Mitochondrial Proliferation Within the Nephron

I. Comparison of Mitochondrial Hyperplasia of Tubular Regeneration with Compensatory Hypertrophy

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Mitochondrial proliferation in the renal proximal tubular epithelium in response to tissue loss has been quantitated by two diverse models: acute tubular necrosis induced by mercuric chloride and unilateral nephrectomy. The increased work demand on the remaining tubular mass in both models has probably stimulated both cell hyperplasia and hypertrophy. In both instances, cell hyperplasia preceeds hypertrophy. During the cellular hypertrophy, mitochondria first proliferate in number and then increase in size; presumably to satisfy the greater need for energy metabolism necessary during increased active transtubular solute transport (Am J Pathol 70:119–130, 1973).

PREVIOUS STUDIES in this laboratory have demonstrated that the proximal tubular epithelium has a marked capability to regenerate following toxic injury with mercuric chloride.[1] An initial burst of cell replication associated with nuclear deoxyribonucleic acid (DNA) synthesis results in complete relining of the injured proximal tubules within 5 days. This early cell replication is followed by reconstitution of the organelles within the new cells in association with the resumption of the transport mechanisms of tubular reabsorption. During this phase of cytoplasmic maturation, grains are observed over the cytoplasm of these cells (in autoradiographs of the kidneys of animals administered tritiated thymidine), suggesting incorporation of thymidine into mitochondrial DNA.[2] Increasing numbers of mitochondria are visualized within these cells by electron microscopy. The present study was undertaken to characterize and quantitate the mitochondrial proliferation within the cytoplasm of these regenerating proximal tubular cells.

Both cellular hypertrophy and hyperplasia are known to occur in the nephron following unilateral nephrectomy.[3–6] Johnson and Amendola have shown that mitochondrial proliferation is present during the com-

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pensatory hypertrophy following unilateral nephrectomy and is associated with an increased rate of tubular reabsorption.[7] They postulated that the compensatory hypertrophy was regulated primarily by the functional demand placed upon the remaining kidney. The present investigation therefore characterizes the mitochondrial proliferation within proximal tubular epithelium in compensatory hypertrophy and compares this phenomenon with that of regeneration following tubular necrosis.

Materials and Methods

Young adult male Sprague-Dawley rats weighing 150 to 200 g were housed in pairs and given Purina rat chow and unrestricted tap water.

Induction of Tubular Necrosis with Mercuric Chloride

Forty-four rats were each injected intravenously with 0.15 mg mercuric chloride per 100 g rat weight[1] and were killed in groups from 1 to 28 days later. Ten rats that were not injected with mercury served as controls. One hour prior to sacrifice each rat received, intraperitoneally, 0.5 μCi of tritiated thymidine (^{3}H-tdr, Schwartz Bio-Research, Inc, Orangeburg, NY) per g rat weight. At autopsy, sections of one kidney were fixed in buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for light microscopy. Portions of the same kidney were diced, fixed in 1% buffered glutaraldehyde, postfixed in 1% osmium tetroxide, embedded in Epon-Araldite, sectioned and photographed in a Hitachi 11-C electron microscope.

Unilateral Nephrectomy

Twenty-eight rats were uninephrectomized through a midline abdominal incision under pentobarbital anesthesia (0.2 ml of 2% solution of sodium pentobarbital per 100 g body weight injected intraperitoneally). The left kidney was removed and the rats were killed in groups from 1 to 28 days later. Thymidine was administered to all rats as described above. At autopsy, tissues were similarly prepared for light and electron microscopy. The nephrectomized left kidneys and both kidneys from an additional 4 rats served as controls.

Autoradiography

Light microscopy autoradiography was performed using Kodak NTB-2 dipping emulsion and 3 weeks' exposure at 4 C. The mitotic index was recorded in each kidney by counting the number of mitoses per 1000 proximal tubular cells. The tritiated thymidine uptake indices were recorded as the number of nuclei containing 10 or more grains per 1000 nuclei in the proximal tubular cells. Electron microscopy autoradiography was performed on selected kidneys using ultrathin sections coated with Ilford L4 emulsion and exposed for 6 weeks at 4 C.

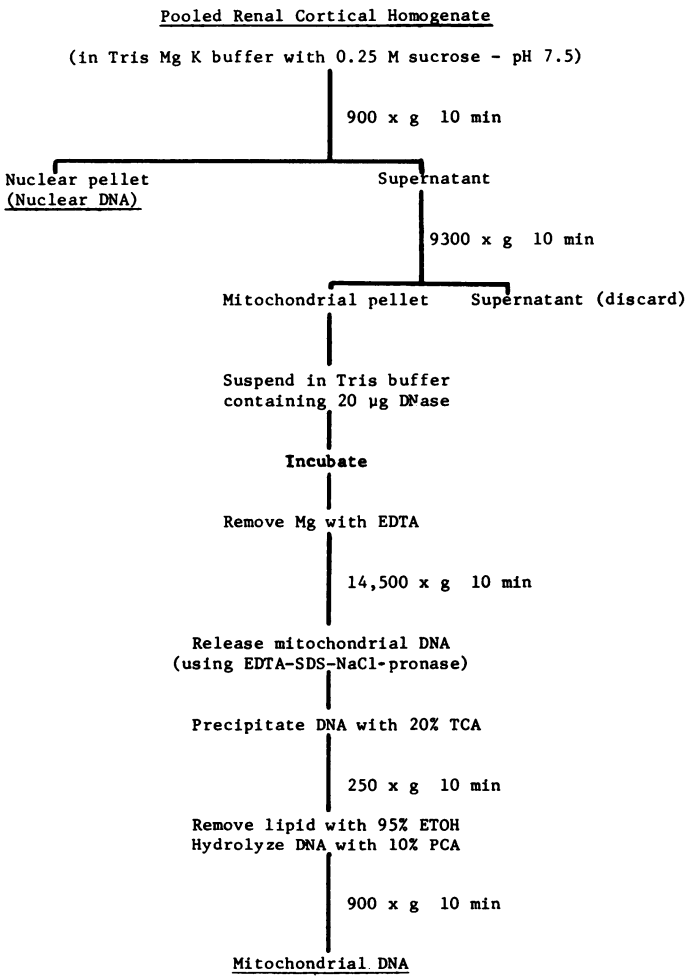
Morphometric Analysis

The Loud technic[8] was used to quantitate the number and size of mitochondria within the cytoplasm of the regenerating and hypertrophic renal tubular cells. Cells were photographed in the electron microscope and printed under constant magnification at 10,000 times. The prints were overlaid with a grid, and measure-

ments were made of cytoplasmic and mitochondrial intercept lengths. Calculations were then made for the number of mitochondria per sq μ of proximal tubular area, the volume ratio of mitochondria to cell and the volume of the average mitochondrion.[9]

Nuclear and Mitochondrial Analysis for DNA

To compare the synthesis of DNA in nuclei and mitochondria during the various stages following recovery from tubular necrosis and following uninephrectomy, estimations of ^{3}H-thymidine incorporation after separation of nuclear and mitochondrial fractions were made according to the scheme on Text-figure 1. The cortices of portions of kidneys not used for microscopy were dissected free from the medullas, weighed and pooled within any one group of animals. Cortical homog-



TEXT-FIG 1—Scheme for preparation of nuclear and mitochondrial fractions.

enates were made from the tissues in Tris buffer with 0.25 M sucrose containing 0.005 M magnesium chloride and 0.025 M potassium chloride at pH 7.5 using a Potter-Elvehjem homogenizer. The nuclear pellet was obtained by centrifuging the homogenate at 900*g* for 10 minutes in a RC-2 refrigerated centrifuge. The supernatant was centrifugated at 9300*g* for 10 minutes to obtain the mitochondrial pellet, which was then resuspended in the buffer and incubated for 30 minutes at 4 C with deoxyribonuclease to remove contaminating nuclear DNA. The reaction was stopped by addition of 0.2 M ethylenediamine tetraacetic acid (EDTA), and the suspension was centrifuged at 14,500*g* for 10 minutes to recover the mitochondrial pellet. Mitochondrial DNA was released by spreading the pellet with a glass rod and mixing it with 2 ml of a solution of 0.15 M sodium chloride with 1% sodium lauryl sulfate containing 3 mg pronase and incubating the mixture for 3 hours at 37 C. An equal volume of 20% trichloracetic acid was used to precipate the DNA. Lipid was removed with equal volumes of 95% ethyl alcohol, and the DNA was hydrolyzed with 10% perchloric acid and collected by centrifugation at 3000 rpm for 5 minutes. The hydrolysed DNA fraction was obtained directly from the time-washed nuclear pellet by omitting DNAse and pronase treatments. The hydrolysed DNA fractions were assayed for DNA content and radioactivity. The specific activity of DNA was determined by standard scintillation technics using a Packard tri-carb liquid scintillation counter;[2] DNA was determined colorimetrically on both pellets using the diphenylamine reaction.[10]

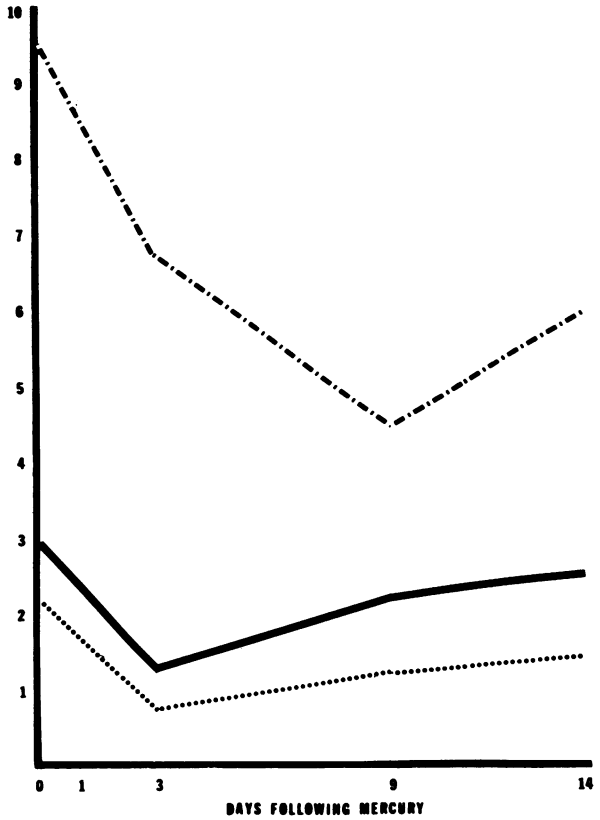
Results

Mitochondrial Hyperplasia Following Tubular Necrosis

Mitochondria contained in proximal tubules from control kidneys were present in their usual number and size along the basilar infoldings and extending up into the apical third of the epithelial cell cytoplasm (Figure 1). A decreased number of smaller mitochondria were present in the cytoplasm of the regenerating cells. Relatively few microvilli, basilar infoldings and arrays of endoplasmic reticulum were identified in the rapidly replicating cells at 3 days. A near complete reconstitution in numbers and size was seen in the cytoplasm within 14 days after injury with mercuric chloride.

Morphometric analysis of these kidneys (Text-figure 2) confirmed that the number of mitochondria per cell area decreased by 3 days and gradually increased toward control levels by 14 days. The ratio of the volume of the mitochondria to the volume of the cell likewise fell to a minimum at 3 days, and subsequently gradually increased toward control levels by 14 days. The average individual mitochondrion volume decreased below control levels by 3 days, continued to fall while the number of mitochondria increased until 9 days and then began to return toward control levels by 14 days.

The specific activity of DNA within the nuclear and mitochondrial pellets (Text-figure 3) revealed a markedly increased incorporation

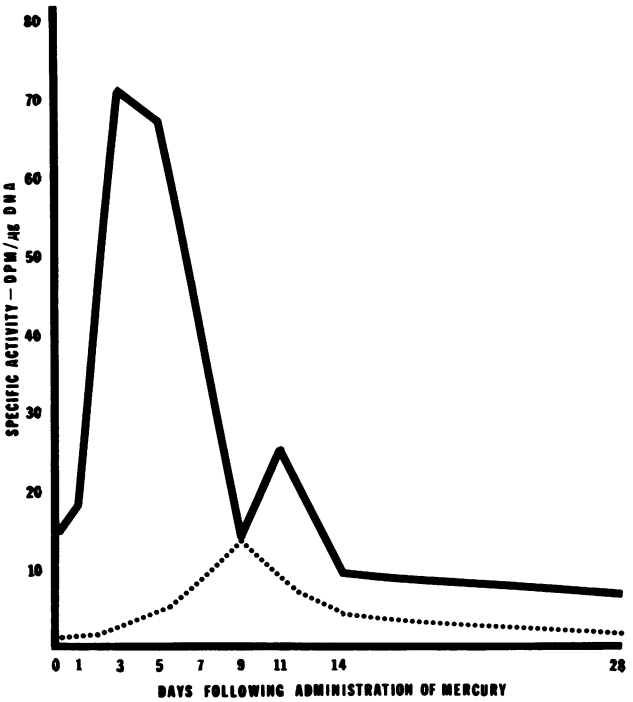


TEXT-FIG 2—Morphometric analysis by Loud technic of mitochondria in regenerating cells following mercury necrosis. The number of mitochondria per sq μ of cell *(solid line)* decreased to a minimum at 3 days and then gradually increased. The ratio of the volume of mitochondria to the volume of the cell *(dotted line)* also decreased to a minimum at 3 days and then increases. The average mitochondrion volume *(dash-dot line)* fell to a minimum at 9 days and then increased toward control levels.

of thymidine into nuclear DNA in the early phase of recovery which reached a peak at 3 days while cells were rapidly replicating. A second, smaller nuclear peak was observed at 11 days, after which the specific activity fell below control values and remained there during the duration of the experimental period. A single, smaller peak of incorporation of thymidine into mitochondrial DNA was observed at 9 days, at a time when the mitochondria were proliferating within the cytoplasm of the maturing tubular epithelium and tracks were present over mitochondria by autoradiography (Figure 2).

Mitochondrial Hyperplasia Following Unilateral Nephrectomy

Within 3 days after removal of the left kidney the mitochondria of the proximal tubular cells in the remaining kidney appeared to decrease in size but increase in number (Figure 3). By 14 days the mitochondria had increased in size. The mitotic index rose rapidly by 1 day, reached a maximum at 2 days and gradually decreased to control levels (Text-



TEXT-FIG 3—The specific activity of nuclear DNA in the regenerating cells following mercury injury *(solid line)* reached a high peak at 3 days, followed by a second peak at 11 days and then returned to control levels. A single smaller peak of mitochondrial DNA specific activity was present at 9 days *(dotted line)*.

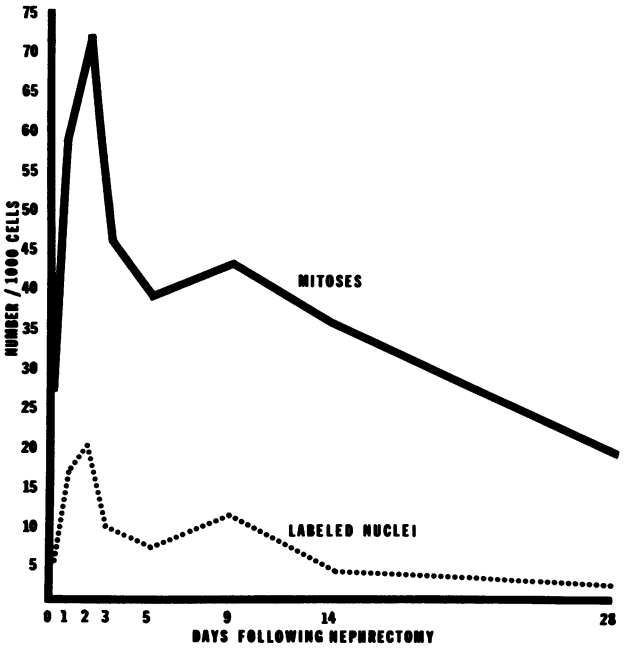
figure 4). The nuclear uptake index also reached a maximum at 2 days and returned to control levels by 14 days.

Morphometric analysis revealed a proliferation of the mitochondria reaching maximum number at 3 days and decreasing toward control levels by 14 days (Text-figure 5). The ratio of the volume of the mitochondria to the volume of the cell gradually increased throughout the experimental period. The average individual mitochondrion volume initially decreased to a minimum at 3 days, followed by a gradual increase toward control levels by 14 days.

Specific activity of DNA within the nuclear and mitochondrial pellets (Text-figure 6) revealed and enhanced incorporation of thymidine into nuclear DNA with an initial peak on day 1, followed by a second, broader and higher peak at 9 days. Mitochondrial incorporation reached a small peak at 1 to 2 days which was followed by a prolonged, marked elevation beginning at 5 days and continuing throughout the experimental period.

Discussion

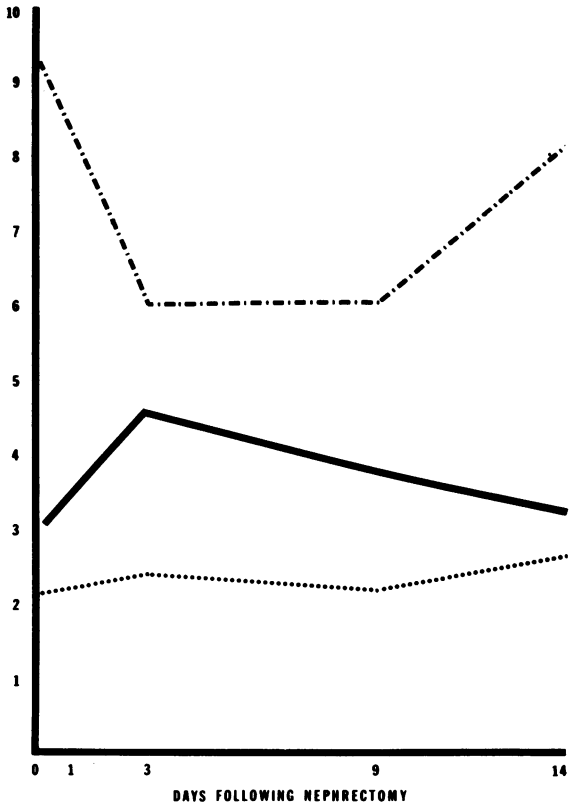
The present investigation affirms the relative dedifferentiation of residual lining cells in the necrotic zone of mercury-induced tubular



TEXT-FIG 4—Mitotic and labeling indices following unilateral nephrectomy. The mitotic index *(solid line)* reached a peak at 2 days and gradually returns to control levels. The nuclear ^{3}H-thymidine uptake index *(dotted line)* also peaked at 2 days, with a second smaller peak at 9 days.

injury. The cells remain relatively dedifferentiated while they are replicating to reline the tubular basement membrane. In order to provide the energy source for active solute transport across the cells, the mitochondrial population increase in regenerating tubules appears to take the form of early mitochondrial hyperplasia, followed by gradual increase in mitochondrial volume and number of cristae. Many mitochondria are elongate and irregular during this phase. Although budding is suggested in some micrographs the method of duplication cannot be established by this study. Within 14 days the proximal tubular epithelial cytoplasm contains the number and size of mitochondria of the controls. At this time the basilar infoldings and microvilli are nearly completely reconstituted.

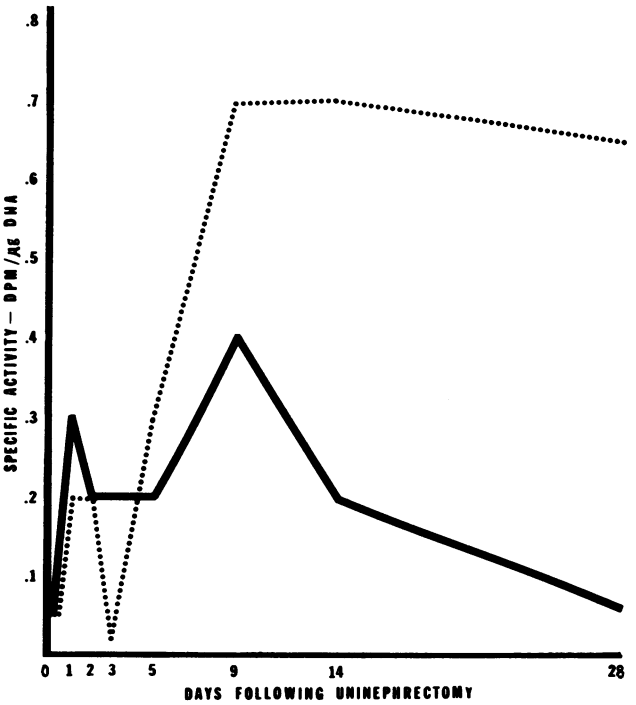
The fact that tritiated thymidine appears to be incorporated into mitochondrial DNA during this proliferative phase suggests that DNA synthesis is intrinsic to the developing mitochondrion. Mitochondrial DNA has been shown to be distinct from nuclear DNA, with properties of synthesis and replication within the mitrochondrion, and it has been suggested that it plays a genetic role.[11] The mitochondrial DNA synthesis demonstrated by this study during this organelle replication suggests that DNA may have a genetic, or at least a regulatory, role in-mitochondrial function. This mitochondrial DNA synthesis was most



TEXT-FIG 5—Morphometric analysis by Loud technic of mitochondria in proximal tubular epithelial cells following unilateral nephrectomy. The number of mitochondria per sq μ of cell *(solid line)* reached a maximum at 3 days and then fell to control levels. The ratio of the volume of mitochondria to the volume of the cell *(dotted line)* increased gradually during the experimental period. The average mitochondrion volume *(dash-dot line)* fell to a minimum at 3 days and then rose to near control levels by 14 days.

likely obscured in previous studies where nuclear DNA synthesis was recorded using whole cortical homogenates rather than nuclear pellets.[2]

In comparison, the mitochondrial proliferation of compensatory hypertrophy after unilateral nephrectomy is somewhat different. Here the cells replicate to a lesser extent prior to their hypertrophy. The initial cellular hyperplasia and elevated mitotic index appears at 1 to 2 days and is at a lower level than it is after tubular necrosis.[2] Less dedifferentiation is needed. Perhaps the increased work demand in some manner stimulates the proximal tubular cells to hypertrophy as suggested by some authors.[6,12] This has been doubted by others [13-14] who postulated that the hypertrophy is perhaps due to some tissue factor released from the remaining kidney [15] or due to an immune mechnism.[16] The cellular hypertrophy, in part, consists of mitochondrial proliferation. Initially, the mitochondria decrease in volume as they increase in number—perhaps by budding, although *de novo* formation of mitochondria from other membrane components of the cytoplasm cannot be excluded.



TEXT-FIG 6—The specific activity of nuclear DNA in the proximal tubular epithelial cells following unilateral nephrectomy *(solid line)* peaked at 1 and again at 9 days and then returned to control levels. Specific activity of mitochondrial DNA *(dotted line)* reached a small peak at 1 to 2 days and a second higher peak at 9 days, which remained elevated.

Biphasic increase in protein synthesis has been reported in unilateral nephrectomy and may correlate with protein needed for cell replication and later for organelle hyperplasia.[17] In contrast to the proliferation following tubular necrosis, the ratio of the volume of mitochondria to the volume of the cell gradually increases throughout the experimental period. As in the necrotic model, the average individual mitochondrion volume initially decreases while the mitochondria are proliferating and then increases. The second peak of nuclear DNA synthesis in unilateral nephrectomy is unexplained, for the mitotic index is low at 9 days. It is unlikely that synthesis for repair of DNA could explain this peak. The prolonged peak of mitochondrial DNA synthesis beginning at 5 days suggests continued buildup or turnover of DNA within the proliferating and enlarging mitochondria.

In both models the work demand on active structural units is increased. In both, cellular hyperplasia is associated with cellular hypertrophy, which in part consists of mitochondrial proliferation. Thus the nephron illustrates a remarkable adaptive role to repair and increased work demand.

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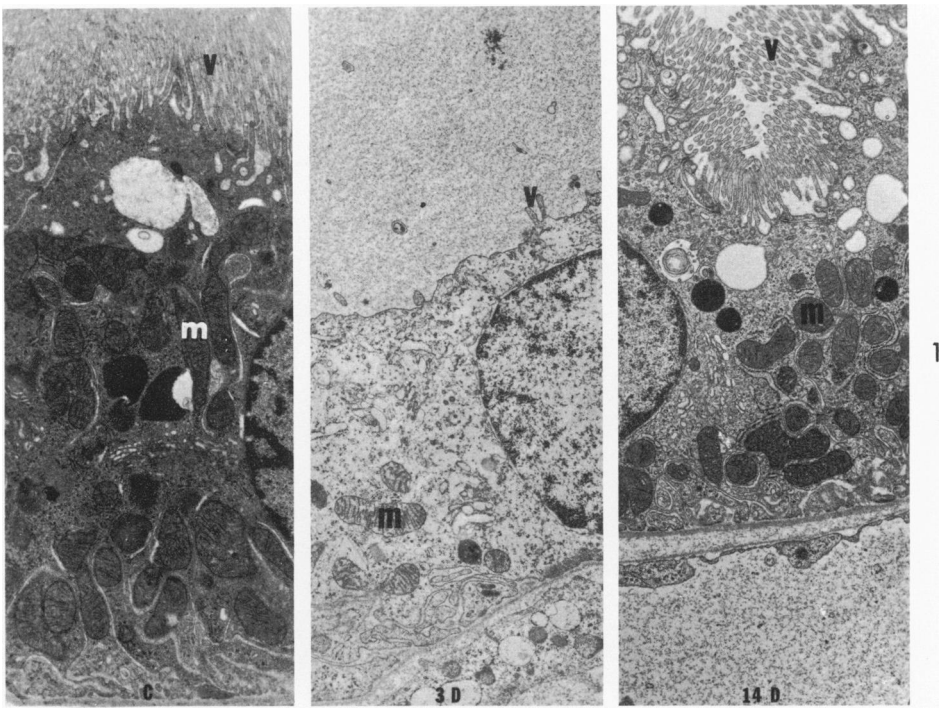

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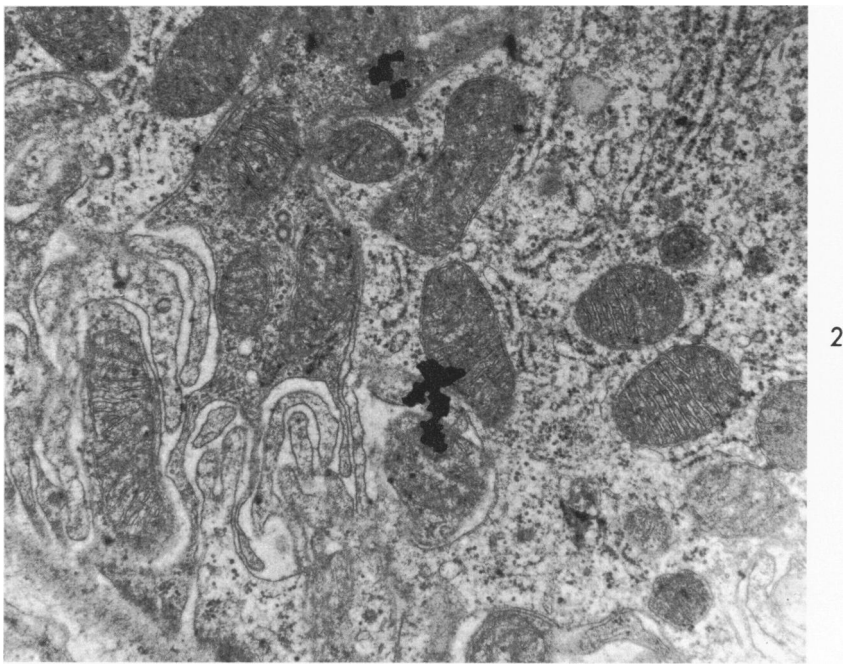

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Fig 1—Electron micrographs depicting regeneration of proximal tubules following mercury necrosis. The control nephron (**left**) contains well-developed microvilli (*v*) at the luminal surface and numerous mitochondria (*m*). At 3 days (**center**) the regenerating epithelium is low cuboidal, with sparse microvilli and only a few mitochondria. Nearly complete regeneration at 14 days (**right**) contains regenerating cells with near normal numbers of microvilli and mitochondria (Lead citrate and uranyl acetate, × 5000).

Fig 2—Electron microscopic autoradiograph of regenerating cell 9 days following mercury necrosis. Tracks are visible over mitochondria. (Lead citrate and uranyl acetate, × 20,000).

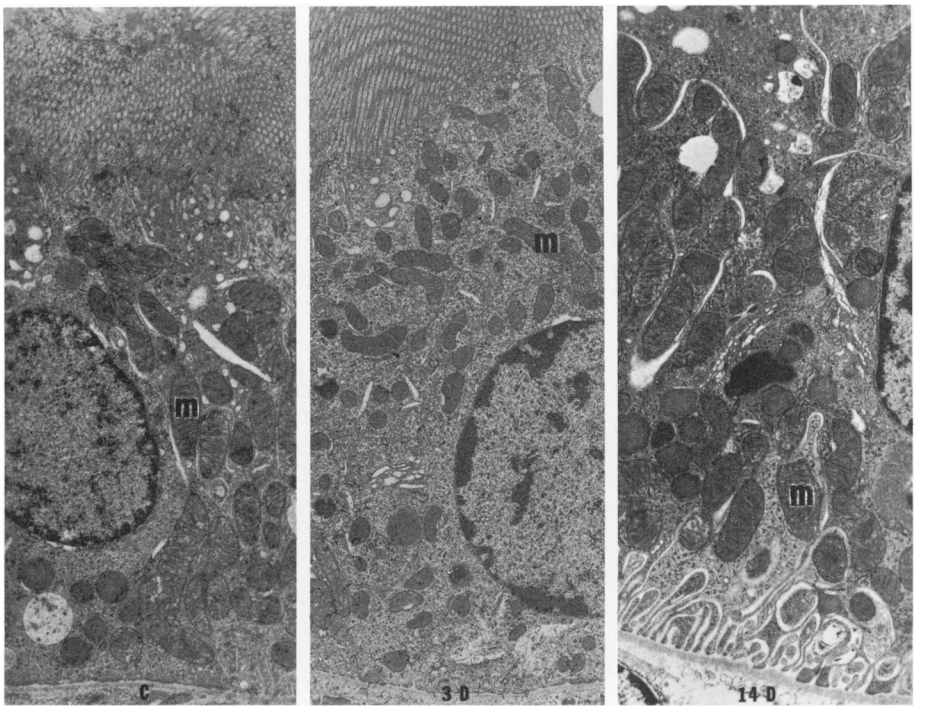

Fig 3—Electron micrographs depicting hypertrophy of proximal tubules following unilateral nephrectomy. The control nephron proximal epithelial cell (**left**) contains a moderate number of mitochondria (*m*). At 14 days (**center**) the cell contains an increased number of smaller mitochondria. By 14 days (**right**) the number and size of mitochondria is similar to those of the control (Lead citrate and uranyl acetate, × 5000).