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# Selective intra-lysosomal concentration of niobium in kidney and bone marrow cells: a microanalytical study

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**Niobium is used as an alloy in the industrial and biomedical fields. The concentration of the toxic element in organs of a number of animal species has been defined by using radioactive niobium ($^{95}$Nb). However, tissue lesions induced by niobium have only been studied at the light microscopy level. In this study, we used an electron probe X-ray analyzer equipped with a transmission electron microscope to define the localization of this element in kidney and bone marrow cells. Results demonstrated that niobium is located in the lysosome and that this element coprecipitates with phosphate. In kidney, lysosomes and precipitates are eliminated in the tubular lumen. In contrast, precipitates appear to be eliminated more slowly from the lysosomes of bone marrow macrophages. These processes therefore correspond to one of the mechanisms by which lysosomes eliminate certain toxic mineral elements and thus play a role in the more general process of the body's defenses.**

**Keywords:** bone marrow, kidney, lysosome, niobium, X-ray microanalysis

## Introduction

Niobium is not a very rare element (Goering & Fowler 1991) that is classified in group Vb on the periodic table of elements. Its physicochemical characteristics lead to numerous industrial applications (Luckey & Venugopal 1977, Haguenoer & Furon 1981, Goering & Fowler 1991) especially in alloys, e.g. in carbon and stainless steels (ferroniobium), as well as in niobium–germanium and nobium–titanium alloys that are super conductive up to 23 $K_a$. In addition, niobium has some biomedical applications, e.g. titanium–aluminium–niobium was developed as a biomaterial for surgical implants (Semlistsch *et al.* 1985). Equally, metal–resin composites have potential use as restorative materials in dentistry (Goering & Fowler 1991). Among niobium salts, pentavalent compounds are the most toxic (Haguenoer & Furon 1981). The intraperitoneal administration of these compounds induces lesions of the renal proximal convoluted tubule cells (Haley *et al.* 1962, Downs *et al.* 1965, Cuddihy 1978, Haguenoer & Furon 1981).

However, these lesions have not been studied at the ultrastructural level and the intracellular localization of niobium has not been determined. Different studies have defined the concentration in organs by using radioactive niobium ($^{95}$Nb) (a by-product of fissionable materials) (Haley *et al.* 1962, Furchner & Drake 1971, Schneidereit *et al.* 1985) The aim of this study, using an X-ray electron probe microanalyzer equipped with a transmission electron microscope, was to investigate alterations induced by the administration of niobium salt at the subcellular level as well as the ultrastructural localization of the salt in intracellular organelles of kidney and bone marrow cells.

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## Materials and methods

The soluble salt niobium pentachloride ($NbCl_5$—Merck) was used. $NbCl_5$ was prepared as an aqueous solution in 0.2 μM sodium citrate buffer at a concentration of 0.3%. The $NbCl_5$ citrate solution was adjusted to pH 6.4–6.9 with NaOH (Downs *et al.* 1965). One group of 12 male Wistar rats, each weighing approximately 200 g, was injected intraperitonally at a dose of 5 mg kg$^{-1}$ day$^{-1}$ of buffered $NbCl_5$ twice weekly for 1 month. Control rats were given an equal volume of a sodium citrate solution in the same pH range. Samples were taken 1 week and 1 month after the start of treatment. The kidneys and bone

marrow were removed and small fragments were prepared for electron microscopy and microanalysis.

Samples were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 1 h. Half the specimens were postfixed in 1% acid osmium in the same buffer. All the fragments were dehydrated in increasing concentrations of alcohol and embedded in epon.

Ultrathin sections (about 100 nm) were cut with an Ultracut E ultramicrotome (Reichert) and collected on titanium or copper grids. Sections for microanalysis were not stained; however, sections for electron microscopy were impregnated with uranium and lead salts.

The ultrastructure of sections was observed using a Philips EM 300 electron microscope. Chemical analysis of the various organelles was performed using a Cameca 'Camebax' electron probe microanalyzer equipped for X-ray spectrometry and specially adapted for examination of ultrathin sections. The electromicroprobe (Camebax) were equipped with the following crystals: TAP (thallium acid phtalate), PET (pentaerthritol), LiF (lithium fluoride).

An electron microscope specially adapted to the apparatus allowed examination of ultrathin sections. A NCS microcomputer was interfaced with the Camebax and permitted automatic counts over a predetermined period. Operating conditions were as follows: electron accelerating voltage, 45 kV; current at the sample, 150 nA; probe diameter, 0.5 $\mu$m.

## Results

*Kidney*

Differences were observed between the samples taken after 1 week (A samples) and 1 month of treatment (B samples).

*A samples: electron microscopy.* The principal alterations were observed in the convoluted proximal tubule cells. Alterations were localized in the intracytoplasmic lysosomes. Many dense deposits were observed in these lysosomes in the form of very dense clusters of granules more or less completely filling the lysosomes. The diameter of small granules was about 30 nm. In these samples, no dense deposits were observed in the tubular lumen or in the other intracytoplasmic organelles.

*B Samples: electron microscopy.* In these samples we observed the same intracytoplasmic lysosomes filled with dense deposits. Similar deposits were also observed in the tubular lumen (Figure 1, panels 1 & 2). Both types of deposits were observed in the same tubule, either within the more or less altered lysosomes or free or grouped in clusters in the lumen (Figure 1, panel 3), which was filled with brush border microvilli. Deposits in the tubular lumen have the same ultrastructural characteristics as those observed in intracytoplasmic lysosomes.

*A sample: microanalysis (Figure 1, inset).* Microanalysis of dense deposits in intracytoplasmic lysosomes demonstrated the $K_\alpha$ line of phosphorus and $L_\alpha$ line of niobium. The number of counts per second was measured on the intralysosomal inclusion (thick arrow). After subtraction of background noise, 24 counts $s^{-1}$ were obtained for the $L_\alpha$ line of niobium. Similar counts were obtained for the other lysosomes studied.

*B samples: microanalysis (Figure 1, inset).* The chemical composition was the same in dense deposits in intracytoplasmic lysosomes, in clusters in the tubular lumen and in free deposits in the lumen. Microanalysis demonstrated the $L_\alpha$ line of niobium and $K_\alpha$ line of phosphorus. The number of counts per second was similar to those of the A sample.

*Bone Marrow samples*

Differences were observed between samples taken after 1 week (A sample) and samples taken after 1 month of treatment (B samples). The changes were more marked in the B samples.

*B samples: electron microscopy.* The alterations were located in bone marrow macrophages (Figure 2, panel 4). These consisted of very small granules about 30–40 nm in diameter that completely filled the lysosome (Figure 2, panel 5). One can also observe other bone marrow cells (erythrocytes and granulocytes).

*B samples: microanalysis (Figure 2, inset).* The $K_\alpha$ line of phosphorus and the $L_\alpha$ line of niobium were detected on dense lysosomal deposits. Counts of 20 and 16 counts $s^{-1}$ were obtained for the $L_\alpha$ line of niobium on the inclusion marked with an arrow (Figure 2, panel 5). Similar counts were obtained for the other inclusions examined.

## Discussion

By a combination of microanalysis and electron microscopy, we were able to clarify the subcellular localization of niobium in renal tissue and bone marrow cells, i.e. two very different types of cell.

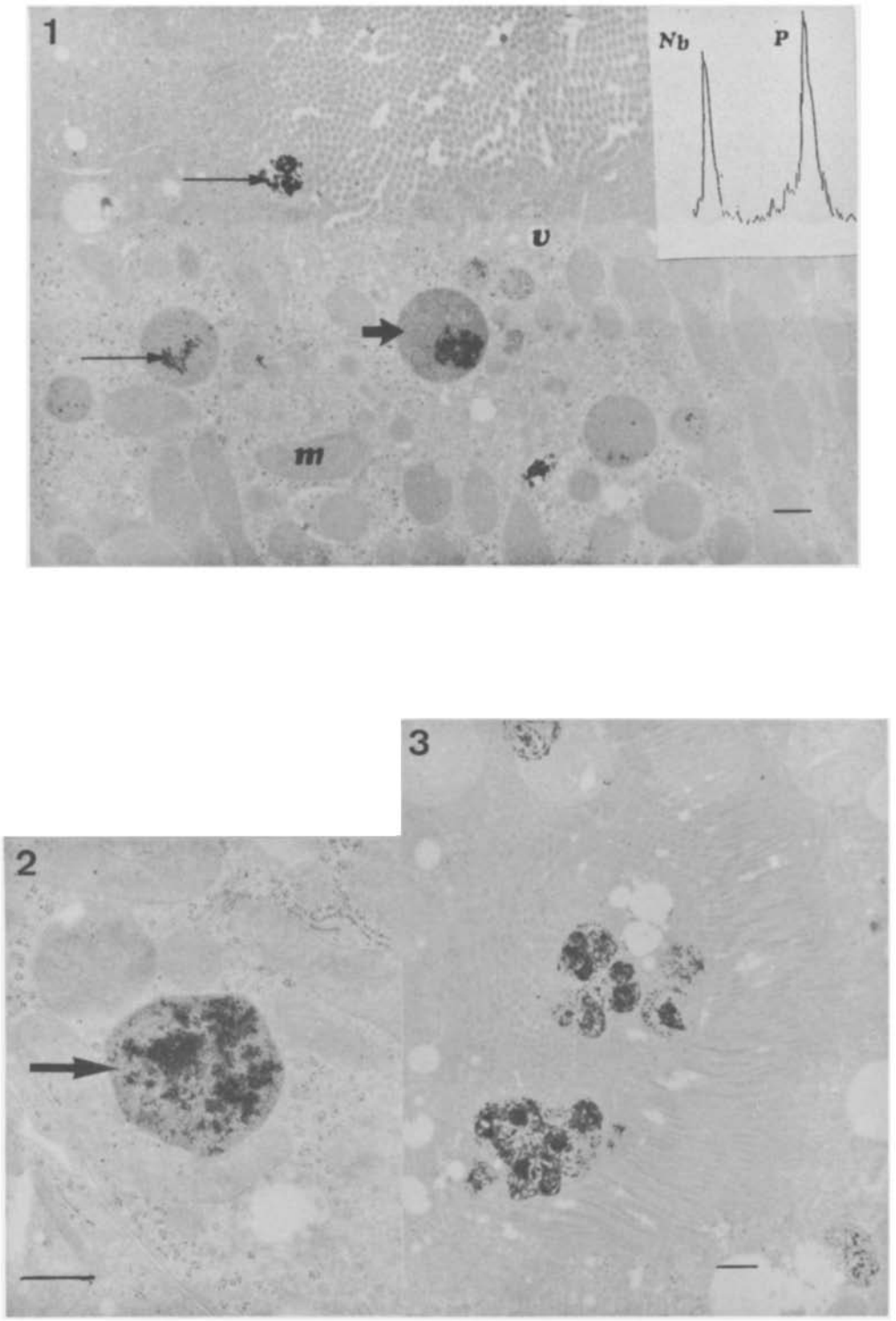
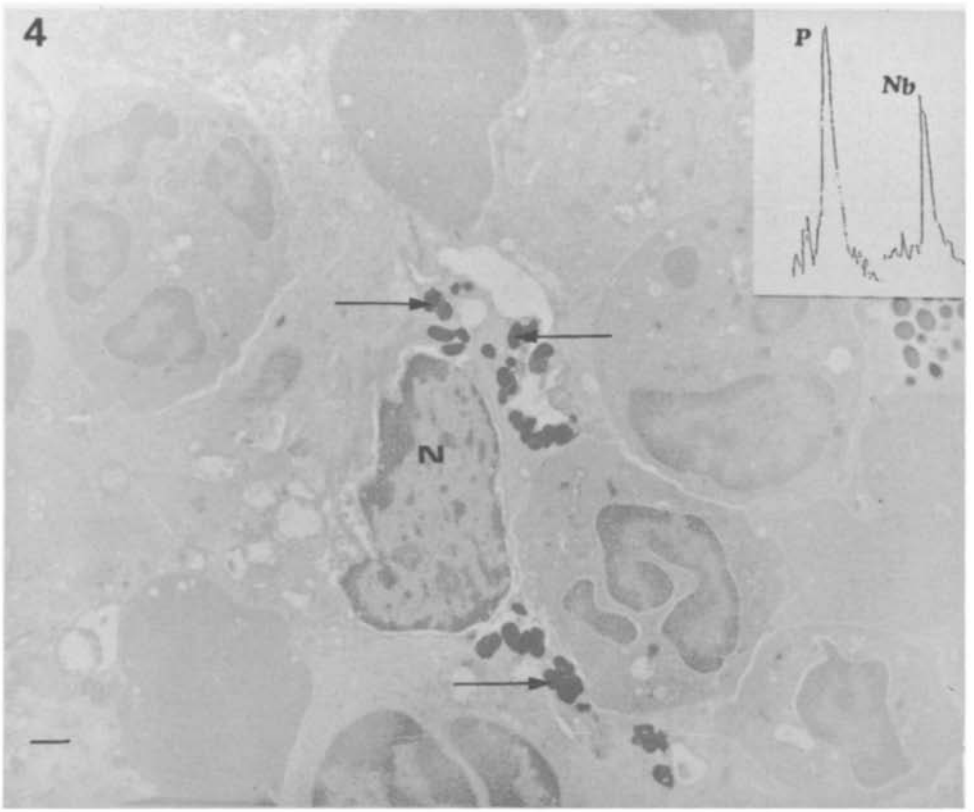



**Figure 1.** Proximal convoluted tubule cell. Sample after 1 month of treatment (B sample). (**1**) Different cells showing some lysosomes containing clusters of dense granules (arrows) which were found to correspond to niobium (Nb) and phosphorus (P) on microanalysis (inset). Lysosomes can be seen in the cytoplasm of the cells and also in the lumen of the tubule, filled with brush border microvilli. (v) Apical vesicles, (m) mitochondria. ×4875. (**2**) Photomicrograph of a lysosome that is nearly filled with clumps of very fine granules containing niobium and phosphorus. × 9375. (**3**) Dense deposits containing niobium and phosphorus can be seen in the tubular lumen which is filled with brush border microvilli. Dense deposits are observed either in the form of free particles or in clusters of more and less altered lysosomes. × 6150.



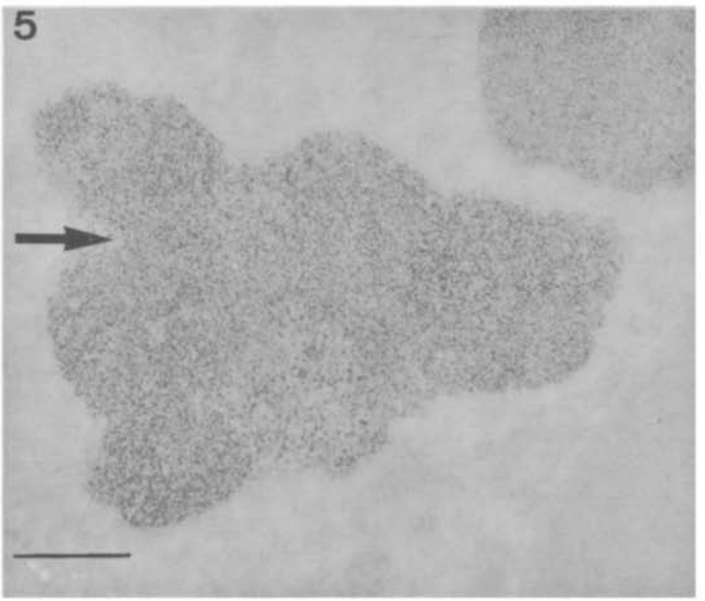




**Figure 2.** Bone marrow cells. (**4**) A macrophage (nucleus = N) can be seen in the center of the figure. Its cytoplasm is flattened against the small blood capillaries. In the cytoplasm there are various lysosomes containing dense deposits in which phosphorus (P) and niobium (Nb) were detected (inset). Other bone marrow cells can also be observed. × 4200. (**5**) High magnification of a lysosome. This organelle is completely filled with clusters of small granules. The wall of the phagolysosome can be clearly distinguished. Niobium and phosphorus were detected on these granules. × 18750.

*Renal tissue cells*

*Comparison with other elements studied.* In renal tissue cells: (i) niobium was detected with phosphorus in granules forming dense intralysosomal clusters; (ii) in the samples taken after 1 week, lysosomes and dense deposits were localized in the cytoplasm of cells of the proximal convoluted tubule; and (iii) in samples taken after 1 month, numerous lysosomes containing dense deposits as well as free deposits were seen in the tubular lumen.

During our previous experiments, the same ultrastructural characteristics described above were observed for numerous elements, i.e. those of group IIIa (aluminum, indium and gallium) (Berry *et al.* 1982a, b, 1984), for some actinides (thorium and uranium) (Galle 1974, Hallegot & Galle 1988), and for some elements such as cerium and chrome (Berry *et al.* 1978, 1988).

We have thus been able to elucidate the mechanism that leads to intralysosomal concentration of these elements and their excretion mechanisms.

*Mechanism of intralysosomal concentration.* This mechanism is linked to the presence of an intralysosomal enzyme that belongs to the hydrolase group, i.e. acid phosphatase. The presence of acid phosphatase can explain the concentration and local precipitation of elements detected with phosphorus (Ericsson & Trump 1965, Galle & Berry 1980, Haguenoer & Furon 1981, Robinson & Karnovsky 1983).

Acid phosphatase enzyme activity can liberate phosphate ions from substrates composed of phosphoric esters (Borel *et al.* 1987). The phosphate ions are then able to precipitate a certain number of mineral elements to form precipitates of insoluble phosphate (Galle & Berry 1980, Galle 1981, Berry *et al.* 1987).

An identical reaction has been devised *in vitro* by Gomori (1952) and developed by cytochemists (Ericsson & Trump 1965, Waters & Butcher 1980, Berry *et al.* 1982a,b, Robinson & Karnovsky 1983) to demonstrate the presence of acid phosphatase in lysosomes. In this reaction, sections of renal tissue are incubated with a mineral salt, generally lead, able to precipitate in the form of insoluble lead phosphate within organelles that contain an enzyme that releases phosphate ions—acid phosphatase. The reaction is as follows:

$$\text{glycerol phosphate} \xrightarrow{\text{acid phosphatase}} \text{glycerol} + \text{phosphate}$$

$$\text{free phosphate} + \text{lead} \rightarrow \text{lead phosphate}$$

forming an insoluble precipitate in the lysosome

The similarity between Gomori's *in vitro* reaction and the phenomenon of precipitation of mineral element in the lysosomes *in vitro* was confirmed in the following fashion. The lead salts were replace by aluminium salts (Galle & Berry 1980, Berry *et al.* 1982a, b) and Gomori's reaction performed in renal tissue sections. A precipitate of aluminum phosphate was observed in the lysosomes of renal cells. These precipitates had the same ultrastructure and chemical composition as those induced *in vivo* by injection of soluble aluminium salts (Galle & Berry 1980). The lysosomes of the cells of the proximal convoluted tubules of the kidney extract this element from the intracellular liquid surrounding them.

*Excretion mechanism.* The lysosome concentrates and precipitates the elements in the form of insoluble phosphate. In the long term, the same precipitates are also present in the lumen of proximal convoluted tubules in the form of fine granules (5–50 nm) or fine needles (about 60 nm long) either free or within lysosomes that have kept their structure (Berry *et al.* 1978, 1988, Galle 1981). These precipitates thus derive from the proximal convoluted tubule after concentration and precipitation of mineral elements.

The following schema can be proposed to explain the excretion mechanism: initially, the mineral elements are filtered in soluble form by the glomeruli. They are then reabsorbed by the tubular cell in ionic form and precipitated as insoluble phosphate thanks to the enzymatic activity of acid phosphatase.

The lysosomes are then released into the urinary lumen where they liberate their contents into the urinary flow. However, other elements are not precipitated in the lysosome according to the Gomori model. Elements like nickel (Berry *et al.* 1985), platinum (Berry *et al.* 1982a, b, Berry 1988), palladium (Berry 1987) and gold (Galle 1974, Rodrigues & Galle 1985) are detected in the presence of sulfur in kidney lysosomes. In this case, a different precipitation mechanism is probably involved. Other intralysosomal enzymes, the arylsulfatases, which hydrolyze sulfuric esters, release sulfate groups that could precipitate elements in the form of insoluble sulfate according to the following schema:

$$ROSO_3 + H_2O \rightarrow ROH + H^+ + SO_4^-$$

$$SO_4^- + RPb \rightarrow SO_4Pb + R$$

The enzymatic activity of the arylsulfatases, like that of acid phosphatase, has been detected *in vitro* in the lysosomes thanks to a cytochemical reaction using lead salts or barium (Hayat 1974).

Sulfur and selenium have very similar physicochemical properties. In some instances, during experiments, the selenium can precipitate certain elements in the lysosomes. For example, we have administered to the same animal a selenium salt followed by an arsenic salt (Berry, personal communication) or, in another experiment, by a platinum salt (Berry 1988). This lead to the formation in the lysosomes of insoluble precipitates of the elements administered (probably in the form of selenide) (Lumbroso 1977). Electron microscopy and microanalysis data show that the excretion mechanism of these intralysosomal precipitates by the renal cell is identical to that described when these elements are precipitated in the presence of phosphate. Lysosomes and their precipitates are eliminated in the urinary lumen.

*Bone marrow cells*

Another subcellular localization site of niobium was found to be in bone marrow macrophages. Niobium was detected in the presence of phosphorus in lysosomes in the form of dense granules. The intralysosomal precipitation mechanism seems identical to that observed in renal cells. The element precipitates in the form of insoluble phosphate in these lysosomes as a result of the activity of acid phosphatase. This mechanism has also been observed for other elements such as aluminum (Watrin & Galle 1986) or gallium (Berry *et al.* 1984) belonging to group IIIa of the periodic table, and for some actinides, e.g. cerium (Berry *et al.* 1988) and thorium (Hallegot & Galle 1988). Some elements such as gold (Rodrigues & Galle 1985) are precipitated with sulfur. In contrast to the excretion process of renal cells, excretion of elements from the bone marrow appears to be very lengthy, blood circulation in this tissue being extremely slow.

In our experiments we have not observed any selenide precipitates induced by administration of selenium or other elements in bone marrow macrophage.

## Conclusion

This study of niobium has allowed us to make an assessment of our experiments concerning certain aspects of lysosomal metabolism, showing the different processes of concentration and elimination of mineral particles, some of which are very toxic. This has confirmed the role of lysosomes in the defense mechanism of organisms.

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