

ROLE OF MITOCHONDRIA IN THE HANDLING OF GOLD BY THE KIDNEY

A Study by Electron Microscopy and Electron Probe Microanalysis

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INTRODUCTION

Gold salts are employed chiefly in the treatment of rheumatic diseases. Their use may cause harmful side effects the most serious of which include various renal lesions (4, 7, 12, 14, 19, 20). Investigation of these lesions has failed to demonstrate the role played by gold itself in their genesis. Light microscope studies do not permit either a detailed description of cellular damage or the determination of the precise nature and intracellular localization of deposits when they are found. Ultrastructural studies have been few. Lee et al. (14) described glomerular irregularities in three patients who developed proteinuria following gold salt injections. Ganote, Beaver, and Moses (11) studied the renal effects in rats of a single subcutaneous injection of gold sodium thiosulfate. They found proximal tubular necrosis with inclusions present in both light and electron microscopic sections. Treatment of the paraffin sections with SnCl_2 stained apical inclusions brown-black, a reportedly specific test for gold (16).

Although the obvious limitations of the light microscope for studying intracellular inclusions were met by the electron microscope, the question of the specific nature of the observed inclusions remained open. Our approach has been to combine electron microscopy with electron probe microanalysis. A combination of a description of ultrastructural changes and an analysis of intracellular inclusions on the same sections is the only method which can provide conclusive information.

In this way, we have been able to localize the subcellular sites of gold concentration in kidney cells.

METHODS

Rats of the Wistar strain were injected subcutaneously with the salt sodium aurothiopropanol sulfonate (Allochrysin, Laboratoires Lumiere, Paris, France). For the acute experiment, three rats (235, 265, and 320 g) were each injected with 1 ml of a 100 mg/ml solution. For the chronic experiment, 10 rats (170–180 g) were given 20 1-ml injections of a 5.0 mg/ml solution at the rate of three injections per week.

Specimens of kidney cortex were taken from the acutely poisoned animals 48 hr after the injection. In the chronic series, two animals were sacrificed after receiving either 15, 45, 75, or 100 mg of Allochrysin, and 7 months after the last injection. Control kidney tissue was taken from uninjected rats of the same age.

The tissues were removed while the animal was under ether anesthesia, fixed in phosphate-buffered osmium tetroxide, embedded in Epon, and stained with uranyl acetate after sectioning.

Electron probe microanalysis of inclusions was performed on the same sections previously photographed with the high-resolution electron microscope (Philips 200). Our instrument couples with the electron probe analyzer itself a system which permits electron microscopic observation of the section. For practical reasons, the magnifying capacity of the microscope was limited to 15,000 times—an amount

which is more than sufficient for our purposes. Allowing one to visualize the precise area analyzed is the keynote of this instrument's value in biological studies. The section is first examined with the instrument acting as an electron microscope. When an interesting inclusion is seen, the path of the electron beam is modified so that it strikes the preparation with an area of less than $1 \mu^2$. Analysis of the element(s) present is based on the principle that atoms of a target element bombarded with a beam of electrons emit X-rays which are specific for the element bombarded. Spectrophotometric analysis of the wavelength of the characteristic "ray" emitted definitively identifies the element in question. For a more complete discussion of the principles and applications of electron probe microanalysis as well as technical specifications of the instrument, see references 5, 8, and 9.

RESULTS

Acute Poisoning

The kidneys removed from these very sick animals were enlarged and pale. Electron micro-

scopic examination showed that the lesions in all animals were identical and consisted of complete destruction of the proximal tubules. The basement membranes of the proximal tubules were intact, but no remnant of cellular architecture remained (Fig. 1). Among the cell debris filling the tubular space were structures containing dense, rounded masses, between 0.1 and 0.5μ , which seemed to be composed of a large number of 40 \AA grains. Microanalysis showed that these masses contained gold and osmium. By the presence of double membranes and cristae, it could be seen that these gold-containing structures had been mitochondria (Fig. 2).

Casts consisting of cell debris and clumps of gold deposits were found in the lumina of the distal tubules.

No deposits large enough for analysis were found in either the glomerulus or distal tubules; however, small masses which corresponded in their morphology to the gold deposits were occasionally encountered in these cells.

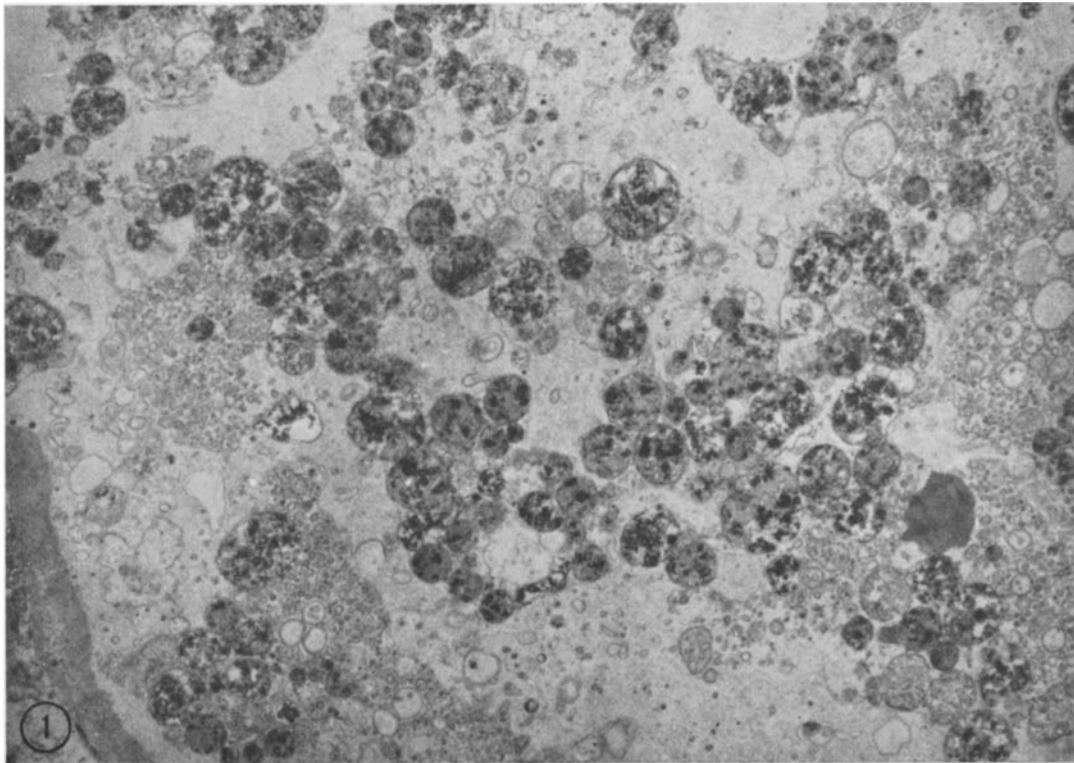


FIGURE 1 Proximal tubule of rat injected 48 hr previously with 100 mg of the gold salt, sodium aurothio-propanol sulfonate. Basement membrane is at left. Tubule cell destruction is complete. $\times 6,700$.

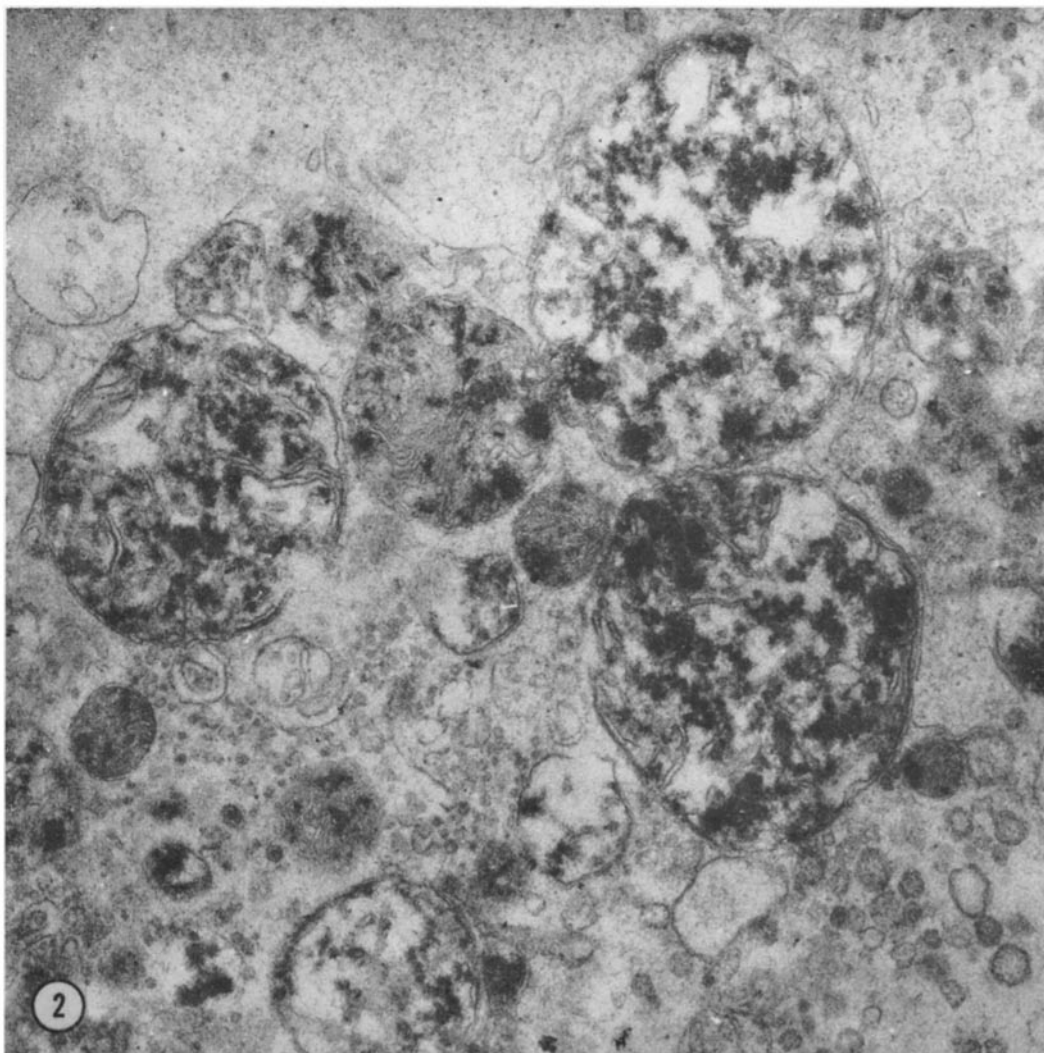


FIGURE 2 Higher magnification of the luminal debris seen in Fig. 1, showing degenerating mitochondria which contain gold deposits. $\times 60,000$.

Chronic Poisoning

The rats supported the injections of Allochrysin very well although the rate of weight gain was less than normal. Grossly, the kidneys had a strictly normal appearance. Only the epithelial cells of the proximal tubules were affected; both glomeruli and distal tubules remained normal at all stages of the intoxication. The severity of the lesion was directly proportional to the amount of gold injected.

Those deposits which were shown by micro-

analysis to be gold formed a group which was morphologically distinct from other kinds of dense inclusions sometimes seen in proximal tubular cells. The gold was found in the form of rounded aggregates of very dense grains, the same as those seen in acute poisoning. The masses varied in size from approximately 0.1 to 0.6μ and were variably condensed. The larger masses had a central region which was very compact and an outer fringe which was less so (Fig. 3).

In the early stages of chronic intoxication, among the majority of normal tubule cells were

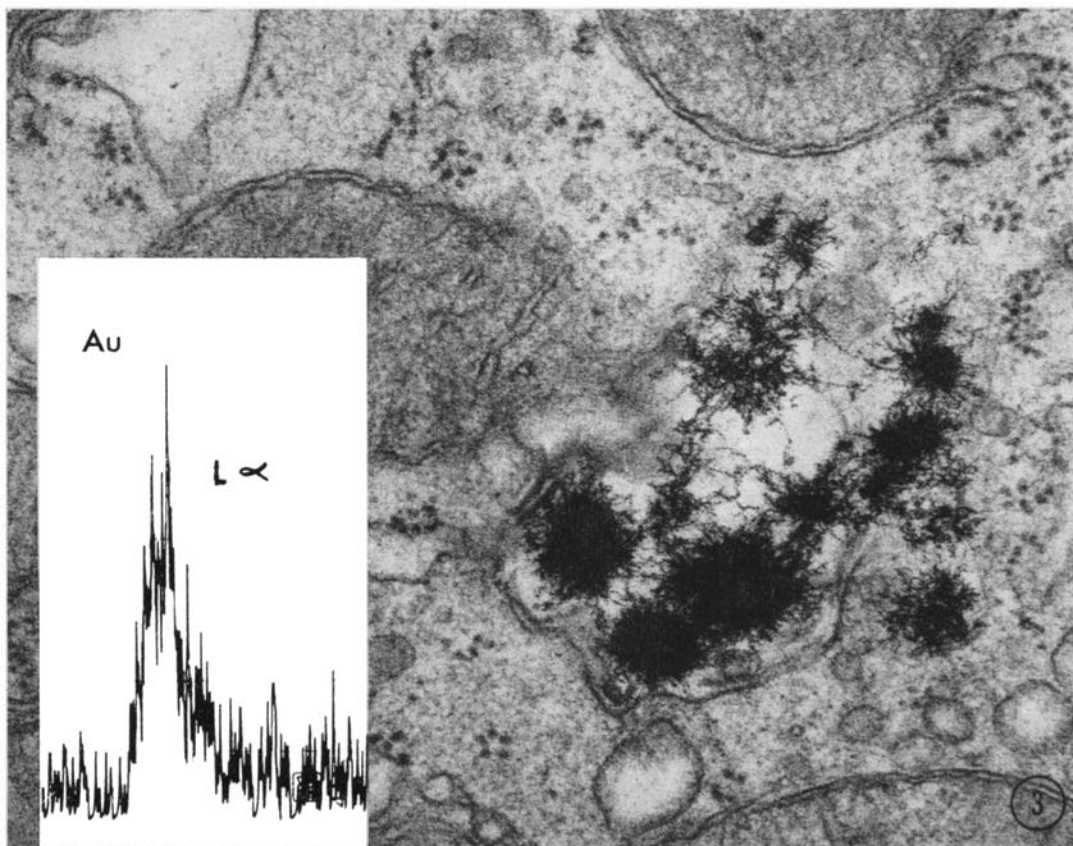


FIGURE 3 The rounded gold deposits seem to be composed of minute grains. The insert shows the characteristic L "ray" of gold which was obtained with electron probe microanalysis on a similar inclusion. $\times 60,000$.

some in which inclusions had formed. These inclusions were found at the bases of the proximal tubular cells and consisted of degenerating mitochondria with gold deposits. The mitochondria varied in size from 1 to 4 μ and had limiting membranes which were often incomplete. Besides the gold deposit, they often contained clumps of less dense material, possibly protein, and fragments of membranes. An affected mitochondrion had from 1 to 10 principal deposits and from a few to innumerable smaller aggregates (Fig. 4).

In the middle stages of chronic poisoning, more of the proximal tubule cells contained inclusions, generally in greater numbers. The over-all size of both the inclusions and the gold deposits remained in the same range; however, there was an increase in the number of deposits per inclusion, resulting in more densely packed inclusions. In addition,

the placement of inclusions was so modified that, in instances in which several inclusions existed in the same cell, some were now found in the apical regions and occasionally among the microvilli of the brush border. A few instances of inclusions in the proximal tubular lumen were observed (Figs. 5 and 6).

In the last chronic stage, most proximal tubule cells contained inclusions. Some of the inclusions had 40 or more gold deposits. The diameter of individual deposits remained approximately 0.55 μ . Many inclusions were found apically in the cell and in the microvilli and tubular lumen. Cells with many inclusions had an altered cytoarchitecture with swollen mitochondria, the formation of many small clear vacuoles, and loss of cytoplasmic organelles (Figs. 7-9).

Deposits of the gold type were seen on rare

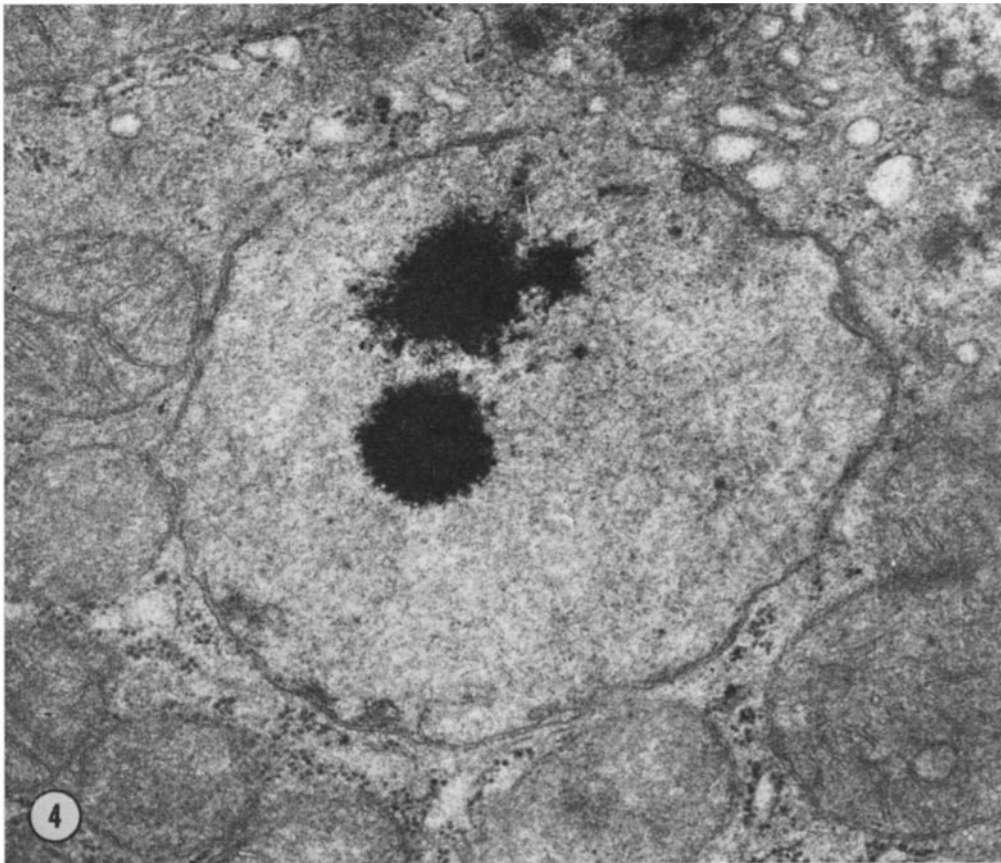


FIGURE 4 An inclusion with two major gold deposits. Only the limiting membranes remain relatively intact. $\times 45,000$.

occasions in an epithelial cell of Bowman's capsule, a glomerular epithelial cell, and an endothelial cell of an intertubular capillary.

7 months after the last injection, there were no gold-containing inclusions in the proximal tubular cells and the kidney structure was normal.

In summary, we found that the greater the quantity of gold injected, the greater was the number of proximal tubular cells found with inclusions containing gold. The inclusions form at the base of the cell from degenerating mitochondria and move toward the apex where they are expelled into the lumen. Tubular damage caused by the accumulation of gold is completely reversible.

DISCUSSION

The study of acute poisoning implicated the importance of mitochondria as sites of gold accumula-

tion in the kidney; however, the extent of the damage to the proximal tubule cells prevented us from being certain that the role played by the mitochondria was a primary one. The chronic poisonings provided us with additional facts and a perspective on the evolution of the cellular damage which lends support to the conclusions of other workers on the nature of cellular ionic regulations.

Studies with radioactive gold-198 have shown that injected gold is completely bound by the plasma proteins (10, 13, 18). Gold may get from its protein-bound state in the circulation into the proximal tubule cells via the tubular capillaries or the glomerular filtrate. A mechanism by which an ion is separated from protein and selectively transported from the tubular capillaries into the cell has not, so far, been demonstrated in the kidney. At the glomerulus, gold may be separated or filtered along with the proteins to which it is

bound. Both ion and protein transport systems exist in the proximal tubule, and the rat kidney normally filters large amounts of protein which are subsequently reabsorbed. A glomerular route has been demonstrated in at least one other case of heavy metal poisoning. Masse and Galle (unpublished observation), using the technique of radioautography on ultrathin sections, showed that uranium is found in the proximal tubular lumen very soon after its injection. They were able to follow its movement from lumen to brush border to cell.

Gold, once inside the cell, probably enters the mitochondrion in ionic form since many mitochondria containing deposits still have intact outer membranes. Even after the destruction of the functional mitochondrion, however, deposits may still continue to form around the centers of precipitation already present.

Mitochondria have been shown by many authors to possess the capacity to accumulate ions. Bartley and Davies (1) showed that isolated mitochondria will take up sodium, potassium, magnesium, and phosphate from the surrounding solution and maintain a concentration gradient. Weiss (21) found that mitochondria in the duodenal absorptive cell contained significantly higher numbers of granules in the case in which the animal had ingested large amounts of sodium or potassium. He noted that mitochondria with many granules were characteristic of cells which transport large amounts of cation, and hypothesized that the mitochondrial granule represented a mechanism for the regulation of the cellular ionic composition. Peachey (15) came to essentially the same conclusions from his studies with isolated rat kidney mitochondria and toad bladder cells. He found that calcium, strontium, and barium were taken up from the medium and accumulated in the mitochondria. In these cases, the mitochondrial granule of low density normally present was replaced by a dense granule. He suggested that the

low-density granule may regulate the internal ionic environment by acting as an ion exchanger. He also speculated that this intramitochondrial binding mechanism played some role in the transcellular movement of ions.

Mitochondria have been shown by Bessis and Breton-Gorius (2, 3) to act as storehouses for ferritin in erythroblasts. The presence of silica in mitochondria has been inferred by Policard et al. (17) in their studies of mitochondrial changes after the injection of silica gel. Finally, rats given a solution of 0.01% silver nitrate to drink were found by Dempsey and Wislocki (6) to have dense deposits in the mitochondria of their proximal convoluted tubules.

If the mitochondria normally act to regulate internal ionic composition by selectively binding and unbinding cations, what we may be seeing here is that system overloaded by the presence of large numbers of gold ions. As more and more mitochondria are disrupted, the cell's energy supply is compromised and structural disintegration begins to occur. Gold granules themselves seem to be inoffensive. A cell which contains a few gold inclusions has an otherwise normal architecture. Large gold deposits may also be found in the lumina of the distal tubules without adversely affecting the tubule cells.

Finally, we should consider whether the 40 A granules seen in the electron micrographs are to be accepted as such. Optical artifacts on ultrathin sections such as the 600 A sections used here are well known. Very dense filaments, for example a protein chain impregnated with gold ions, could produce the same effect.

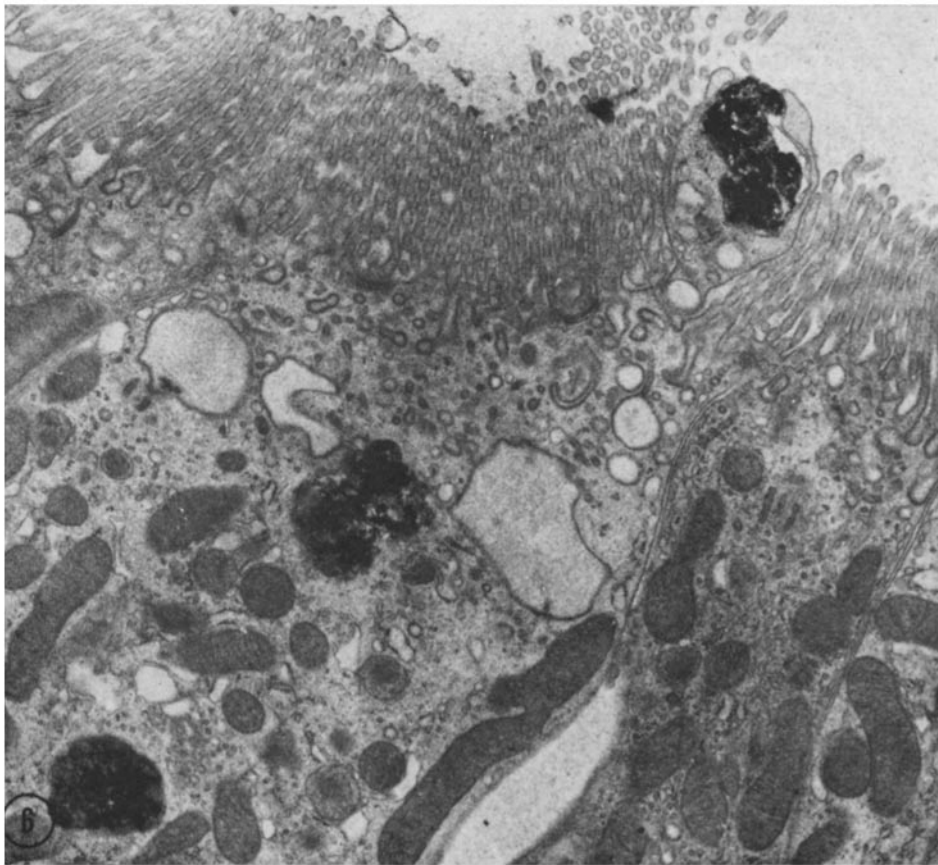
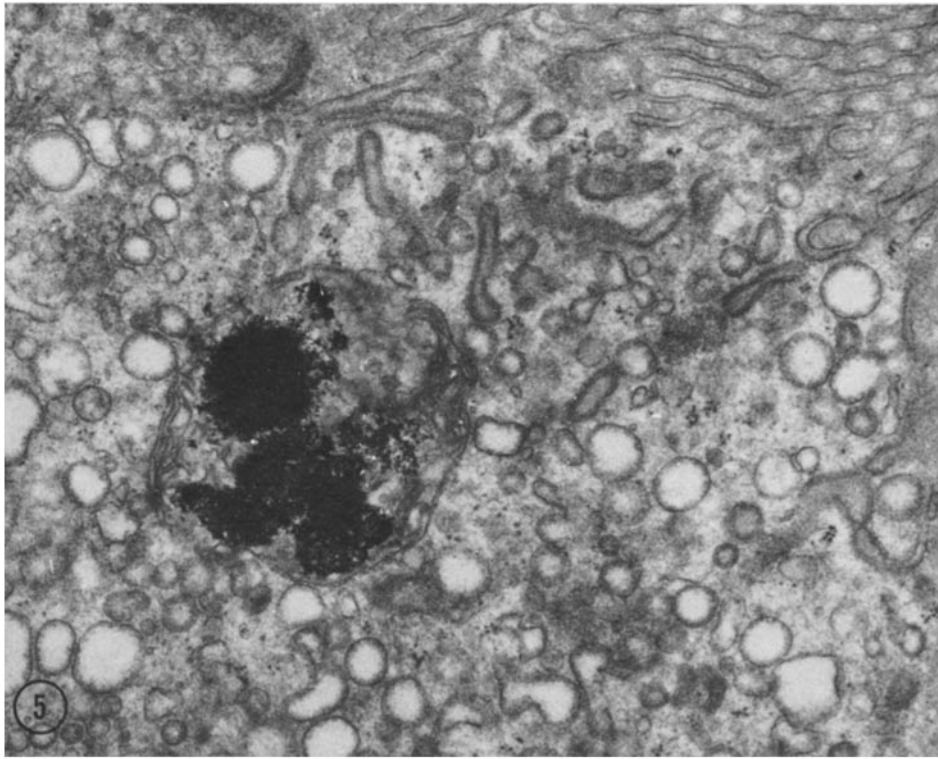
SUMMARY

A water-soluble gold salt, sodium aurothiopropanol sulfonate, was injected subcutaneously into albino rats according to two experimental pro-

FIGURES 5 and 6 In later stages of chronic poisoning, the cell contains a greater number of inclusions and more of them are found in and near the brush border. At this time, there are more gold deposits present in each inclusion although the over-all size of the inclusions is not greatly changed.

FIGURE 5 An inclusion located near the brush border. $\times 34,000$.

FIGURE 6 Three inclusions progressing toward the brush border. One is being extruded from the cell. $\times 17,000$.



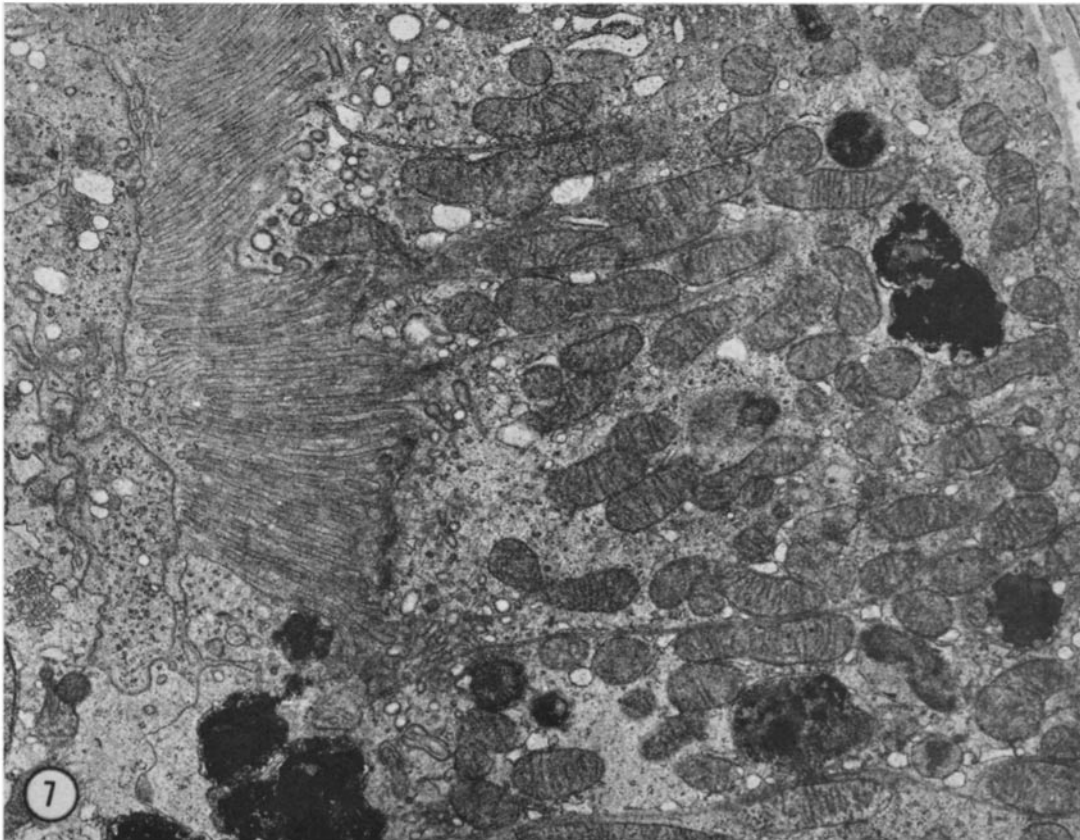


FIGURE 7 Cells in different stages of formation and migration of inclusions. $\times 12,300$.

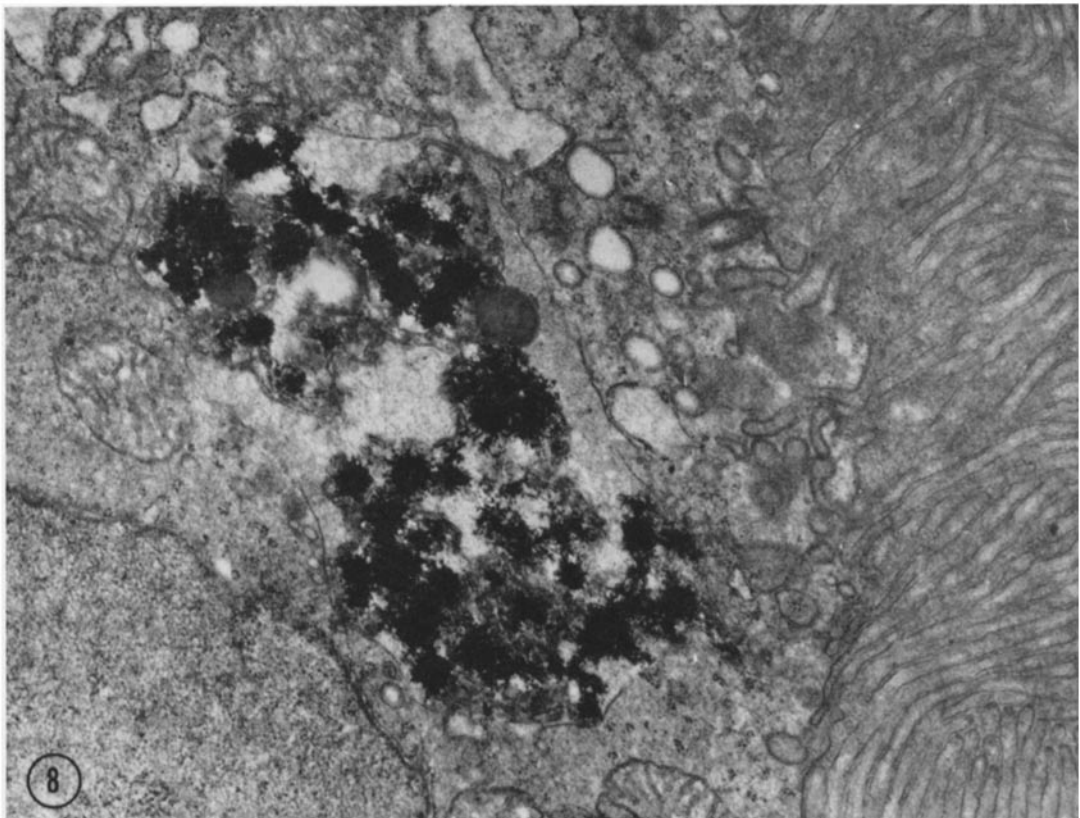


FIGURE 8 A large group of gold deposits located near the brush border. The mitochondrial membranes have almost completely disappeared. $\times 21,000$.

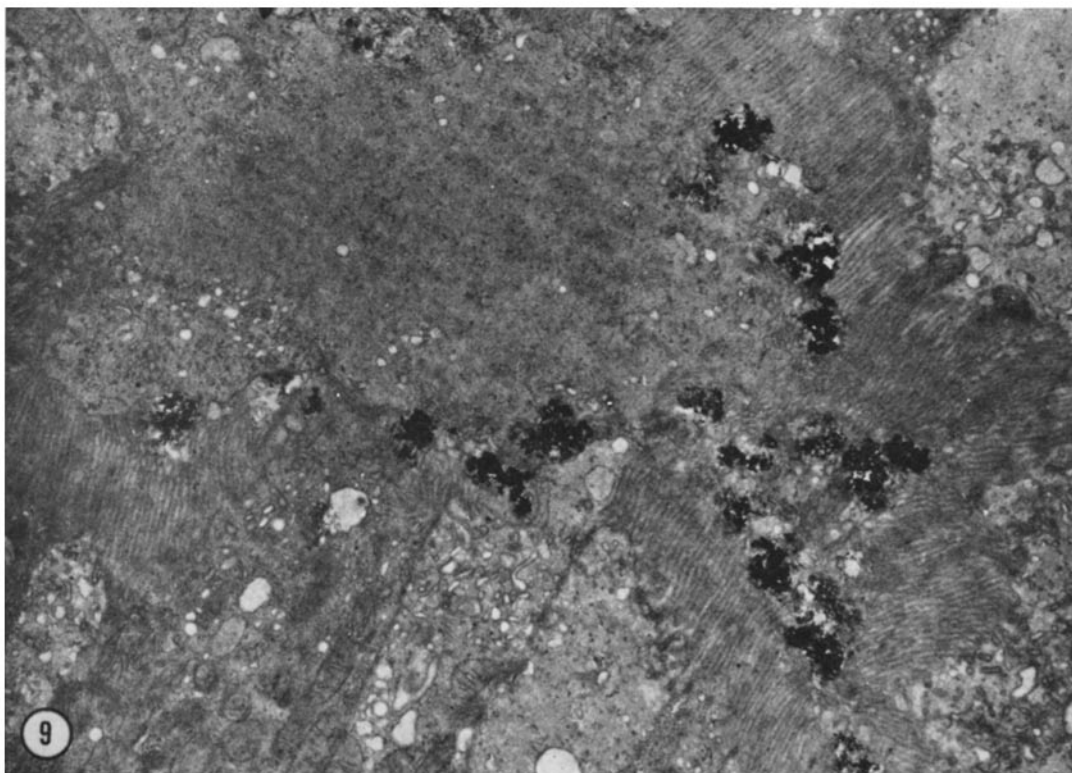


FIGURE 9 Proximal tubule cells extruding their gold deposits into the tubular lumen. $\times 8,400$.

tocols. One group received a single injection of 100 mg while a second received the same total amount in doses of 5 mg administered three times a week. Ultrathin sections of kidney cortex were examined by electron microscopy and electron microprobe analysis. By these means it has been shown that: (a) the primary site of concentration of gold in the kidney is the mitochondrion of the proximal tubule

cell; (b) the number of mitochondria affected is proportional to the amount of gold injected; (c) accumulation of gold results in the destruction of the mitochondrion and its expulsion into the tubular lumen; and (d) tubular structure returns to normal after the cessation of gold injections.

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