Ultrastructural Evidence for Nephropathy Induced by Long-Term Exposure to Small Amounts of Methyl Mercury

Abstract. *A low dose of methyl mercuric chloride fed to female rats for 12 weeks caused extrusion of numerous cytoplasmic masses from kidney proximal tubule cells of the pars recta segment. These masses were characterized ultrastructurally by the presence of a smooth endoplasmic reticulum aggregate. The in vivo metabolism of methyl mercury to inorganic mercury may produce this effect and account for the proteinuria observed in persons occupationally exposed to organic mercury compounds.*

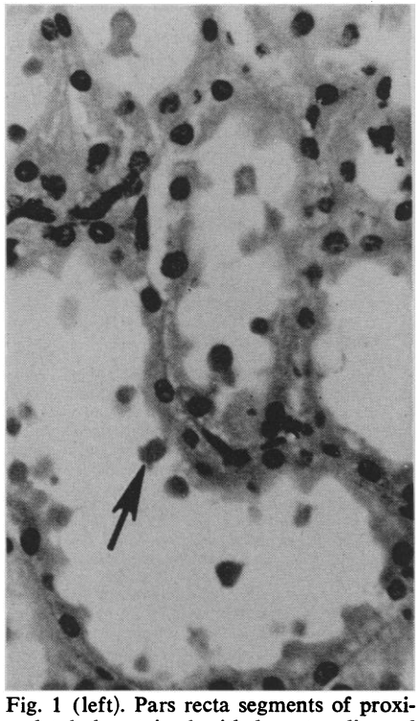
Organo-mercury compounds are metabolized in mammalian kidney and liver to inorganic mercury (*1*). The kidney is a major site of concentration and storage of mercury derived from these chemicals (*2*). Several investigators (*3*) have noted proteinuria in persons occupationally exposed to organo-mercury compounds. Histological and ultrastructural changes in the kidney associated with long-term exposure to small amounts of ingested methyl mercury have not been reported. The effects of long-term exposure of low doses of methyl mercuric chloride (CH_3HgCl) on rat nephron are now described.

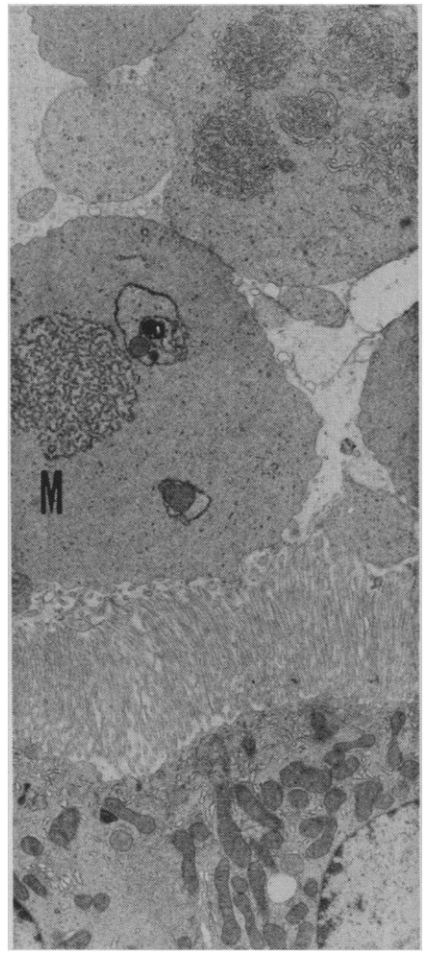
Twenty-five Oregon State University Wistar rats were fed a high-protein diet (*4*). When the animals were 28 days old, mercury (2 ppm) as CH_3HgCl was added to the rations of three male and six female rats. Sixteen animals served as controls. The average food intake for both control and experimental male rats was 19 g per rat per day. Both control and experimental female rats consumed an average of 15 g of food per rat per day. All rats were housed in sterile chambers (*5*) and fed for 12 weeks.

The animals were killed, and the right kidney of each was excised. A coronal section was fixed in 10 percent formalin, dehydrated in a graded series of alcohols, and embedded in paraffin. Adjacent sections 5 nm thick were stained with hematoxylin and eosin and the periodic acid–Schiff technique for examination by light microscopy.

Tissue blocks for electron microscopic examination were excised from both the inner and outer renal cortex and placed in a fixative solution containing 2 percent formaldehyde, 2.5 percent glutaraldehyde, and 250 mg of $CaCl_2$ per liter in 0.085*M* cacodylate buffer at *p*H 7.4. Kidneys of four female control and four female experimental rats were perfused via cardiac puncture with a Ringer-procaine solution (*6*) and then with the above fixative in 3 percent sucrose. All blocks were finally fixed for 1 to 2 hours in OsO_4 (*7*).

All kidneys of female experimental animals exhibited a marked change in the pars recta segments of the proximal tubules. Large numbers of spherical masses were present in tubule lumens (Fig. 1). These masses stained moderately with hematoxylin but not with PAS. The masses were most clearly seen by electron microscopy in patent tubule lumens of perfused kidneys.





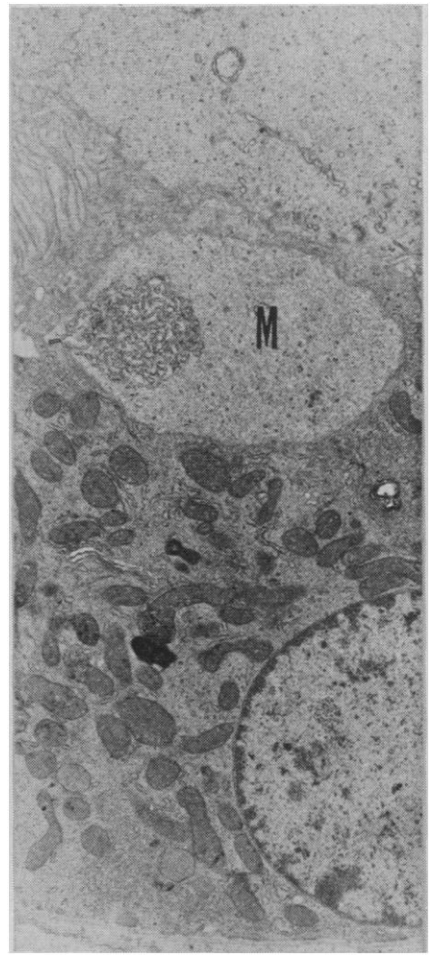


Fig. 1 (left). Pars recta segments of proximal tubules stained with hematoxylin and eosin, from a female rat given CH_3HgCl (2 ppm); the arrow indicates spherical masses in tubule lumens (× 480). Fig. 2 (center). Patent lumen of a pars recta segment displaying spherical masses (*M*) containing bundles of SER in lumen (× 7830). Fig. 3 (right). Isolated spherical mass (*M*) in apical cytoplasm of pars recta cell (× 8700).

They appeared as round blebs of cytoplasm containing a few ribosomes and a single compact bundle of smooth endoplasmic reticulum (SER), sometimes enveloping a microbody (Fig. 2). Similar blebs were occasionally observed in control animals and treated males, but these usually contained degenerating mitochondria and cytosomes. Isolated, spherical masses containing a single bundle of SER were noted in the apical cytoplasm of pars recta cells from affected kidneys (Fig. 3).

In this study, the glomeruli of all animals appeared structurally normal. No alteration of epithelial cell foot processes, basal lamina, or capillary endothelium was noted. This observation indicates that the animals were not losing abnormal amounts of serum protein; rather, it suggests that any increased protein excretion was the result of cytoplasmic blebs extruded from proximal tubule cells.

The presence of these cytoplasmic blebs in this portion of the nephron is significant in light of the known conversion of organo-mercury compounds to inorganic mercury in the kidney (*1*) and the described toxic effect of inorganic mercury (that is, mercuric chloride) on the pars recta (*8*). Mercury derived from CH_3Hg has been reported to concentrate in the microsomal fraction of rat kidney (*9*). Microsomal enzyme systems are closely associated with the SER (*10*). The SER is a logical site for the conversion of CH_3Hg to inorganic mercury because of its detoxification activity. Cleavage of the carbon-mercury bond would release inorganic mercury, which could react with microsomal enzymes as a noncompetitive inhibitor. Mercurials strongly inhibit liver enzymes of sterol biosynthesis and these enzymes are present in the microsomal fraction (*11*).

If mercury inhibits these enzyme systems in the kidney the selective extrusion of SER bundles by pars recta cells of female animals may represent the removal of nonfunctional organelles through the process of potocytosis (exocytosis) (*12*). The more prominent effect of CH_3Hg on the pars recta of female rats in comparison to males is probably due to known sex differences in the activities of kidney enzymes (*13*). Enzymes responsible for the metabolism of organic or inorganic mercury in female rat kidneys may be less efficient than those of males.

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References and Notes

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Improved Flow Rates with Porous Sephadex Gels

Abstract. *The use of an internal support of siliconized glass beads 6 millimeters in diameter was found to improve markedly the flow rates obtainable with porous Sephadex gels without significant alteration of other properties of the gels. The method may be generally applicable in situations where rapid separations on Sephadex are required.*

The speed with which separations can be performed on porous Sephadex gels is severely limited by the development of compression in the gel bed and the consequent diminution of flow rate when the operating pressure head is increased (*1*). We report here a simple modification of column design which greatly increases the maximum flow rates obtainable with such gels without significant impairment of resolution. The modification consists in providing the gel with an internal support of glass beads which enables the gel to withstand much greater operating pressures without being compressed. The "bead column" thus operates in a fashion similar to a series of very short columns linked end to end.

Any conventional column used for gel filtration can be converted to a bead column provided that the column diameter is greater than 1.5 cm (a limit imposed by the diameter of the glass beads). One fills the column about half full with solvent and then adds a sufficient number of glass beads to fill the column to the desired height, taking care to add the beads slowly enough so as not to trap any air between the beads as they settle through the solvent. Solid 6-mm glass beads (Propper Manufacturing Company) provide the optimum support. Smaller beads cause difficulties in the packing of the gel, whereas larger sizes cause impaired resolution and are not as effective in preventing gel compression. The beads are siliconized with Siliclad (Clay Adams) before use.

We then allowed the swollen Sephadex gel to pack between the glass

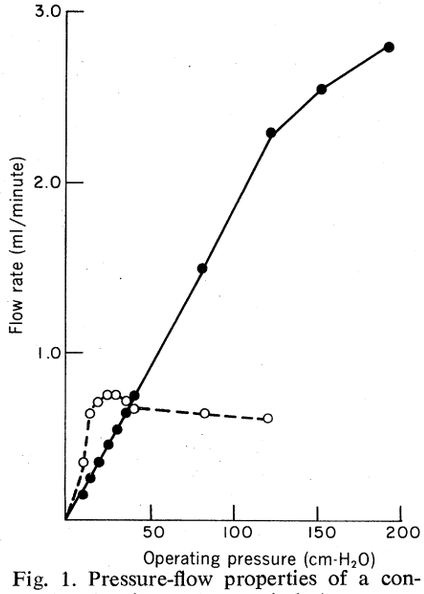

Fig. 1. Pressure-flow properties of a conventional column (open circles) as compared to those of a bead column (solid circles); both columns were of the same diameter and were packed with the same quantity of Sephadex G-200 (the bed of the bead column being approximately twice as long as that of the conventional column).