individuals who smoke may have blood cadmium levels as high as 4–5 times greater than that of non-smokers. This increased exposure to cadmium may lead to an increased susceptibility of smokers to the nephrotoxic effects of cadmium. Furthermore, increased exposure to cadmium may eventually play a role in the development of chronic kidney disease.

Aging kidneys are often characterized by areas of glomerular and tubular hypertrophy, hyperfiltration, and glomerulosclerosis. This pathological scenario is somewhat similar to the experimental model created by a uninephrectomy. In uninephrectomized animals, the contralateral kidney undergoes significant compensatory hypertrophy in order to maintain normal fluid and solute homeostasis. As part of the compensatory mechanism, the transcription and translation of numerous proteins, including membrane transporters and metal-binding proteins (MT1 and MT2), are upregulated significantly. An increase in the number and/or activity of transport proteins involved in the proximal tubular uptake of cadmium may enhance the nephropathy induced by this metal. When uninephrectomized and sham Sprague-Dawley rats were exposed to cadmium, the renal burden of cadmium was greater in the contralateral kidney of uninephrectomized rats than in the corresponding kidney of sham rats. In addition, urinary excretion of NAG and cadmium was greater in uninephrectomized rats than in sham rats. Furthermore, when a toxic dose of cadmium was administered to each group of rats, it was found that uninephrectomized rats were more susceptible to the toxic effects of cadmium than corresponding sham rats. Considering these data, it is logical to suggest that exposure to cadmium following a reduction in functional renal mass may lead to more severe nephropathy.

Diseases that affect renal health, such as hypertension and diabetes, are common in elderly and aged individuals. Therefore, it is important to understand the relationship between aging, cadmium exposure, and
superimposed diseases. A link between aging and body levels of cadmium following chronic exposure has been clearly established. Similarly, numerous epidemiological and animal studies have provided evidence suggesting an association between exposure to cadmium and the occurrence and severity of diabetes.\textsuperscript{145,146} While diabetes alone may lead to decreased GFR, albuminuria, and morphological alterations along the nephron,\textsuperscript{147} chronic exposure to cadmium may enhance the onset of these negative renal effects.\textsuperscript{145} Indeed studies in which normal Sprague-Dawley rats were injected intraperitoneally with cadmium showed that administration of cadmium induced hyperglycemia.\textsuperscript{148,149} This hyperglycemia may be the result of increases in levels of the gluconeogenic enzymes, glucose-6-phosphatase, fructose-1,6-diphosphatase, phosphoenol pyruvate carboxykinase, and pyruvate carboxylase.\textsuperscript{149} Exposure of rats to cadmium chloride also appears to decrease the gene expression and release of insulin.\textsuperscript{150–152} Taken together, these studies suggest that exposure to cadmium may increase one's susceptibility of developing diabetes. Exposure to cadmium may also promote the development of signs and symptoms in a diabetic patient. Diabetes-induced renal pathology may be observed earlier in patients that are exposed chronically to low levels of cadmium compared with un-exposed patients. This theory is supported by studies comparing streptozotocin-induced diabetic Wistar rats and non-diabetic Wistar rats.\textsuperscript{153} The findings from these studies showed that urinary levels of protein, NAG, and $\gamma$-glutamyltransferase were greater in diabetic rats than in controls, suggesting that renal damage was more extensive in diabetic rats.\textsuperscript{153} In addition, diabetic rats were found to excrete less cadmium in urine and consequently had a greater renal burden of cadmium than that of non-diabetic rats, suggesting the presence of glomerular damage.\textsuperscript{153} In a similar study it was found that exposure of diabetic Sprague-Dawley rats to cadmium significantly increased the urinary excretion of albumin, transferring, and IgG.\textsuperscript{154} In a separate study, varying concentrations of CdMT were injected into normal or obese hyperglycemic (ob/ob) mice.\textsuperscript{155} Pathological signs of nephron damage (proteinuria and calciuria) were observed at lower concentrations of cadmium in the ob/ob mice than in normal mice, suggesting that the hyperglycemic state increases susceptibility to cadmium-induced nephropathy.\textsuperscript{155} The results of multiple epidemiological studies correlate well with the aforementioned animal studies and provide additional support for the notion that exposure to cadmium enhances the renal pathology associated with diabetes. In a cross-sectional study carried out in the Torres Strait Islands, located between Australia and New Guinea, investigators identified a strong positive correlation between urinary markers of cadmium exposure and diabetic nephropathy.\textsuperscript{156} Similarly, a cross-sectional study of 1699 Belgian subjects suggested that diabetic patients may be more susceptible to the nephrotoxic effects of cadmium.\textsuperscript{157} Moreover, Åkesson and colleagues assessed the effect of cadmium exposure on diabetes-induced renal dysfunction in 10 766 subjects and reported that the nephrotoxic effects of cadmium exposure could be observed at lower levels in diabetic patients compared with non-diabetic patients.\textsuperscript{121}
Interestingly, cadmium levels in men and women appear to differ significantly. The body burden of cadmium in women tends to be significantly greater than that in men. In a study of healthy Thai men and women, it was found that the average urinary excretion of cadmium in non-smoking women was similar to that of men who smoked cigarettes. In a study of 57 non-smoking women, it was found that urinary and blood levels of cadmium correlated with age and body iron stores. Women with lower serum ferritin were found to have higher levels of cadmium. In general, women have lower iron stores than men; when iron stores are low, the divalent metal transporter 1 (DMT1) in the intestine is upregulated to facilitate increased intestinal uptake of ferrous iron (Fe$^{2+}$). DMT1 has also been shown to mediate the intestinal uptake of cadmium; therefore, upregulation of this carrier could potentially increase the absorption of dietary cadmium ions from the lumen of the intestine. Indeed, it has been proposed that an increase in DMT1 expression and consequent increase in cadmium absorption is the primary reason for the greater levels of cadmium detected in women.

13.4.2 Mercury

Mercury is a toxic metal found in many environmental and occupational settings. It exists in elemental (metallic), inorganic, and/or organic forms. Elemental mercury (Hg$^0$) is unique in that it exists as a liquid at room temperature. Inorganic mercury may be found as mercurous (Hg$^{1+}$) or mercuric (Hg$^{2+}$) ions, which are usually bonded with anionic species of chlorine, sulfur, or oxygen to form mercurous or mercuric salts. In the environment, inorganic mercury is usually found in the mercuric form. Organic forms of mercury include phenylmercury, dimethylmercury, and monomethylmercury. Of these forms, methylmercury (CH$_3$Hg$^+$) is the most frequently encountered in the environment. It is formed primarily when inorganic mercuric ions are methylated by microorganisms present in soil and water.

Humans may be exposed to mercury in occupational and environmental settings, as well as through dental amalgams, and medicinal and dietary sources. The majority of human exposure, however, results from the ingestion of food and water contaminated with CH$_3$Hg$^+$. Much of the dietary intake of this metal is via ingestion of large predatory fish, such as northern pike, salmon, swordfish, and shark, which may contain high levels of CH$_3$Hg$^+$. Upon ingestion, CH$_3$Hg$^+$ is absorbed readily by the gastrointestinal tract of humans and other mammals. Mercuric ions can then enter systemic circulation where they can be delivered to target organs.

13.4.2.1 Renal Handling of Mercury

Inorganic and organic forms of mercury accumulate readily in the kidney. While the kidney is the primary site of accumulation of and intoxication by inorganic forms of mercury, organic forms of mercury, which primarily affect the central nervous system, also have serious toxicological effects in the
Within this organ, the primary site of uptake and accumulation of mercuric species is the proximal tubule. It should be noted that following exposure to CH$_3$Hg$^+$, a fraction of absorbed CH$_3$Hg$^+$ will be oxidized within tissues and cells to form Hg$^{2+}$. It is also important to note that within biological systems, mercurous, mercuric, or methylmercuric ions do not exist as inorganic salts, or in an unbound, “free” ionic state. Rather, mercuric ions are found bonded to one or more thiol-containing biomolecules, such as GSH, Cys, Hcy, N-acetylcysteine (NAC), and albumin. For Hg$^{2+}$, this bonding occurs in a linear II, coordinate covalent manner while thiol-conjugates of CH$_3$Hg$^+$ form linear I, coordinate covalent complexes.

Renal accumulation of as much as 50% of a nontoxic dose of Hg$^{2+}$ occurs rapidly in the kidneys within a few hours of exposure. The vast majority of this Hg$^{2+}$ accumulates in the epithelial cells of the proximal tubule. A preponderance of data collected in the past decade indicates that mercuric ions gain access to proximal tubular cells via mechanisms present on both the luminal and basolateral plasma membranes. Early studies utilizing isolated perfused proximal tubules indicate that the primary transportable, biological form of mercury across the luminal membrane of the proximal tubule is a Cys S-conjugate (Cys-S-Hg-S-Cys). Additional studies in isolated perfused tubules indicate that amino acid transporters located in the luminal plasma membrane are likely involved in the uptake of mercuric conjugates from the tubular lumen. It has been hypothesized that since Cys-S-Hg-S-Cys is similar in size and shape to the amino acid cystine, this mercuric conjugate may be a substrate of a cystine transporter. Studies using Madin-Darby Canine Kidney (MDCK) cells stably transfected with the sodium-independent cystine transporter, system b$_0$, provide strong evidence implicating this carrier in the uptake of Cys-S-Hg-S-Cys from the lumen into the proximal tubular cell. Similar studies have also identified the Hcy S-conjugate of Hg (Hcy-S-Hg-S-Hcy) as a substrate for system b$_0$. To our knowledge, a sodium-dependent mechanism for the luminal uptake of Cys-S-Hg-S-Cys and Hcy-S-Hg-S-Hcy by proximal tubular cells has not yet been identified. In contrast, experimental evidence from Xenopus laevis oocytes indicates that Cys- and Hcy-S-conjugates of CH$_3$Hg$^+$ (Cys-S-CH$_3$Hg$^+$ and Hcy-S-CH$_3$Hg$^+$ respectively) are substrates of the sodium-dependent amino acid carrier, system B$_0$. Currently, there are no experimental data supporting a role for system B$_0$ in the uptake of Cys-S-Hg-S-Cys or Hcy-S-Hg-S-Hcy.

Approximately 40–60% of the mercury that accumulates in proximal tubular cells is taken up at the basolateral plasma membrane. Numerous in vitro studies using cultured MDCK cells stably transfected with the organic anion transporter (OAT)-1 provide strong evidence indicating that mercruic conjugates of Cys, Hcy, and NAC (NAC-S-Hg-S-NAC) are taken up by this carrier. Cys-S-Hg-S-Cys has also been shown to be a transportable substrate of another isoform of OAT, namely OAT3. Both, OAT1 and OAT3 are localized in the basolateral plasma membrane of proximal tubular epithelial cells. Based on published reports, it appears that OAT1 is the primary mechanism involved in the basolateral transport of...
Cys-S-Hg-S-Cys, NAC-S-Hg-S-NAC, and Hcy-S-Hg-S-Hcy into proximal tubular cells.\textsuperscript{180,181,185,189–192,204–206} In addition to conjugates of inorganic mercury, Cys-, NAC-, and Hcy-S-conjugates of CH\textsubscript{3}Hg\textsuperscript{+} have also been identified as substrates for OAT1.\textsuperscript{201–203} Collectively, these data provide strong support for a role of OAT1 and OAT3 in the basolateral uptake of certain mercuric complexes.

Once mercuric ions gain access to the intracellular compartment of cells, they form strong bonds with protein and non-protein thiol-containing biomolecules. Intracellular mercuric ions also induce, and bind to, MT and/or GSH.\textsuperscript{207,208} Bonding to these biomolecules often prevents or reduces greatly the export of mercuric ions from the cell. However, it is well-documented that mercuric ions can be extracted from renal tubular cells following treatment with a metal complexing agent, such as 2,3-bis(sulfanyl)propane-1-sulfonic acid (formally known as 2,3-dimercaptopropane-1-sulfonic acid, DMPS)\textsuperscript{209} or 2,3-dimercaptosuccinic acid (DMSA).\textsuperscript{209–214} It is thought that DMPS and DMSA gain access to proximal tubular cells at the basolateral membrane via OAT1, OAT3 and/or the sodium-dependent dicarboxylate transporter (NaC2).\textsuperscript{215–218} It is hypothesized that once internalized, DMPS and DMSA form complexes with intracellular Hg\textsuperscript{2+} and these complexes are then exported across the luminal membrane via the multidrug resistance-associated protein 2 (MRP2). In a recent study, TR\textsuperscript{−} rats, which lack Mrp2, were exposed to a non-nephrotoxic dose of HgCl\textsubscript{2} and treated subsequently with DMPS or DMSA. The results of this study provided strong evidence for a role of Mrp2 in the DMPS- and DMSA-mediated extraction of mercuric ions from proximal tubular cells.\textsuperscript{219,220} In addition, \textit{in vitro} studies utilizing inside-out, brush-border membrane vesicles prepared from Sf9 cells transfected with human MRP2 provided direct evidence indicating that DMPS- and DMSA-S-conjugates of Hg\textsuperscript{2+} are transportable substrates of MRP2.\textsuperscript{219,220} Mrp2 also appears to play a role in the NAC-mediated extraction of mercuric ions following exposure to CH\textsubscript{3}Hg\textsuperscript{+}.\textsuperscript{221} \textit{In vivo} studies in TR\textsuperscript{−} rats suggest that Mrp2 plays a role in the transport of CH\textsubscript{3}Hg-S-NAC from within proximal tubular cells into the tubular lumen.\textsuperscript{222} The ability of Mrp2 to utilize CH\textsubscript{3}Hg-S-NAC as a substrate was also shown directly via studies using inside-out, brush-border membrane vesicles prepared from the kidneys of TR\textsuperscript{−} rats.\textsuperscript{222} Similarly, Mrp2 has been implicated in the DMPS- and DMSA-mediated elimination of CH\textsubscript{3}Hg\textsuperscript{+} from proximal tubular cells using TR\textsuperscript{−} rats and inside-out, brush-border membrane vesicles from MRP2-expressing Sf9 cells as experimental models.\textsuperscript{223} Collectively, these data provide strong support for the hypothesis that MRP2 plays an important role in the renal elimination of mercuric ions following exposure to forms of Hg\textsuperscript{2+} or CH\textsubscript{3}Hg\textsuperscript{+}.

### 13.4.2.2 Renal Effects of Mercury Exposure

Exposure to all forms of mercury can have nephrotoxic effects;\textsuperscript{168–171} however, exposure to conjugates of inorganic mercury induces the most severe acute nephropathy. Following exposure to a low dose of HgCl\textsubscript{2}, cellular damage can be observed in the \textit{pars recta} of the proximal tubule, suggesting that this region
of the nephron is the most sensitive to the toxic effects of mercury. Exposure to higher doses of HgCl₂ not only results in more rapid induction of cellular necrosis, but also leads to necrosis in the pars convoluta and distal segments of the nephron. Following exposure to a nephrotoxic dose of mercury, injury can be detected at the cellular level in pars recta segments in as little as three hours. Changes in mitochondrial structure are evident and pyknotic nuclei can be identified. After six hours, cells begin to lose microvilli, mitochondrial swelling worsens, and dilation of the endoplasmic reticulum can be detected. Reductions in enzymatic activity in the pars recta have also been reported. Twelve hours after exposure to HgCl₂, electron microscopic analyses of cells reveal rupture of the plasma membrane, loss of microvilli, decreased contact with the basement membrane, and loss of cell shape. After 24 hours, cellular fragments can be identified in the tubular lumen, junctional complexes between cells are absent, and nuclear structure is compromised. When tubular epithelial cells are injured and die, numerous brush-border and intracellular enzymes, such as alkaline phosphatase, γ-glutamyltransferase, lactate dehydrogenase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) can be detected in urine. As the extent of mercury-induced renal injury progresses, there is also a simultaneous increase in the urinary excretion of mercuric ions.

Exposure to HgCl₂ can also have detrimental effects on glomeruli. Hall and colleagues exposed rats to a non-nephrotoxic dose of HgCl₂ for 21 weeks. At the end of the exposure period there were no significant differences in the parameters of renal function (i.e., plasma urea nitrogen, plasma creatinine, protein excretion); yet significant histological changes were noted in the kidneys. Tubular, interstitial and glomerular lesions were found to be significantly worse in rats exposed to HgCl₂ than in control animals. Similarly, in rats exposed to methylmercury over a two-year period, fibrotic changes were identified in a fraction of glomeruli. In addition, deposits of IgG, IgM, and C3 were detected along the glomerular basement membrane, suggestive of the development of membranous glomerulonephritis. Glomerular changes such as fibrosis and glomerulonephritis often lead to reductions in GFR. Therefore, it is not surprising that following exposure to mercury, reductions in GFR have been observed.

Based on these studies, it is clear that acute and chronic exposure to mercuric compounds has numerous, deleterious effects in the kidneys. Considering that aged and elderly individuals have reduced functional renal mass (due to glomerulosclerosis, tubular atrophy, and interstitial fibrosis), it is possible that exposure of these individuals to mercuric compounds may be even more detrimental to renal health.

13.4.2.3 Aging and the Effects of Mercury Exposure on the Kidney

Although the aged and elderly make up a significant percentage of the population, and exposure to mercury and mercuric compounds appears to be
increasing, little is known about the relationship between aging kidneys and the renal effects of mercury exposure. Several in vitro studies suggest that accumulation of mercuric ions is greater in aged animals than younger animals. Hirayama and Yasutake\textsuperscript{242} exposed C57BL/6N mice, ages two weeks to 45 weeks, to a single dose of methylmercury and found that accumulation of mercuric ions was greater in older mice than in younger mice. This study also found that urinary excretion of mercury was lower in older mice, suggesting that more mercury was retained within the kidneys and other organs.\textsuperscript{242} Interestingly, increases in the expression of MT and GSH, which have been shown to occur with aging, may lead to increased binding and retention of mercuric ions within target cells.\textsuperscript{243}

As the kidneys age, the total number of functioning nephrons decreases significantly. This decline in functional renal mass is somewhat similar to an experimental model in which animals are uninephrectomized. Although the uninephrectomized rat model does not mimic exactly the changes that occur in an aging kidney, similarities exist between the two systems. In each, a significant number of nephrons are lost and remaining nephrons must go through a compensatory, hypertrophic phase in order to maintain normal fluid and electrolyte homeostasis.\textsuperscript{51,52,143} It has been suggested by a number of investigators that hyperfiltration and hyperperfusion occurring in hypertrophied nephrons may result in these nephrons being exposed to higher levels of potential nephrotoxicants such as mercury. This increased exposure may increase the susceptibility of these nephrons to the deleterious effects of mercury or other nephrotoxicants.\textsuperscript{231,244–250}

Several studies in uninephrectomized rats support the idea that hypertrophied nephrons are more sensitive to the toxic effects of mercury. Ramos-Frendo and colleagues\textsuperscript{246} exposed uninephrectomized and sham rats to a nephrotoxic dose of HgCl\textsubscript{2} and found that the development of acute renal failure was more pronounced in uninephrectomized animals than in sham animals. Similarly, Houser and Berndt\textsuperscript{244} exposed uninephrectomized and sham Sprague-Dawley rats to a nephrotoxic dose of HgCl\textsubscript{2}, following which they assessed renal susceptibility to mercuric ions. Exposure to HgCl\textsubscript{2} included glomerular and tubular dysfunction, which appeared to be more severe in uninephrectomized rats than in sham rats.\textsuperscript{244} In a separate, more detailed study using uninephrectomized and sham Sprague-Dawley rats exposed to a nephrotoxic dose of HgCl\textsubscript{2}, it was discovered that mercuric ions were redistributed within the kidney following uninephrectomy. The concentration of mercuric ions was greater in the renal cortex and outer stripe of the outer medulla in uninephrectomized rats than in sham animals.\textsuperscript{245} Not surprisingly, the urinary excretion of mercury (per kidney) was greater in uninephrectomized rats than in shams.\textsuperscript{244,245} A similar study was carried out in uninephrectomized and sham Long Evans hooded rats, which were exposed to nephrotoxic doses of HgCl\textsubscript{2}.\textsuperscript{231} It was found that mercury-induced proximal tubular necrosis was more extensive in uninephrectomized animals than in sham animals. In addition, the urinary excretion of cellular enzymes and plasma proteins, including lactate dehydrogenase, $\gamma$-glutamyltransferase and albumin, was greater in
uninephrectomized animals than in sham animals. Collectively, the results of these studies indicate that kidneys of animals with reduced renal mass are more susceptible to the toxic effects of mercury. Similarly, elderly and aged individuals who have reduced renal function, due to normal aging processes and/or superimposed disease processes may be more susceptible to renal injury following exposure to a nephrotoxicant such as mercury.

Chronic kidney disease, which may lead to end-stage renal failure, frequently affects the elderly and aged population. This disease, which is often caused by uncontrolled diabetes and hypertension, is characterized by a progressive and permanent loss of functioning nephrons. In the United States, which has one of the highest rates of chronic kidney disease in the world, nearly 50% of patients with this disease are over the age of 65. Because many patients in the early stages of chronic kidney disease are asymptomatic, a diagnosis is often not made until renal function has been compromised significantly and GFR is well below the normal range. During this period, patients may continue to be exposed to nephrotoxicants such as mercury. Such exposure may enhance morbidity and mortality as these patients may be more sensitive to the toxic effects of mercury. In rat models of chronic kidney disease (i.e., 75% nephrectomy), the disposition of mercuric ions differs significantly from that in sham or uninephrectomized animals. The renal accumulation of mercuric ions has been shown to be significantly lower in 75% nephrectomized rats compared with uninephrectomized rats. This finding is likely related to the fact that GFR is reduced more in 75% nephrectomized rats than in uninephrectomized rats. Based on these data, it has been suggested that the uptake of a large fraction of the mercuric ions that accumulate within the kidney is dependent upon filtration of these ions at the glomerulus. A reduction in GFR would be expected to decrease the amount of mercury filtered at the glomerulus and presented to the tubular epithelial cells. This theory is consistent with the finding that the concentration of mercuric ions in blood is significantly greater in 75% nephrectomized rats than in uninephrectomized or sham rats. Interestingly, the hepatic accumulation and fecal excretion of mercury have been shown to be significantly greater in 75% nephrectomized rats than in sham or uninephrectomized rats. The increased concentration of mercuric ions could enhance the injurious effects of mercury in hepatocytes, which may lead to a decline in overall health.

13.5 Summary

The aging process in the kidneys has been studied and characterized extensively. It is well known that glomerulosclerosis leads to decreases in GFR and RBF. However, there is little information regarding the response of aged kidneys to environmental toxicants such as cadmium or mercury. Because of the prevalence of these metals in the environment, human exposure is nearly unavoidable. Furthermore, it is well-known that acute and chronic exposures to each of these toxic metals can be detrimental to the kidneys of normal adults, thus it can be postulated that exposure of the elderly and aged to these metals
may lead to additional reductions in renal function. Individuals with compromised renal function, either from aging, disease, or a combination of aging and disease, are especially susceptible to nephrotoxicants. The few studies available have demonstrated an association between exposure to mercury or cadmium and an increase in the incidence and severity of renal disease. It is important to note that early signs of renal dysfunction often go unnoticed, thus individuals with reduced renal function are often unaware that they are at risk during the early stages of disease. Exposure to nephrotoxicants may occur during this early period, and this exposure may be especially detrimental to these individuals. Therefore, a thorough and complete understanding of the way in which nephrotoxicants are handled by aging kidneys is of utmost importance. Because of the paucity of data available on this topic, additional studies are clearly necessary.

References


