# INTERACTIONS OF MERCURY, SELENIUM, TELLURIUM AND ARSENIC

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## INTRODUCTION

The purpose of this paper is to present some of the published data and some unpublished findings on the biological interactions of selenium (Se) with arsenic (As), mercury (Hg) and tellurium (Te).

From the point of view of classical toxicology the experimental data arising from the studies of these interactions appear contradictory and confusing. In particular is the observation that the combined administration of two highly toxic salts, mercuric chloride ($HgCl_2$) and sodium selenite ($Na_2SeO_3$) results in a reduction in toxicity of both chemicals. Not only is that unusual, but at the same time the tissue concentrations of these two elements are not decreased as one might expect, but are greatly increased above the levels achieved by injecting either one alone. Combined with this are the many variables that influence the toxicity of chemicals, namely, (1) nutrition, (2) animal species, (3) route of administration, (4) dose, (5) duration of exposure, (6) sequence of administration, (7) interval between injections, and (8) the chemical compound in which the element occurs.

Not all of these variables will be covered in this brief article, but the data will be presented in categories of the effect of each element on the other element's toxicity, tissue concentration and metabolism.

## ARSENIC

### A. Effect of Arsenic on Selenium Toxicity

Within a few years of the discovery that selenium was the principle toxicant in feed responsible for blind staggers and alkali disease in range animals (1) was the equally important discovery that 5 mg of As as sodium arsenite ($NaAsO_2$) per liter of drinking water (5 ppm As) completely protected rats from selenosis produced by 11 ppm Se in a seleniferous wheat diet over a period of 60 days (2). It was also found that $NaAsO_2$ not only protected rats from seleniferous wheat, but was equally effective in inhibiting the effects of added $Na_2SeO_3$. When the Se content of the wheat was 14 ppm, however, 5 ppm As provided only partial protection. Sodium arsenate had the same protective effect as $NaAsO_2$, whereas $AsS_2$ and $As_2S_3$ were ineffective (3). Subcutaneously injected $NaAsO_2$ also protected from the lethal effects of dietary $Na_2SeO_3$ (4). The addition of $NaAsO_2$ to drinking water 20 days after initiating the feeding of seleniferous wheat reversed the toxic effect of Se (3). Excellent reviews of these and similar interactions have been published (5,6). In a one-year study in rats in the authors' laboratory, no gross or microscopic lesions were seen in 30 rats imbibing 10 ppm Se as $Na_2SeO_3$ with an equimolar amount of As as $NaAsO_2$ in their drinking water that were different from control rats on distilled water.

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However, there are some experiments which show that arsenic does not always protect from selenosis. Levander and Argrett (7) found that rats ingesting 10 ppm Se with 10 ppm As had mild liver damage after 7 weeks. Ganther and Baumann (8) found no statistically significant differences in weight gains between groups of rats ingesting 5 ppm Se as $Na_2SeO_3$ or the same amount of Se plus 5 ppm As as $NaAsO_2$. In addition, the arsenite only partially protected the rats from the liver damage, and the degree of protection varied considerably from one experiment to another. Carlson, et al., (9) reported that, whereas, 15 ppm As protected chicks from 10 ppm Se on one type of diet, the As actually increased the toxicity of Se on another type of diet. It would appear that an unidentified dietary factor is necessary for As to prevent Se toxicity. It would also appear that there is an endogenous factor in the rat which limits the total combined dose of As + Se that can be given without toxic effects, since Palmer and Bonhorst (10) reported that any combination of arsenite and selenite in which the combined total injected dose of the elements exceeded 11 mg/kg was lethal to rats. In their experiments 2 mg of As/kg protected against a lethal dose of Se (5 mg Se/kg).

The chemical compound in which the Se exists also effects the interaction. Obermeyer, et al., (11) found that injections of 1 mg As/kg as $NaAsO_2$ increased the toxicity of sub-lethal doses (40 mg/kg) of trimethylselenonium chloride in rats. The toxic effects of the combination were similar to those obtained by the trimethylselenonium chloride alone.

B. Effect of Arsenic on Tissue Selenium Concentration

In a review of the literature Rosenfeld and Beath (6) stated that the incorporation of As into a Se-containing diet did not significantly alter the tissue deposition of selenium. However, Carlson, et al., (9) found a 5-fold increase in Se concentration in the livers of chickens receiving As + Se as opposed to those receiving Se alone on one type of diet and only a 1.5-fold increase in Se concentration on another type of diet. The diet that resulted in the highest concentration of Se in the livers was also the diet that provided better protection from Se toxicity. In an article by Hill (12), the work of Klug, et al., was reviewed. They showed that As in drinking water caused increased retention of Se in livers and kidneys of rats fed a seleniferous corn diet. However, when rats were fed 10 ppm Se in a semi-purified diet and 10 ppm As in drinking water, their livers contained about one-half and their kidneys one-third as much Se as when Se was given alone in a 7-week experiment (7).

Several short-term studies with $^{75}Se$ have consistently shown that $NaAsO_2$ causes a decrease in the concentration of Se in rat livers from 30 minutes to 12 hours after the injections (7, 10, 13, 14). In these experiments $NaAsO_2$ caused an increase in the concentration of Se in blood from 30 minutes to 3 hours after the injections, but at 10 and 12 hours the concentration of Se fell below those levels produced by injecting Se alone. In three of the studies As caused an elevation of Se in kidneys (7, 13, 14), whereas, in one study the As had no effect (10). These effects were also demonstrated with sodium selenate (14), but the differences in concentration were not as great as they were with sodium

selenite. The feeding of $NaAsO_2$ with trimethylselenonium chloride, $(CH_3)_3$ SeCl, had very little effect on the concentration of Se in rat livers (11).

C. Effect of Arsenic on Metabolism of Selenium

In this section the effect of As on Se excretion in animals and chemical reactivity in vitro will be considered.

Selenium compounds in rats are excreted primarily in exhaled breath, urine and feces. The volatile product has been identified as dimethyl selenide (15). Several authors (7, 13, 16, 17) have reported that the injection of $NaAsO_2$ reduces the amount of Se exhaled. However, the percentage of the dose exhaled within 10 hours after the injection of $Na_2SeO_3$ alone varied by more than 600% from one experiment to another, depending upon the age of and the diet given to the animals. Ganther and Baumann (13) found that weanling rats ingesting a semi-purified diet exhaled only 6% of the injected Se in 24 hours and that the $NaAsO_2$ injection only reduced this to 5.2%, whereas, 176-gram rats fed a commercial diet exhaled 39% of the dose, and the $NaAsO_2$ reduced this to 11%. It is reasonable to expect that some nutrient was directly or indirectly responsible for the formation of dimethyl selenide, and that this nutrient was present in lower concentrations in the tissues of weanling rats than in adult rats, and that it was also present in lower concentrations in the semi-purified diet than in the commercial diet. This activity of the commercial diet was lost upon ashing, and neither the methionine, protein or selenium content of the diet could explain it (18). The authors also noted that the Se was more toxic to rats on the semi-purified diet; thus it is conceivable that the nutrient responsible for promoting the formation of dimethyl selenide also protected the rats from the lethal effects of $Na_2SeO_3$.

Levander and Baumann (16), on the other hand, found that an organic arsenical, sodium arsanilate, increased the formation of volatile Se compounds in rats. Also, when trimethyl selenonium chloride [$(CH_3)_3$ SeCl] was given to rats, injections of $NaAsO_2$ increased the amount of exhaled Se (11).

Another route of excretion is the gastrointestinal tract, and the major source of Se in this route is the bile. Levander and Baumann (14) showed in a series of dose-response experiments that $NaAsO_2$ increased the excretion of Se in bile from 0.77% of the injected dose when Se was given alone as $Na_2SeO_3$ to 24% when 2 mg of As/kg were given with it. When Se was given as sodium selenate, a similar response was observed. This excretion occurred within one hour after subcutaneous administration of the chemicals. Since no difference in bile flow could be detected, the increase in Se excretion was due to an increased concentration of Se in the bile. The intraduodenal injection of dehydrocholate almost completely blocked the As effect, even though the flow of bile was increased.

The third major route of excretion is the urinary tract. Approximately 12-16% of injected Se was excreted in rat urine within 10 hours after injection of $Na_2SeO_3$, and the injection of $NaAsO_2$ had no significant

effect on the quantity of Se eliminated by this route (7, 13, 16). However, As did effect the amount of Se excreted in urine when $(CH_3)_3Se$ Cl was injected (11). Sodium arsenite reduced the Se excretion from 81% to 23% when 0.75 mg Se/kg was given.

Palmer, et al., (19) found that trimethyl selenonium ions were one of the major urinary metabolites of injected $Na_2SeO_4$, and that $NaAsO_2$ had no effect on the total amount of $(CH_3)_3Se+$ excreted by this route (11). Thus it would appear that although $NaAsO_2$ decreased the production of $(CH_3)_2Se$ from $Na_2SeO_3$ in rats, it had very little effect on the generation of $(CH_3)_3Se+$ from $Na_2SeO_4$.

Ganther in a series of publications (20, 21, 22, 23) has presented evidence for the chemical pathways for the generation of dimethyl selenide from $Na_2SeO_3$ in liver and kidney homogenates. In that system selenite is first reduced by and combines with glutathione to form a selenotrisulfide derivative of glutathione (GSSeSG). This chemical was reduced by glutathione reductase to glutathione persulfide (GSSeH). It was then postulated that this compound proceeded to react in vitro with S-adenosyl -L-methionine to form $(CH_3)_2Se$ or was reduced to $H_2Se$, which was then methylated. He found that $1x10^{-6}$ M $NaAsO_2$ produced a 50% inhibition of the $(CH_3)_2$ Se production. In addition, a slight excess of the substrate, $Na_2SeO_3$, was also inhibitory. A concentration of $4\text{-}6x10^{-5}$ M $Na_2SeO_3$ was necessary for maximum evolution of $(CH_3)_2$ Se, whereas, $10x10^{-5}$ M $Na_2SeO_3$ produced an approximately 40% inhibition. Although the inhibition of dimethyl selenide synthesis was well demonstrated by excess $SeO_3^=$ or $AsO_2^-$, there was no evidence presented to show what the other products of the reaction were under those circumstances. Since in <u>in vivo</u> systems the synthesis of $(CH_3)_3Se^+$ is not inhibited by $AsO_2^-$, it can be concluded that $AsO_2^-$ does not inhibit the methylation of Se, but just the formation of dimethyl selenide.

Another effect of $AsO_2^-$ on Se includes the ability of $AsO_2^-$ to inhibit the swelling of Se-deficient mitochondria caused by selenite and glutathione (24). Although the following is not an effect of As on Se, it should be noted that Little and O'Brien (25) found that $NaAsO_2$ did <u>not</u> inhibit a Se-containing enzyme, glutathione peroxidase.

D. <u>Effect of Selenium on Arsenic Toxicity</u>

The authors were able to find only one paper that was specifically designed to test the effect of Se on the toxicity of an arsenical. Holmberg and Ferm (26) showed that the simultaneous administration of 2 mg Se/kg as $Na_2SeO_3$ with sodium arsenate reduced the incidence of malformed and resorbed fetuses produced by injecting 20 mg As/kg into pregnant hamsters.

Perhaps the reason for the lack of experiments on the effect on arsenicals is the relatively low toxicity of arsenicals in chronic animal experiments. In a two-year feeding study Byron, et al., (27) found no toxic effect of 62.5 mg As/kg diet as $NaAsO_2$ or 125 mg As/kg diet as $Na_2HAsO_4$ in rats. Considering the numerous epidemiological studies and human case reports that have implicated inorganic arsenicals as being

much more toxic in humans, it would be advisable to devise animal models to duplicate these toxic effects and then evaluate the effect of Se on As toxicity.

E. Effect of Selenium on Arsenic Concentrations in Tissue and Metabolism

Only two articles were uncovered that demonstrated the effect of Se on As concentration and metabolism. In an article by Hill (12) the work of Klug, et al., was reviewed. They showed that a seleniferous corn diet increased the concentration of As in livers and kidneys of rats given 5 ppm As in their drinking water. The increase in the livers was approximately 19 times and in the kidneys 2.8 times that in the rats receiving the non-seleniferous diet. Levander and Baumann (16) showed that the injection of $Na_2SeO_3$ increased the concentration of As in gastrointestinal contents and kidneys, but had little effect on the As levels in urine, blood and livers of rats injected with 3 mg As/kg as $NaAsO_2$. They were unable to detect any volatile arsenic compounds in their experiments.

F. Effect of Selenium on the Toxicity of Mercury

An experiment that revealed the biological interaction of Hg and Se was reported as early as 1941 (28). Not until 1967, however, was it proven that Se could prevent the lethal effects of Hg. Parizek and Ostadalova (29) reported that injections of 2.4 mg Se/kg as $Na_2SeO_3$ into rats provided almost complete protection from the $LD_{100}$ of $HgCl_2$ (4 mg Hg/kg), when the selenite was injected one hour after the $HgCl_2$. Shortly thereafter, the same authors reported that reversing the sequence of administration of these two chemicals increased their toxicities (30). When dimethyl-selenide, a metabolite of $Na_2SeO_3$, was injected with the $HgCl_2$, there was also an enhancement of toxicity (30, 31).

Gunn, et al., (32) found that the injection of 0.072 mmoles Se/kg (3.6 mg Se/kg) as $Na_2SeO_3$ did not protect mice from an injection of 0.090 mmoles Hg/kg (18 mg Hg/kg) as $HgCl_2$. There are three reasons for that. The dose of Hg was excessive, i.e., 18 mg Hg/kg is approximately 7 times the $LD_{50}$ for Hg in rats and probably also greatly exceeds the $LD_{50}$ in mice. The difference between the dose of Hg (0.090 mmoles/kg) and the dose of Se (0.072 mmoles/kg) is 0.018 mmoles, or an excess of 3.6 mg Hg/kg, which is greater than the $LD_{50}$ for Hg in rats. In addition, there are probably endogenous factors in the animals which are required for Se to inhibit Hg, just as in the case of the Se - As interaction, and when these endogenous factors are overwhelmed by excessive doses of Hg and Se, the mutual inhibition will not occur.

In chronic experiments Groth, et al., (33) showed that 15 ppm Se as $Na_2SeO_4$ in drinking water decreased the proteinuria, prevented the lowered serum albumin/globulin ratio and elevated diastolic blood pressure produced by 50 ppm Hg as $HgCl_2$ in drinking water. In addition, Se decreased the incidence and severity of chronic nephritis produced by the Hg (34).

In 1972, Ganther, et al., (35) reported that as little as 0.5 ppm Se in a purified diet protected rats from the lethal effects of 10 ppm Hg as methylmercury in drinking water. Very little protection was provided from 25 ppm Hg in the same 6-week experiment. Iwata, et al., (36) found that

daily subcutaneous injections of 0.17 mg Se/kg as $Na_2SeO_3$ partially protected rats from the neurological effects produced by the daily oral intubation of 5.9 mg Hg/kg as methylmercuric iodide. The molar ratio of Hg/Se was 14/1. Johnson and Pond (37) showed that 3 ppm Se as $Na_2SeO_3$ in diets partially reversed the lack of weight gain produced by 320 ppm Hg as $HgCl_2$ or 25 ppm Hg as methylmercury dicyandiamide.

In general, it can be concluded that dietary Se protects from the toxic effects of dietary Hg, either inorganic or organic, and that in some cases very little Se is needed to provide this protection. However, careful dose-response studies are needed to clarify this relationship. The inhibition of the toxicity of $HgCl_2$ when selenite is given after the Hg, and the increased toxicity when the sequence of administration is reversed, is a phenomenon that should be extensively investigated.

G. Effect of Selenium on the Tissue Concentration of Mercury

Several authors have shown that Se increases the tissue retention of Hg. In 1969 Eybl, et al., (38) using $^{203}HgCl_2$, examined the retention of Hg in several tissues of mice. Simultaneous, subcutaneous injections of equimolar amounts of $Na_2SeO_3$ with $HgCl_2$ produced concentrations of Hg in livers, kidneys, spleens, lungs, brains, femurs, testicles and blood, that were 55, 11, 41, 13, 3.5, 4, 4 and 5 times respectively greater than those obtained by injecting $HgCl_2$ alone, 28 days after the administrations. They also found that the concentrations of Hg in most of the tissues decreased between one and 28 days after the injections, either with or without the Se. Moffitt and Clary (39), however, in a similar experiment found that the concentrations of Hg in livers, kidneys and spleens increased from 1 to 28 days post-injections when $Na_2SeO_3$ was given with the $HgCl_2$, whereas, when $HgCl_2$ was given along the Hg concentrations in these same organs decreased. In the latter experiment the molar ratio of Hg/Se was 1/2.5 and the salts were given intraperitoneally into rats, whereas, in the former experiment equimolar doses were used and the salts were injected subcutaneously into mice. In both experiments the initially high blood levels of Hg induced by Se fell rapidly. Parizek, et al., (30, 40), have also shown that subcutaneous injections of $Na_2SeO_3$ increase the Hg levels in blood and testes, and decrease them in kidneys and intestines. In a 20-month experiment in which 50 ppm Hg as $HgCl_2$ in drinking water was given to rats, $Na_2SeO_4$ increased the concentration of Hg in the livers 125 times (to 301 ppm) and in the kidneys 5 times (to 500 ppm) above those produced by $HgCl_2$ alone (34). The molar ratio of Hg/Se in the drinking water was 1.3/1. Johnson and Pond (36) found that as little as 3 ppm Se as $Na_2SeO_4$ in the diet increased the concentration of Hg in the livers of rats when the Hg was fed as $HgCl_2$, phenylmercury or methylmercury dicyandiamide. However, Iwata, et al., (36) showed that subcutaneous injections of $Na_2SeO_3$ (0.5 mg/kg/day) decreased the tissue concentrations of Hg given orally as methylmercuric iodide (10 mg/kg/day). It is possible that these differences in results could be explained on the basis of the different compounds of Hg that were used and/or the routes of administration.

H. Effect of Selenium on the Excretion of Mercury

Very little information is available on the effect of selenium on the excretion of Hg. Moffitt and Clary (39) found that the accumulative excretion of $^{203}Hg$ in urine and feces of rats was decreased from 65% to 12% of the injected dose 28 days after administration when $Na_2SeO_3$ was injected with the $HgCl_2$. Parizek, et al., (30) also showed that Se decreased the excretion of Hg in urine from 22 µg to 1 µg within 20 hours after the injections in rats. Selenite was also reported to decrease the transmission of Hg from mothers to fetuses and to reduce the excretion of Hg in the milk of lactating females (41).

I. Effect of Mercury on the Toxicity of Selenium

Fewer studies have been performed on the effect of Hg than the effect of As on Se toxicity.

In a 20-month experiment Groth, et al., (33, 34) reported that although 10 ppm Se as $Na_2SeO_4$ in drinking water was lethal to rats, 15 ppm Se plus 50 ppm Hg as $HgCl_2$ did not increase mortality and there were no histopathological alterations in hepatic cells. Hill (42) in several experiments on chicks showed that the incorporation of equimolar amounts of Hg as $HgCl_2$ with 20 ppm Se as $Na_2SeO_3$ in the diets either completely or partially prevented the lack of weight gain produced by 20 ppm Se alone. In a 7-week experiment on rats Levander and Argrett (7) showed that 10 ppm Hg as $HgCl_2$ only partially reversed the lack of weight gain and did not prevent the liver damage produced by 10 ppm Se as $Na_2SeO_3$. The molar ratio of Se/Hg was 2.5/1.

In contrast to the protective effect that equimolar amounts of $HgCl_2$ have on $Na_2SeO_3$ toxicity are the observations made by Parizek, et al. (30, 31). They found that injections of 0.2 mg Hg/kg as $HgCl_2$ increased the toxicity of 0.04 mg Se/kg as $(CH_3)_2$ Se. It should be noted that although the $LD_{50}$ of injected $(CH_3)_2$ Se has been reported to be over 1 gm/kg (43), that Parizek, et al., (30, 31) found no clear dose-response relationship when they injected several different doses into rats. The doses of Se as $(CH_3)_2$ Se were 0.4, 1.58, 15.8 and 158 mg/kg. The mortality varied between 40 and 80% and was not dependent upon the dose. However, when 0.2 mg Hg/kg was injected with the $(CH_3)_2$ Se there was a clear-cut dose-response relationship, 0.04 mg Se/kg producing a 30%, 0.08 mg Se/kg producing 60% and 0.4 mg Se/kg producing 100% mortality within 24 hours. This combination of chemicals clearly represents one of the most toxic substances known. The effect of Hg on the toxicity of other methylated inorganics, e.g., tetramethyl lead and dimethyl sulfoxide (DMSO) should certainly be investigated as soon as possible.

J. Effect of Mercury on the Tissue Concentration and Excretion of Selenium

Levander and Argrett (7) found that 12 mg Hg/kg increased the retention of Se in kidneys, spleens, livers and blood and decreased elimination in exhaled breath, urine and feces 12 hours after the subcutaneous injections of $HgCl_2$ and $Na_2SeO_3$ in rats. In a 7-week study by the same authors, 10 ppm Hg as $HgCl_2$ in drinking water decreased the concentration

of Se in kidneys and had little effect on liver Se levels when 10 ppm Se as $Na_2SeO_3$ was fed in the diet. In contrast to that, Johnson and Pond (37) showed that 320 ppm Hg as $HgCl_2$ increased the concentration of Se in rat kidneys 2.5 times the level achieved by feeding 3 ppm as $Na_2SeO_3$ alone in the diet at the end of 4 weeks. There was little effect of Hg on the concentration of Se in livers, brains and muscles. In this study the molar ratio of Se/Hg was 1/43, whereas, in the former experiment it was 2.5/1. Parizek, et al., (41) discovered that injected $HgCl_2$ increased the retention of Se in whole pregnant rats 3 times, in blood 5 times, and in livers 3 times that produced by $Na_2SeO_3$ injections alone, 20 hours post-administration. The $HgCl_2$ also decreased the transmission of Se into fetuses and into the milk of lactating rats. In another study, Parizek, et al., (42) showed that injections of 8 mg Hg/kg as $HgCl_2$ decreased the exhalation of Se from 1% to 0.08% of the injected dose of Se 5 hours after the administration of 0.04 mg Se/kg as $Na_2SeO_3$. Mercuric chloride was also reported to decrease the exhalation of Se when selenomethionine was given.

## K. Biochemical Interactions of Mercury and Selenium

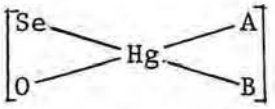
Other than the effects that each element has on the other's excretion, little is known about their mutual metabolism. Although it is not definitely known whether the altered toxicities, excretion and tissue retention can be explained solely on the basis of a direct reaction between Hg and Se, there are several experiments that suggest this possibility.

As observed by Hill (43), an equimolar or greater amount of $HgCl_2$ was necessary to inhibit the toxicity of $Na_2SeO_3$ in chicks. Levander, et al., (24) found that equimolar amounts of $HgCl_2$ were necessary to inhibit swelling of mitochondria induced by $Na_2SeO_3$. Burk, et al., (44) determined the relative concentrations of $^{203}Hg$ and $^{75}Se$ in plasma proteins by column chromatography. When $HgCl_2$ and $Na_2SeO_4$ were injected separately into rats, the Hg and Se concentrated in two different, but closely spaced peaks. However, when they were injected together, they appeared in the same peak, widely separated from the aforementioned peaks, and the molar ratio of Hg/Se in the peak was 1/1. This ratio was 1/1 even when the molar ratio of the injected Hg/Se was 2/1. Dialysis of the pooled peak against mercaptoethanol or $HgCl_2$ resulted in loss of 53% and 66%, respectively, of the $^{203}Hg$ from the protein. At the same time only 32% and 11%, respectively, of the $^{75}Se$ was lost. The authors suggested that the Se was attached to a sulfhydryl group and that the Hg was attached to the Se.

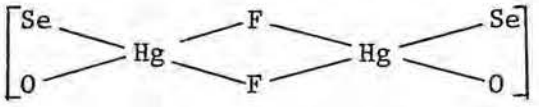
Experiments in the authors' laboratory which have been reported elsewhere (34), have shown that at least in the livers of rats exposed to $HgCl_2$ and $Na_2SeO_4$ in drinking water for 20 months, the Hg and Se were present in approximately equimolar concentrations. Microscopic examinations of the tissues revealed black particles in phagocytic cells in every organ examined including livers (Fig. 1), lymph nodes (Fig. 2), spleens (Fig. 3), kidneys and lungs.

These particles were analyzed semiquantitatively with the electron microprobe, and contained equimolar amounts of Hg and Se. Particles as small as 50 angstroms in diameter were found in the lymph nodes and lungs. These particles were randomly aggregated within cellular phagosomes.

Another finding was intranuclear inclusion bodies in the renal proximal tubular cells (Fig. 4). These inclusions measured about 2.5 microns in diameter and were visible on unstained and stained sections. Electron microscopic examination showed these to contain several smaller bodies about 800 angstroms in diameter, each of which was composed of structured, dendritic arrays of particles measuring about 50 angstroms in diameter (Fig. 5). Quantitative analysis of 25 of these intranuclear inclusions, utilizing the wave-length dispersive system on the electron microprobe showed that the mean molar ratio of Hg/Se was 1.008 with a standard deviation (S.D.) of ± 0.056 and a range of 0.913-1.117. The mean molar ratio of S/Hg was 0.213 with a S.D. ± 0.055 and a range of 0.134-0.331, resulting in a molar ratio of Hg/Se/S of 5/5/1. The mass concentrations of Hg and Se in these inclusions were approximately 15-30% and 6-11%, respectively. Excluding the elements from the tissue staining media (Os, U) and that from the transmission grid (Cu), as well as those for which this detection system is insensitive (H, Li, Be, B, C, N, O and F), no elements other than Hg, Se and S were present in these inclusions. However, since the sum of the concentrations of Hg, Se and S accounted for only 24 to 48% of the mass, it is unlikely that the particles are totally inorganic. Also, since the molar concentration of S is one-fifth that of Hg and of Se, it is unlikely that the Hg and/or Se are bound to sulfhydryl groups. It is more likely that the Se is present in a selenoprotein bound to a carbon atom, and serves as one of the ligands in complexing divalent Hg as represented graphically as follows:




or




where Se O represents the selenoprotein and A B is another organic compound.

The only other indirect evidence that supports this hypothesis is the work performed by Schneider and Flohe (46). They found that $HgCl_2$ only weakly inhibited glutathione peroxidase in vitro, whereas, the $HgCl_2$-EDTA complex was strongly inhibitory. It can be reasonably postulated that the EDTA maintained Hg in the divalent state and that the ligands on the selenoprotein, glutathione peroxidase, substituted for one or more of the EDTA ligands to form a more stable chemical complex with $Hg^{++}$. However, the $HgCl_2$ added by itself, without an organic chelator to stabilize it, was probably reduced to monovalent Hg which reacted to form a different and less stable compound. It can be inferred that the in vivo complex of Hg and Se in rats is very stable, since the intranuclear inclusions and other particles were still visible in the tissues 8 months after Hg and Se were discontinued (34). It is possible that this chemical complex of Hg is responsible for the reported biological half-time of Hg of one year or more in fish.

The evidence strongly suggests that $HgCl_2$ and $Na_2SeO_3$ act to inhibit each other's toxicities by forming stable molecules that in chronic experiments appear as dense particles in phagosomes of phagocytic cells and intranuclear inclusion bodies in renal proximal tubular cells.

L. Interactions of Tellurium with Selenium, Arsenic and Mercury

Groth, et al., (34) studied the gross and light microscopic tissue changes in rats exposed to varying concentrations and combinations of Hg, Se, As and Te in drinking water. They found that the addition of $Na_2TeO_4$ to $HgCl_2$ and $Na_2SeO_4$ prevented the formation of intranuclear inclusions and other particles. The mechanism for this interaction is unknown, but apparently all three inorganics inhibited each other since there were no toxic effects from either the $HgCl_2$ or $Na_2SeO_4$, and there were no particles in the tissues.

Extension of these studies in the authors' laboratory have shown that when $Na_2TeO_4$ was given alone (15-30 ppm Te in drinking water), no gray discolorations or particles appeared in the tissues. However, when equimolar $Na_2SeO_4$ was given with it, the cerebral cortices and testicles assumed a dark gray color and there were black areas in the renal papillae. The only particles seen by light microscopy that could explain the color change were in the cells and lumina of the collecting ducts of the renal papillae. Evidently the chemical complexes formed were not large or dense enough to be seen by light microscopy in the other tissues. The combination of $SeO_4^=$ & $TeO_4^=$ in the drinking water also resulted in the complete elimination of chronic nephritis seen in the control rats.

CONCLUSION

In conclusion it can be said that this is the beginning of a new era in the study of trace metals, their essentiality and toxicity. It is apparent that we can no longer speak of an element producing a specific effect, but must refer to specific inorganics, organometallics or metalloproteins as exerting specific effects. Because of the dramatic alterations in toxicities, tissue concentrations and metabolism that can occur when compounds of these elements interact, it is not possible to predict the effects of exposures to complex mixtures of these chemicals in food, water and air at the present time. This is particularly true when analyses of environmental samples are confined to only a few elements or chemicals.

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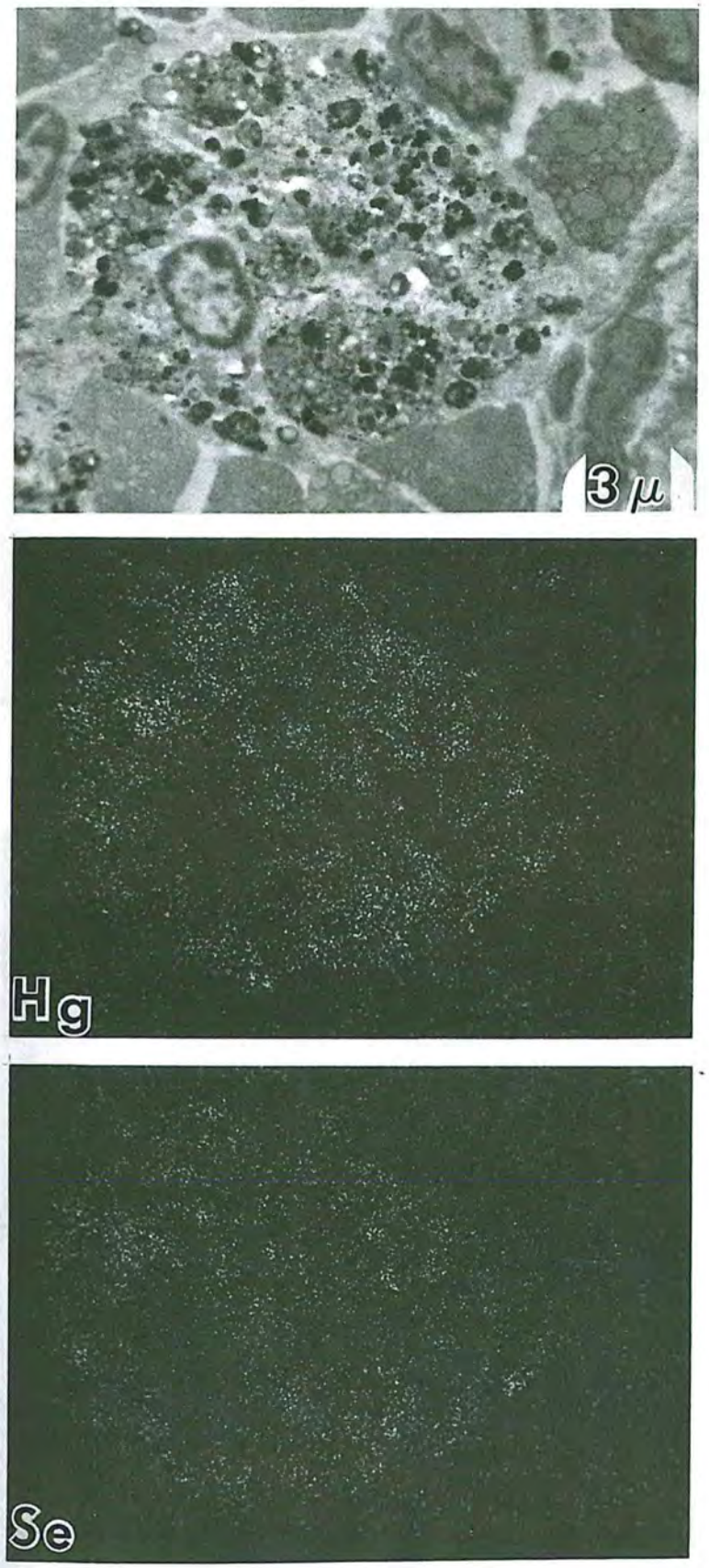


Figure 1. Scanning transmission electron micrograph of a 0.5 μm-thick section of liver from a rat given mercury (Hg) and selenium (Se) along with the corresponding x-ray distribution maps.

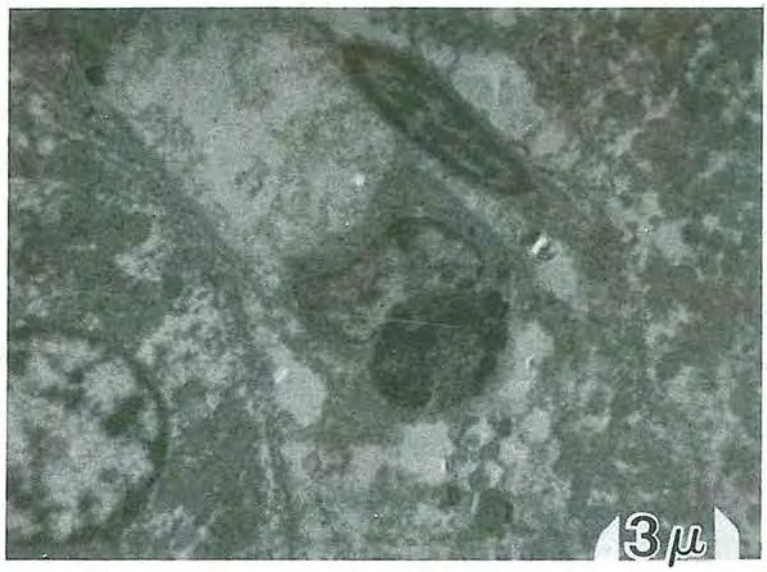




Figure 2. Scanning transmission electron micrograph of a 0.15 μm-thick section of mesenteric lymph node from a rat given mercury (Hg) and selenium (Se) along with the corresponding x-ray maps.

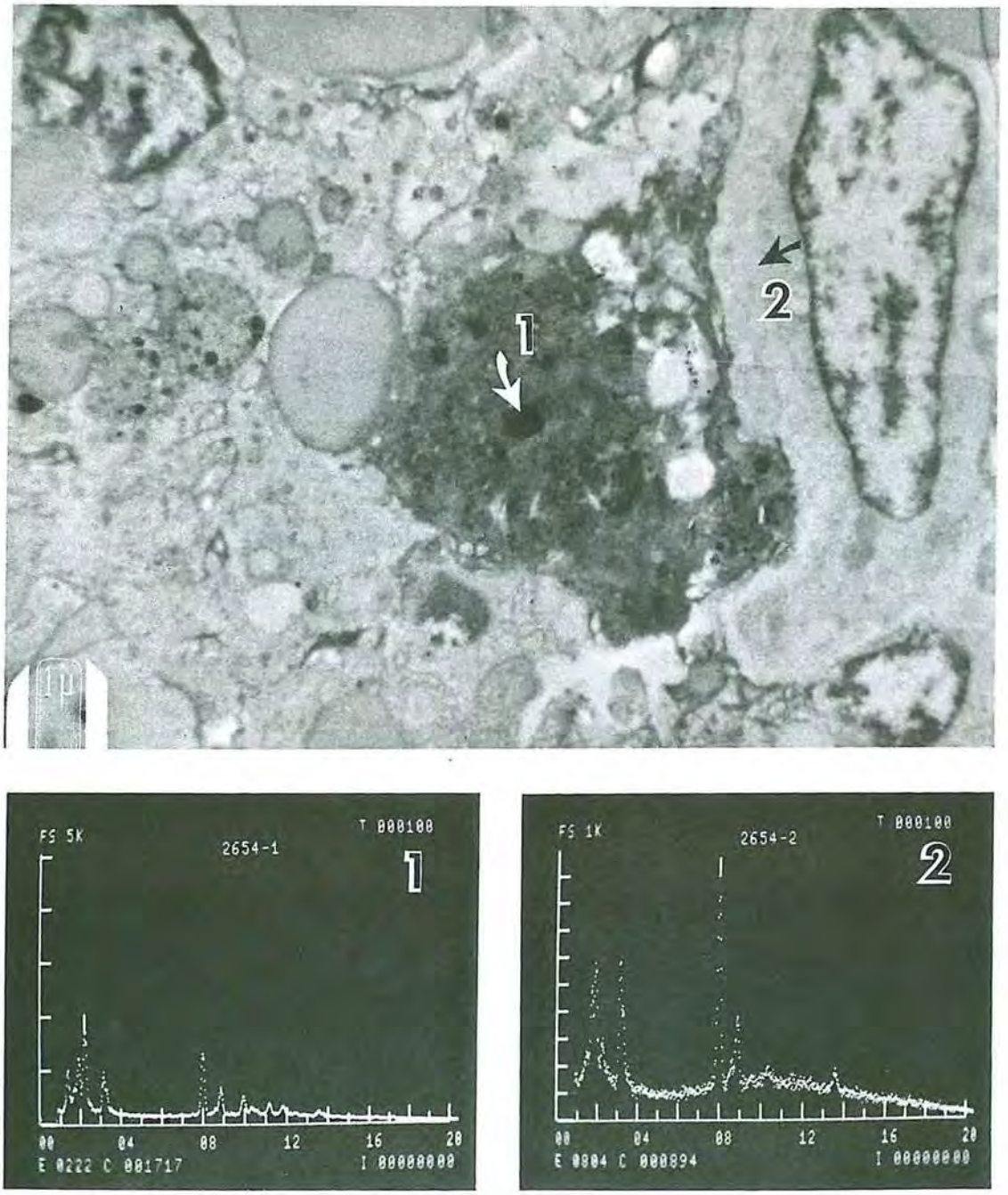


Figure 3. Scanning transmission electron micrograph of a 0.25 μm-thick section of spleen from a rat given mercury and selenium along with the energy dispersive spectra obtained from the two indicated spots. Spectrum 1 contains peaks for mercury (Mα-2.2 Kev, Lα-9.9 Kev, Lβ-11.8 Kev) and for selenium (Lα-1.4 Kev, Kα-11.2 Kev). Sulfur may be present but the sulfur x-ray peaks would be hidden by the mercury Mα peak. The remaining peaks are from the tissue staining chemicals (osmium and uranium) and from the copper transmission grid. Spectrum 2 shows the osmium, uranium, and copper peaks as well as a small sulfur peak (Kα-2.3 Kev).

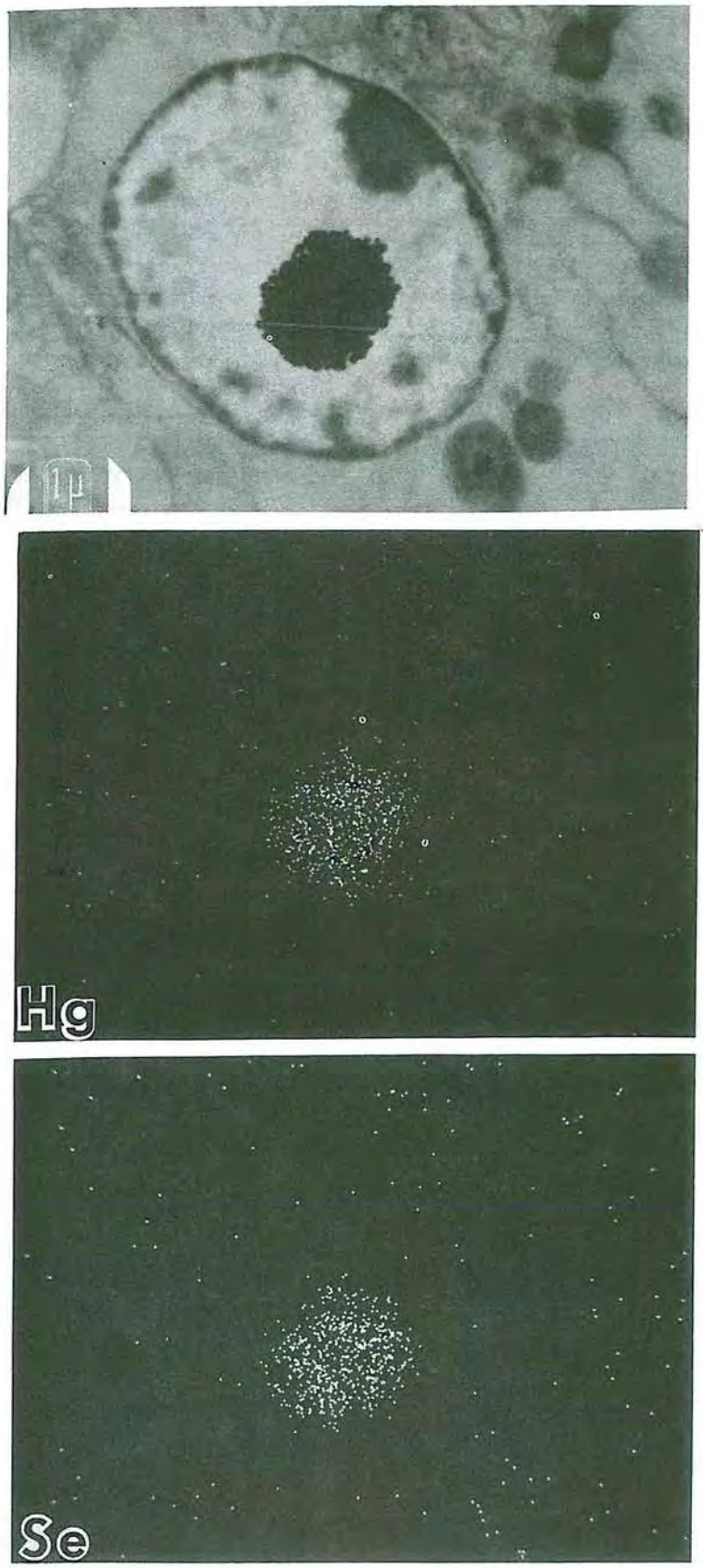


Figure 4. Scanning transmission electron micrograph of a 0.5 μm-thick section of kidney from a rat given mercury (Hg) and selenium (Se) along with the corresponding x-ray distribution maps. The particulate is contained within the nucleus of a proximal convoluted tubule cell.

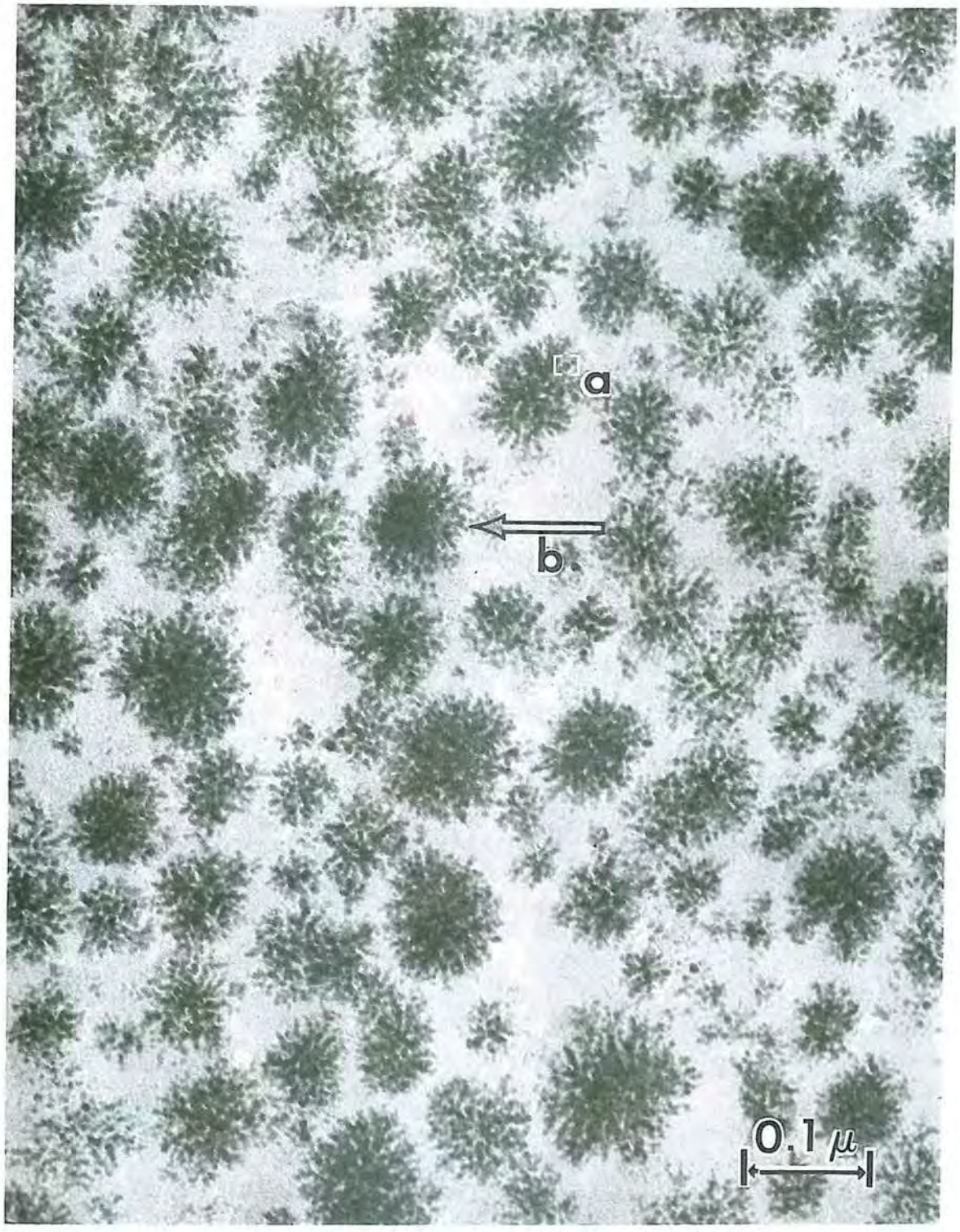


Figure 5. High magnification transmission electron micrograph of a 0.1 μm-thick section of rat kidney from an animal given mercury and selenium. The substructure of an intranuclear inclusion body from a proximal convoluted tubule cell is shown to be dendritic. The primary unit (a) and the secondary unit (b) are shown.

# PROCEEDINGS OF THE SYMPOSIUM

on

# SELENIUM-TELLURIUM IN THE ENVIRONMENT

University of Notre Dame
Notre Dame, Indiana
May 11-13, 1976

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