

## 老齡マウス視床における鉍物質沈着症のX線微小部分析

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## X-Ray Microanalysis of Mineralization in the Thalamus of Aged Mice

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The incidence and histopathology of spontaneous vascular mineralization occurring in the thalamus have been reported in several strains of mice [1-3, 5-9]. This report is to describe ultrastructure and X-ray microanalysis on the lesions.

Four male and three female 4-week-old B6C3F1 [(C57BL/6N × C3H/HeN) F1] mice were obtained from a commercial breeder (Charles River Japan, Atsugi) and were housed individually in polypropylene cages with hardwood bedding in a conventional animal facility. They were fed a commercial diet (NMF: Oriental Yeast Co., Tokyo) and tap water *ad libitum*. At 120 weeks of age the animals were sacrificed by exsanguination under ether anesthesia and were perfused with 4% glutaraldehyde in 0.1 M cacodylate buffer for 10 min. The thalamus was sampled and cut into 0.5 mm-cubes, some of which were postfixed with 1% osmium tetroxide in 0.1 M cacodylate buffer for 2 hrs at 4°C. The remaining tissue cubes were subjected to X-ray microanalysis. The tissue specimens were dehydrated and embedded in epoxy resin. Semithin sections were made and stained with 1% toluidine blue and examined by light microscopy. Thin sections were made and stained with uranyl

acetate and lead citrate and examined with a JEM 100CXII electron microscope. Unstained 100-nm sections on a beryllium grid from tissues without postfixation were subjected to X-ray microanalysis with an electron microscope equipped with a tilt goniometer and an energy dispersive X-ray microanalyzer (EDX system, Model 7000-77: Kevex Co., Foster, Calif.) at 20 Kev using a probe size of 200 nm.

Four (two males and two females) of seven mice were shown to have vascular mineralization varying in size (60  $\mu$ m in diameter on the average) and shape in the thalamus (Fig. 1).

Electron microscopy showed electron-dense depositions mostly in the moderately or severely thickened basement membrane of small blood vessels (Fig. 2). They sometimes had an electron-dense core with a lamellar cover (Fig. 3). Radially arranged crystalline structures, 70 to 140 × 1000 nm in size, which were located in the thickened basement membrane, might represent an early stage of the deposition. Severe deposits induced narrowing of the lumen of blood vessels (Fig. 2).

Using X-ray microanalysis, prominent peaks of calcium as well as phosphorus were detected with the deposits. Small peaks of iron, copper, zinc and magnesium were also found (Fig. 4). The quantitative analysis on five specimens revealed that the deposits contained 61% calcium and

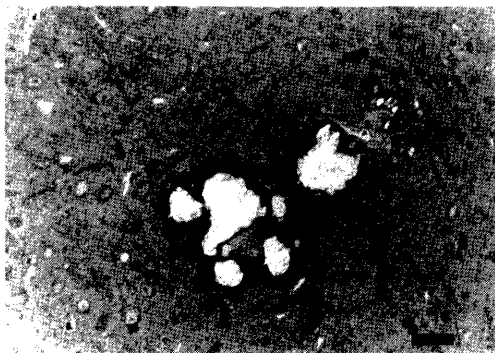


Fig. 1. Fragmentation of mineralized foci in the thalamus. Toluidine blue-stained epoxy section. Bar=50  $\mu$ m.

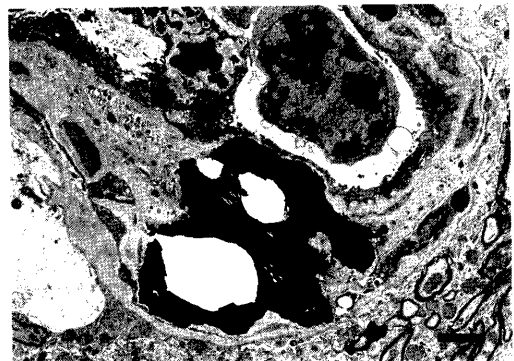


Fig. 2. Electron microscopy of nonlamellar deposits in the thickened basement membrane of small blood vessel. Bar=2  $\mu$ m.

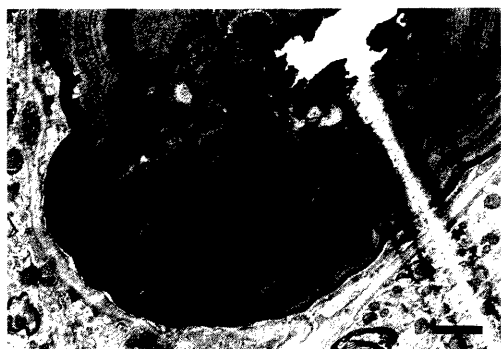


Fig. 3. Electron microscopy of a deposit with lamellar structure. Bar=2  $\mu$ m.

29% phosphorus in weight percent on the average. The vascular basement membrane without deposits had neither calcium nor phosphorus.

Although qualitative X-ray microanalysis had revealed the presence of both calcium and phosphorus in deposits on specimens postfixed with osmium tetroxide and stained with uranyl acetate and lead citrate [4], quantitative analysis had not been attempted with the deposits. Since osmium ( $M\alpha$ ) gives a peak very close to those of phosphorus ( $K\alpha$  and  $K\beta$ ), the specimens used in the present study were not immersed in the osmium tetroxide solution in order to obtain clear peaks of phosphorus for the quantitative analysis on mineral contents of the deposits.

Histochemically the mineralized lesions in the thalamus have been reported to be intensely positive for calcium with von Kossa's silver nitrate stain [1, 4, 8]. Some deposits were found to be weakly positive for iron with Perls' stain [8]. The present study supported these histochemical findings.

Thalamic mineralization in older B6C3F1 mice has been thought to be age-related and aging is the most important factor [8], while the time-course pathogenesis of the lesions remains unclear.

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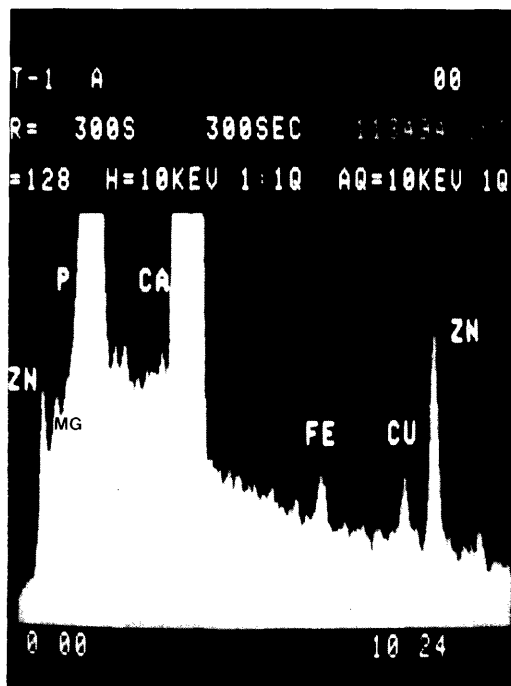


Fig. 4. X-ray microanalysis of a deposit. CA: calcium, P: phosphorus, ZN: zinc, FE: iron, CU: copper and MG: magnesium.

#### REFERENCES

1. Fraser, H. 1968. *J. Pathol. Bacteriol.* 96: 220-222.
2. Frith, C. H., Highman, B., Burger, G., and Sheldon, W. D. 1983. *Lab. Anim. Sci.* 33: 273-286.
3. Jortner, B. S., and Percy, D. H. 1978. pp. 319-421. *In: Pathology of laboratory animals* (Benirschke, K., Garner, F. M., and Jones, T. C. eds.). Springer-Verlag, New York.
4. Morgan, K. T., Johnson, B. P., Frith, C. H., and Townsend, J. 1982. *Acta Neuropathol.* 58: 120-124.
5. Sheldon, W. D., and Greenman, D. L. 1979. pp. 155-167. *In: Innovations in cancer risk assessment (ED<sub>01</sub> study)*. (Staffa, J. A., and Mehlman, M. A. eds.). Pathotox Publ., Illinois.
6. Toker, M. J., and Baker, S. B. 1967. pp. 787-824. *In: Pathology of laboratory rats and mice* (Cortchin, E., and Roe, F. J. C. eds.). Blackwell Scientific Publications, Oxford.
7. Ward, J. M., Goodman, D. G., Squire, R. A., Chu, K. C., and Linhart, M. S. 1979. *J. Natl. Cancer Inst.* 63: 849-854.
8. Yanai, T., Kudo, K., Manabe, J., and Matsunuma, N. 1984. *Jpn. J. Vet. Sci.* 46: 761-765.
9. Yoshii, Y., and Phillips, T. L. 1983. *Lab. Anim. Sci.* 33: 66-69.

## 要 約

老齡マウス視床における鉍物質沈着症のX線微小部分析（短報）：柳井徳磨・矢本 敬・真鍋 淳・高岡雅哉・松沼尚史（三共(株)安全性研究所）——120日齡 B6C3F1 マウスの視床に認められた鉍物質沈着症についてX線微小部分析を行い，沈着巣にカルシウム，燐のほか，少量の亜鉛，鉄，銅およびマグネシウムが検出された。