

Figure 7.18a

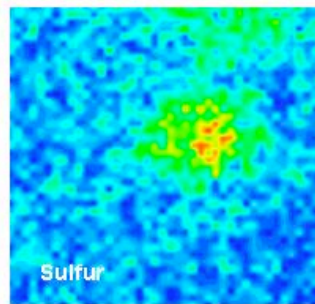


Figure 7.18c

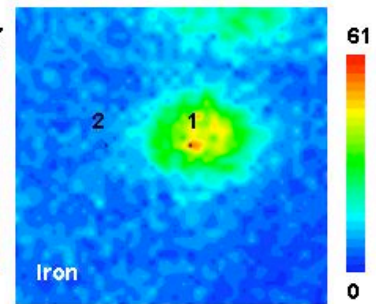


Figure 7.18e

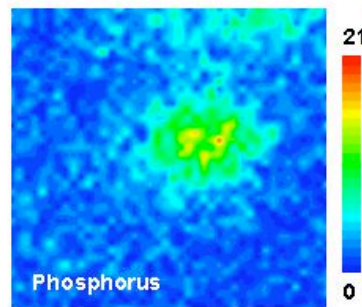


Figure 7.18b

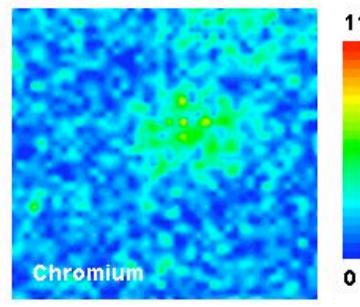


Figure 7.18d

**Figure 7.18a-e.** A SEM photograph of a neuron dried on a PET film is shown in figure 7.18a. The elemental distribution of P, S, Cr and Fe within a single cell that was cultured in a 0.04 g/L Cr solution environment for 0.5 h. are shown in figure 7.18b, c, d and e, respectively. These images are matrices of  $43 \times 43$  pixels of  $1 \mu\text{m}$  resolution. In figure 7.18e, the plotted points named No. 1 and 2 are defined as the center of cell body and junction between axon or dendrites and cell membrane.

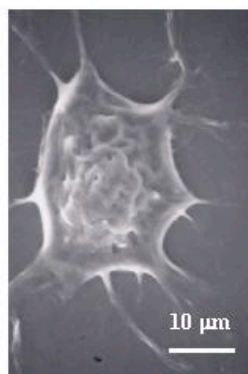


Figure 7.19a

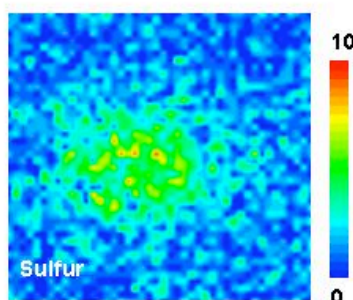


Figure 7.19c

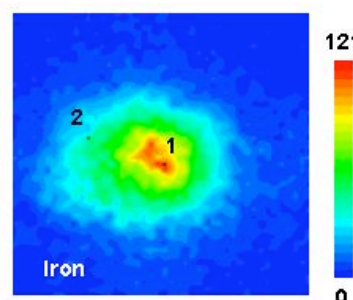


Figure 7.19e

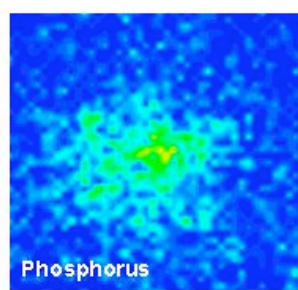


Figure 7.19b

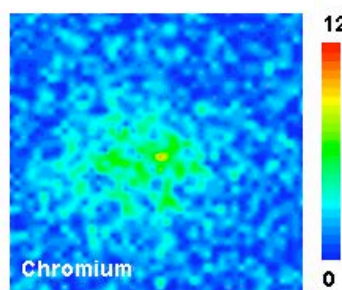


Figure 7.19d

**Figure 7.19a-e.** A SEM photograph of a neuron dried on a PET film is shown in figure 7.19a. The elemental distribution of P, S, Cr and Fe within a single cell that was cultured in a 0.04 g/L Cr solution environment for 4 h. are shown in figure 7.19b, c, d and e, respectively. These images are matrices of  $45 \times 45$  pixels of  $1 \mu\text{m}$  resolution. In figure 7.19e, the plotted points named No. 1 and 2 are defined as the center of cell body and junction between axon or dendrites and cell membrane.

### **Center of cell body**

In the cell body of the control cell, a large amount of Fe was detected and its peak intensity was detected at the center of the cell body. After exposing the neuron to Cr oxide solution for 0.5 h., the density of Fe at the center of the cell body in the neuron decreased a little, as shown in table 7.13. On the other hand, the densities of P, S, Cl and Ca were almost identical to those in the control cell. The densities of P and S increased a little, but they should not be considered to be significant changes because these values of fluorescence intensities were small. After exposing the neuron to a Cr oxide solution environment for 4 h., the density of Fe at the center of the cell body increased. The densities of P and S were almost identical to those in the control cell. On the other hand, the depletion of Ca was observed at this point.

Compared with the increase of the Fe density in the neuron cultured in a V solution environment, the increase of Fe is considered to be remarkable in this case.

**Table 7.13-7.16.** The intensities and relative densities of the elements in the treated cells with Cr oxide solution environment for 0.5 h. are shown in tables 7.13 and 7.14. The results of the treated cells with Cr oxide for 4 h. are shown in tables 7.15 and 7.16.

Center of cell body		
Element	Intensity	Relative
Ar	1	
P	0.100647	1.741677
S	0.103085	1.579532
Cl	0.05455	0.581597
K	0	0
Ca	0.054138	0.80439
V	0	
Cr	0	
Fe	0.4531	0.772545
Cu	0	
Zn	0	

**Table 7.13**

Junction		
Element	Intensity	Relative
Ar	1	
P	0.017523	1.242193
S	0.032155	1.753855
Cl	0.049477	0.655502
K	0.028245	0.654538
Ca	0	0.80439
V	0	
Cr	0	
Fe	0.134177	1.023294
Cu	0	
Zn	0	

**Table 7.14**

Center of cell body		
Element	Intensity	Relative
Ar	1	
P	0.064836	1.121975
S	0.089172	1.366348
Cl	0.060065	0.640391
K	0.030943	0.66468
Ca	0.023493	0.349062
V	0	
Cr	0	
Fe	1.717469	2.928321
Cu	0	
Zn	0	

**Table 7.15**

Junction		
Element	Intensity	Relative
Ar	1	
P	0.037062	2.627299
S	0.042529	2.319692
Cl	0.063234	0.837763
K	0.013518	0.31326
Ca	0.016628	1.072062
V	0	
Cr	0	
Fe	0.710059	5.41523
Cu	0	
Zn	0	

**Table 7.16**

### **Junction of dendrites and cell membrane**

For the 0.5 h. exposure sample, the density of Fe at the junction of dendrites and cell membrane in the neuron was almost identical to that in the control cell, as shown in table 7.14. The densities of P and S did not display any significant changes. The depletion of Ca was observed. For the 4 h. sample, the density of Fe at the junction of dendrites and cell membrane increased remarkably. Furthermore, the densities of P, S and Ca also increased at this point. The relative density of Fe at the junction of dendrites and cell membrane is calculated by using the value of the control cell at the same point. Thus, it is plausible that the high density of Fe detected at the junction point between dendrites and cell membrane may be attributed to the redistribution of Fe.

## **Summary**

When trypan blue dye exclusion test was performed to the macrophage cells cultured in a metallic solution environment, toxicity of chromium oxide ( $\text{CrO}_3$ ) solution to the cell was the strongest of all metallic solutions. After exposing the neuron to a chromium oxide solution environment, the increase of the density of iron within the cell was relatively higher than that within the cell cultured in a vanadium solution. Therefore, it is probable that toxicity of the metal is related to the internalization of iron into the neuron. The internalization of vanadium by neurons was not observed, but that of chromium was observed. It is unclear why neurons did not internalize vanadium.

## **5.2. Result of X-Ray Absorption Fine Structure Analysis**

In order to compare the chemical state of Fe before and after exposing the neurons to a Cr oxide solution, XANES spectrometry was carried out. The spectra shown in figure 7.22 were obtained from the different cells shown in figure 7.20 and 7.21. These spectra were obtained in fluorescence mode. The cells shown in figure 7.20 and 7.21 were cultured in a Cr oxide solution environment (0.04 g/L) for 4 h. and in non-metallic solution (normal condition) respectively. The reference spectrum, which is iron oxide ( $\text{Fe}_2\text{O}_3$ ), is also shown in figure 7.22. If the chemical structure around the element changes, it is reflected in the structures of the x-ray absorption spectrum. The XANES spectra indicated that there was virtually no change of chemical state of Fe within the cells shown in figure 7.20 and 7.21. From the results of the elemental distribution images, it can be observed that the distribution patterns and the densities of many intracellular elements changed significantly. Yet, the chemical structures around Fe atoms were stable before and after internalization into the cell.

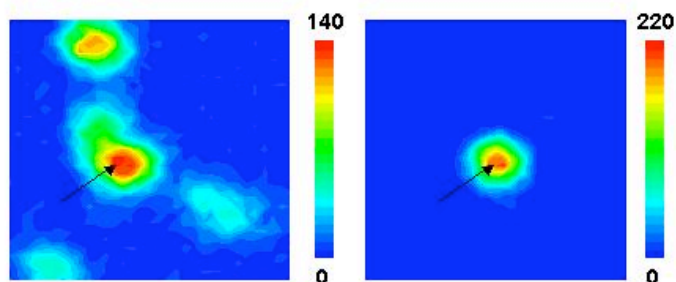


Figure 7.20

Figure 7.21

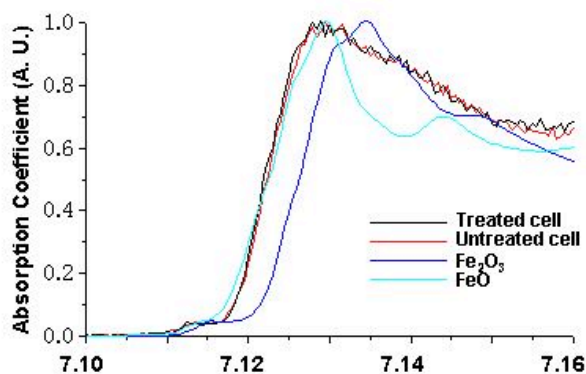


Figure 7.22

**Figure 7.20, 7.21, 7.22.** XANES spectra were obtained from the cells shown in figure 7.20 and 7.21, which were cultured in a 0.04 g/L Cr oxide solution environment and non-metallic solution for 4 h., respectively. XANES spectra of the cells and reference spectra of FeO and Fe<sub>2</sub>O<sub>3</sub> are shown in figure 7.22.

### 5.3. Results of EPMA Imaging

#### 5.3.1. Experimental Set-Up and Sample Preparation

The elemental images of single cells using EPMA-EDX were measured in the Department of Psychiatry in Nagoya University School of Medicine and on the JEOL-2010 electron microscope, equipped with a Norran Voyager energy dispersive x-ray micro-analyzer with a Si(Li) detector.

For EPMA measurement, the cells were immersed in glutaraldehyde and cacodylic acid. The thin section sample having 250 nm thickness was obtained from freeze-dried neurons.

#### 5.3.2. Results

The neurons measured by EPMA were cultured in a V solution (0.04 g/L) environment for 4 h. The TEM photograph of the sample is shown in figure 7.23. The

state of the sample was not good. It is unclear whether the toxic effect of V broke the cell membrane or whether the procedures of the sample preparation affected the membrane state. The elemental distribution patterns of P, S and V are shown in figure 7.24, 7.25 and 7.26, which were prepared similarly to the previous figures. The population of V within the neuron was located along the nuclear membrane. The density of P was also high in the same area. P and S displayed relatively uniform distribution patterns, but V was concentrated along the nuclear membrane of the neuron.

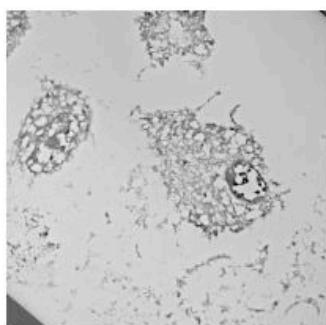


Figure 7.23

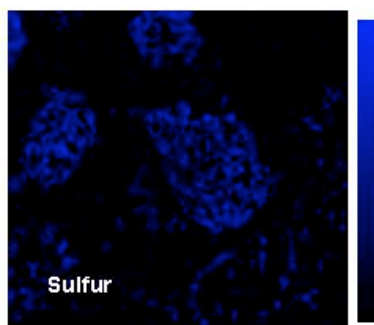


Figure 7.25



Figure 7.24

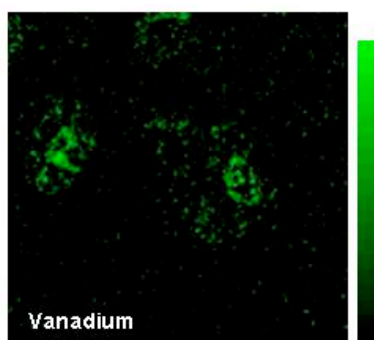


Figure 7.26

**Figure 7.23, 7.24, 7.25, 7.26.** The TEM photograph of the neurons cultured in 0.04 g/L V solution environment for 4 h. is shown in figure 7.23. The elemental distribution patterns of P, S and V measured with EPMA are shown in figures 7.24-7.26, respectively

## 6. Discussion About The Interactions Between Neurons And Foreign Metal Elements

After exposing neurons to vanadium chloride and chromium oxide solution environments, the immediate depletion of calcium was observed. Furthermore, the subsequent internalization of iron had occurred at the center of the cell body and axon

hillock of the neuron. It appeared that chromium oxide may induce a larger amount of the internalization of iron than vanadium.

Iron is essential for the normal neurological function. It is said that iron uptake in most mammalian cells occurs via the transferrin cycle. Iron is normally transported in the plasma in the ferric state by transferrin [15]. Transferrin subsequently binds onto the transferrin receptor on the cell surface, which then undergoes endocytosis, generating endosomal vesicles within the cell. In a normal tissue, iron rarely exists as a free ion but rather is bound to a variety of active proteins including hemoglobin and myoglobin, transport proteins such as transferrin, and storage proteins such as ferritin [16]. Intracellular iron is usually tightly regulated, being bound by ferritin in an insoluble ferrihydrite core. When the intracellular ferritin iron was released, it was observed that it contributed to a free radical-induced cell damage *in vivo* [17]. Iron catalyzes lipid peroxidation and free radical production with results that could be especially destructive to a lipid-rich structure, such as the brain [12].

Why is the subsequent internalization of iron observed after exposing neurons to metallic solutions, such as vanadium and chromium? The SEM photograph of the neuron cultured in a chromium oxide solution environment displays that many of the thin branches of dendrites and axon were lost by a certain effect of chromium. It can be assumed that the first effect of the foreign metal to the neuron appears at the tip of the dendrites and axon. Proteins and membranes that are required for renewal of the axon are synthesized in the cell body. In the cell body, they are assembled into membranous vesicles or multi protein particles, which are then transported along microtubules down the length of axon to the terminals. Axonal microtubules also are the tracks along which damaged membranes and organelles move up the axon toward the cell body. Biomedical processes in the brain that are dependent on iron include neurotransmitter synthesis, myelin production and maintenance, and basic cell functions such as energy production [18]. It is probable that the internalization of iron would be induced by the functional disorder of these transportation systems because of the injuries on the axon terminals.

It follows that the excessive accumulation of iron within the cell leads to an increased production of free radicals via the Fenton reaction [17]. Furthermore, free radicals cause the mitochondrial dysfunction and the subsequent excessive production of reactive oxygen species, such as  $O_2^-$ ,  $H_2O_2$  and  $OH \cdot$ . It has been suggested that the excessive formation of hydroxyl free radicals ( $OH \cdot$ ) is cytotoxic and initiate lipid peroxidation and consequent cell damage [19].

In the case of the cell cultured in a chromium oxide for 4 h., the chemical state of

iron within the cell did not change compared with that in the control cell. Therefore, it can be assumed that almost all of the internalized iron was converted to ferritin or other proteins at this density level. However, the more excessive accumulation of iron is considered to lead to production of reactive oxygen species.

It has been suggested that the production of nitric oxide is closely related to the accumulation of iron within the cell [19]. The amount of the free iron within the cell is assumed to be controlled by the production of nitric oxide. However, the excessive iron, which cannot be fixed as ferritin or other proteins, may form a large amount of  $O_2^-$  within the cell and result in the production of cytotoxic  $ONOO^-$  and  $OH \cdot$  after the interaction between nitric oxide and  $O_2^-$ . If the excessive accumulation of iron within the cell induces this process, further injurious reactions may be accelerated.



## References

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