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Ultrastructural Localization of Superoxide Dismutase in Human Skin

T. KOBAYASHI[1], N. SAITO[2], N. TAKEMORI[2], S. IIZUKA[3], K. SUZUKI[4], N. TANIGUCHI[4] and H. IIZUKA[1]

[1] Department of Dermatology, Asahikawa Medical College, [2]Third Department of Internal Medicine, Asahikawa Medical College, [3]Department of Pediatrics, Sapporo National Hospital and [4]Department of Biochemistry, Osaka University Medical School, Japan

The ultrastructural localization of copper and zinc superoxide dismutase (Cu,Zn-SOD) and manganese superoxide dismutase (Mn-SOD) was investigated in normal human skin by the post-embedding immunogold staining method. Epidermal keratinocytes were positive for both Cu,Zn-SOD and Mn-SOD. The Cu,Zn-SOD was predominantly located in the cytoplasm with a slight labelling on the mitochondria. The basal cells were remarkably positive for Cu,Zn-SOD and the upper spinous or granular cells faintly positive for this enzyme. In contrast to Cu,Zn-SOD labelling, Mn-SOD labelling was mostly restricted to the mitochondria. A similar labelling pattern was observed in dermal fibroblasts, endothelial cells, melanocytes, and in various epidermal appendageal cells (i.e. outer root sheath cells, sweat ducts and eccrine sweat gland cells). Thus the subcellular localization of Cu,Zn-SOD and Mn-SOD in the skin was similar among various cell types. Although no evidence for the peroxisomal localization of Cu,Zn-SOD was obtained in the present study, this could be due to a marked fragility of peroxisome that has not been identified in epidermal keratinocytes. ***Key words: Cu,Zn-superoxide dismutase; Mn-superoxide dismutase.***

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H. Iizuka, Department of Dermatology, Asahikawa Medical College, 3–11 Nishikagura, Asahikawa, 078 Japan.

Superoxide dismutases (SODs; superoxide: superoxide oxidoreductase, EC 1.15.1.1) are a family of enzymes, which catalyze the dismutation of superoxide anion into O_2 and H_2O_2, thereby protecting cells against toxic reactive oxygen species (1). Three types of SOD – Cu,Zn-SOD, Mn-SOD, and extracellular Cu,Zn-SOD (EC-SOD) – have been identified in mammalian cells. They are encoded by three separate genes and differ in their amino acid sequences and their localization in tissues (2–4).

It has been reported that the epidermis contains the former two enzymes: Cu,Zn-SOD and Mn-SOD (5, 6). These can be distinguished by the sensitivity to cyanides (5). We have demonstrated the presence of SOD in epidermis by immunohistochemistry (6). Cu,Zn-SOD is present in the basal cell layer, whereas Mn-SOD is present in the whole epidermis. Deranged Cu,Zn-SOD activity as well as altered staining patterns have been described in various types of pathological skin (6, 7).

It has been reported that EC-SOD exists in significant amounts only in exceptional tissues (8). Although skin might contain EC-SOD, without an appropriate antibody that recognizes this type of enzyme, the presence of EC-SOD in the skin cannot be justified at present.

The intracellular localization of Cu,Zn-SOD is controversial. Although it is usually assumed to be a cytosolic enzyme, a recent study suggests that it is located in peroxisome (9). Typical peroxisomes are known to be abundantly present in the liver and kidneys (10). However, the existence of peroxisome in keratinocytes has not been confirmed.

Although keratinocytes are known to contain Cu,Zn-SOD and Mn-SOD, the exact subcellular localization of these enzymes has not been reported. We have developed highly specific antibodies against Cu,Zn-SOD and Mn-SOD (11, 12). In this paper, we describe the ultrastructural localization of Cu,Zn-SOD and Mn-SOD in various cells in human skin.

MATERIALS AND METHODS

The characterization of the antibodies specific for Cu,Zn-SOD and Mn-SOD has previously been reported (11, 12). Five normal skin specimens were obtained from patients undergoing plastic surgery. For light immunohistochemical studies, the avidin-biotin-peroxidase method was employed by using frozen sections (6).

For immunoelectron microscopy normal human skin was fixed for 2 h at 4°C in a mixture of 1% paraformaldehyde and 1% glutaraldehyde in 0.05 M phosphate buffer (pH 7.4), to which 1.5% sucrose was added. Specimens were washed with 0.1 M phosphate buffer and were embedded in Lowicryl K4M (Chemische Werke Lowi, Waldkraiburg, Germany) according to the method of Kellenberger et al. (13). Briefly, after dehydration in 80% ethanol for 30 min at 4°C, the specimens were infiltrated with increasing concentrations of Lowicryl K4M (K4M:80% ethanol = 1:1 for 1 h, then 2:1 for 1 h) at 4°C and finally infiltrated with Lowicryl K4M for 24 h at −20°C. Polymerization was performed by ultraviolet irradiation (wavelength peak at 360 nm) for 24 h at 4°C. Ultrathin sections were mounted on nickel grids.

A modified procedure of Roth (14) was carried out for immunogold electron microscopy. Ultrathin sections were rinsed in 0.02 M phosphate buffered saline (pH 7.2) containing 1% bovine serum albumin (BSA) for 30 min, and then reacted with an appropriately diluted antiserum for 1 h at 4°C. The antiserum used in this study was mouse IgG fractions specific for human Cu,Zn-SOD or human Mn-SOD. The ultrathin sections were washed with distilled water and reacted with 5 or 10 nm gold particle-labelled goat antiserum specific for mouse IgG (GAM G5 and G10, Amersham, Amersham; U.K.). After having been washed with distilled water, the ultrathin sections were treated with both uranyl acetate and lead citrate and then observed with an electron microscope (Hitachi HU-12A). As a control, normal mouse serum was substituted for the specific antiserum.

RESULTS

Immunohistochemical study

Cu,Zn-SOD was detected in the lower part of epidermis especially in the basal cells (Fig. 1-A) Cu Zn-SOD was also detected in the basal cells of the upper part of the outer root sheath of hair follicle (Fig. 1-A), eccrine sweat ducts, and on eccrine sweat glands (Fig. 1-C). Several suprabasal layers of the outer root sheath were also positive for Cu,Zn-SOD (Fig. 1-A, B). Mn-SOD was faintly detected in the whole

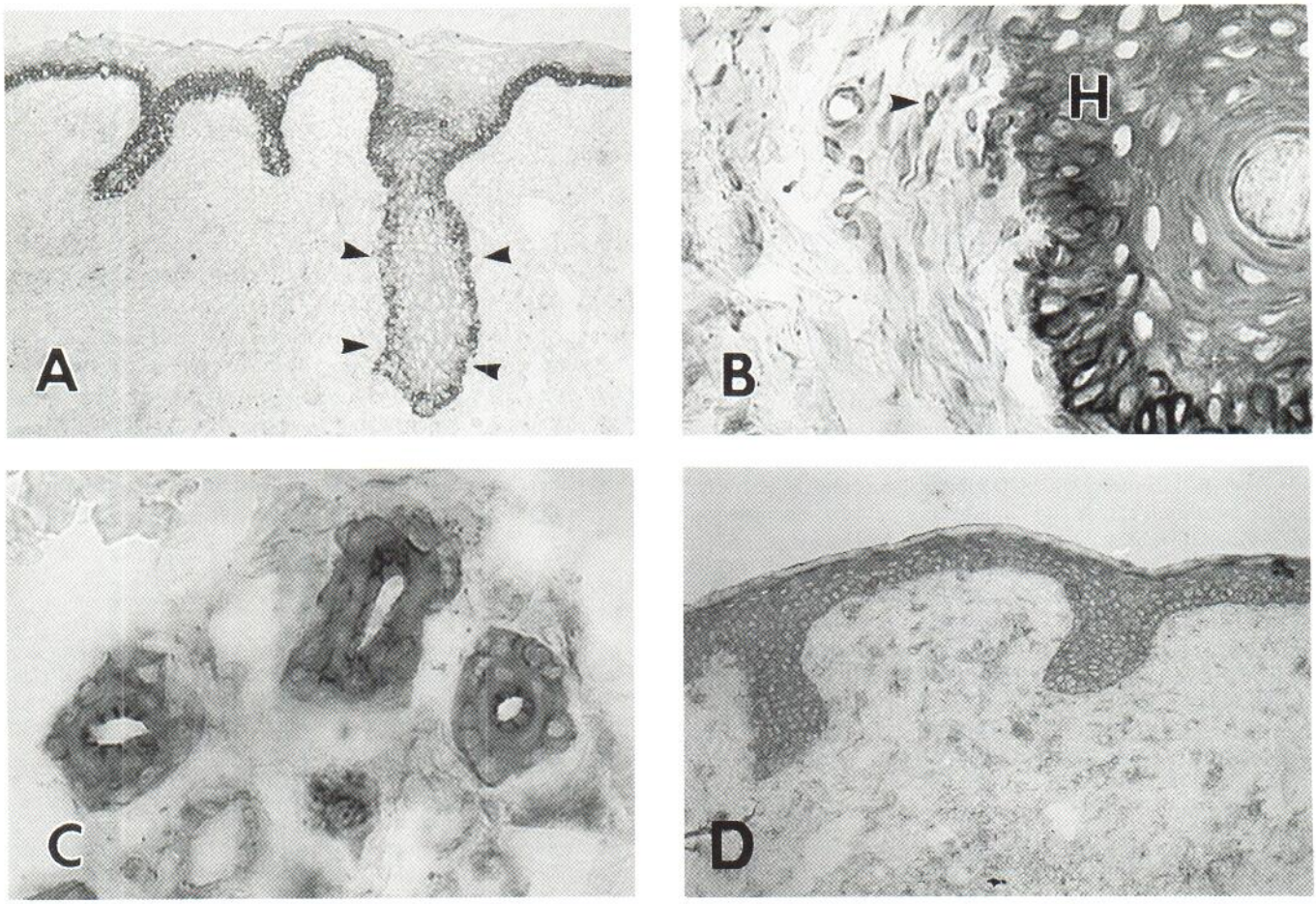


Fig. 1. Immunohistochemical staining for Cu,Zn-SOD, and Mn-SOD. (A) Cu,Zn-SOD staining (×200). Lowermost epidermal cells are intensely stained. Basal cells of upper part of hair follicle were also stained (*arrowheads*). (B) Cu,Zn-SOD staining. *H*: hair follicle (×400). Dermal fibroblasts are faintly positive (*arrow*). Note the positive staining of hair follicle. (C) Cu,Zn-SOD staining (×400). Eccrine glands are faintly positive. (D) Mn-SOD staining. Whole epidermal layers are stained (×200).

epidermis (Fig. 1-D). Dermal fibroblasts (Fig. 1-B) and endothelial cells were faintly positive for both Cu,Zn-SOD and Mn-SOD.

Immunogold electron microscopy study

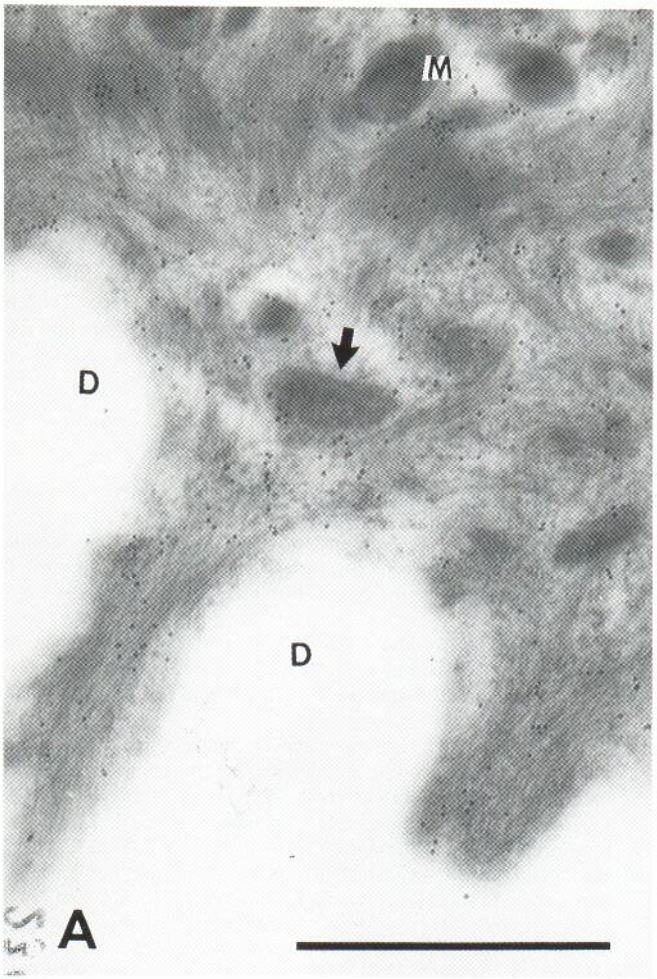
Positive labelling for Cu,Zn-SOD was seen in keratinocytes diffusely in the cytoplasm, particularly associated with tonofibrils (Fig. 2-A). No labelling was seen on either melanosomes or desmosomes. The labelling was more remarkable in basal cells than in the upper spinous or granular cells (Fig. 2-A, B). The cytoplasm of dermal fibroblasts (Fig. 2-C), endothelial cells, melanocytes or various epidermal appendageal cells (outer root sheath cells, eccrine sweat glands and eccrine sweat duct cells) was diffusely labelled for Cu,Zn-SOD.

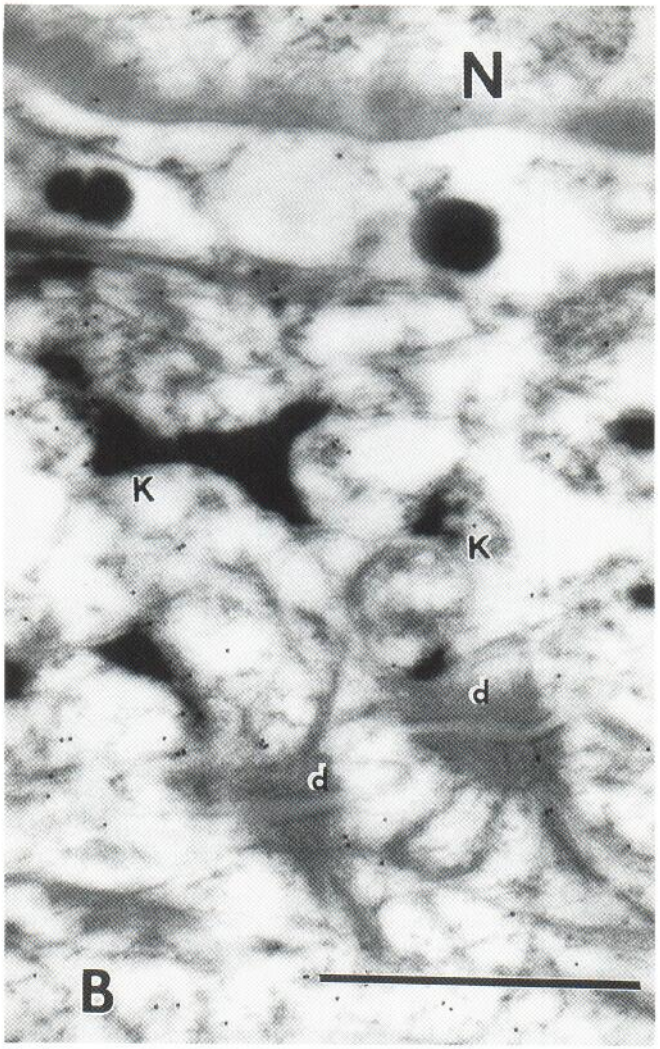
On the other hand, positive labelling for Mn-SOD was seen almost exclusively in the mitochondria of basal keratinocytes (Fig. 3). No labelling was apparent on tonofibrils or on melanosomes. The similar labelling of mitochondria was observed in dermal fibroblasts (Fig. 4), endothelial cells, and other cells studied.

DISCUSSION

To our knowledge this is the first report regarding the ultrastructural localization of Cu,Zn-SOD and Mn-SOD in human skin tissue. Cu,Zn-SOD was detected in the cytoplasm and Mn-SOD predominantly in the mitochondria. No evidence for peroxisomal localization of Cu,Zn-SOD was obtained. Keratinocytes, melanocytes, fibroblasts, endothelial cells, and epidermal appendageal cells showed a similar immunogold labelling pattern; this enzyme does not seem to be specific for cell types.

Our findings are consistent with the reports that Cu,Zn-SOD is a cytoplasmic enzyme and Mn-SOD a mitochondrial enzyme (15, 16). Although Keller et al. (9) reported that Cu,ZnSOD was located in peroxisomes, this was not supported by the present study. It is well known that peroxisomes are highly sensitive to hydrostatic pressure damage as well as mechanical stress and are easily destroyed during a conventional fractionation process (17). This kind of fragility is observed even after the fixation (18). Possibly the fragile nature of peroxisomes might affect the results, masking the actual peroxisomal location of Cu,Zn-SOD. It is noteworthy that successful immunogold labelling of peroxisomes has been re-





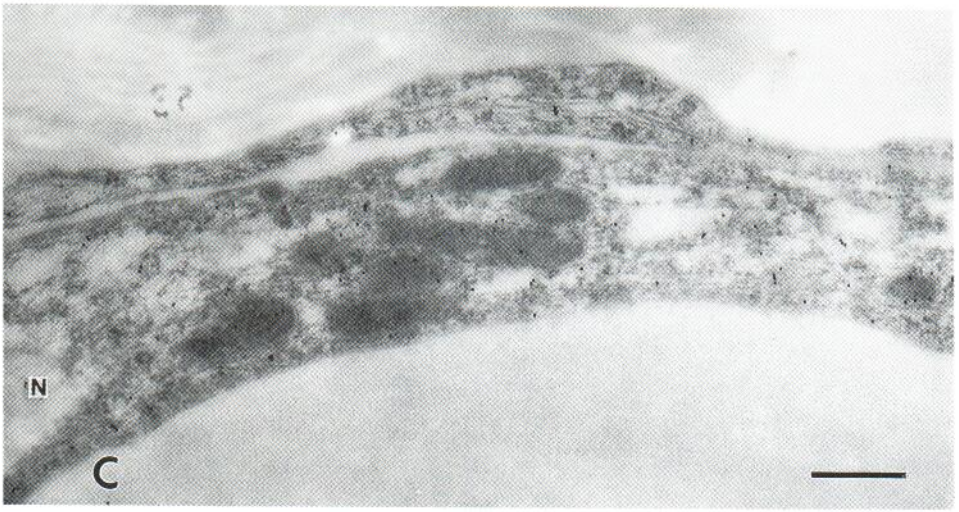


Fig. 2. Immunogold labelling (10 nm) for Cu,Zn-SOD. (A) Epidermal basal cell. The cytoplasm which contains abundant tonofibrils is labelled. A mitochondrion (*arrow*) is faintly labelled. Melanosomes (*M*) are negative. *D*; dermis (Bar, 1 μm). (B) Upper epidermal keratinocytes. The cytoplasm is faintly labelled. Desmosomes (*d*) are not labelled. *N*; nucleus; *k*; keratohyalin granules (Bar, 1 μm). (C) Dermal fibroblast. Gold particles are evenly distributed in the cytoplasm. Some gold particles are also seen on the mitochondria (Bar 1 μm).

ported only in the liver and kidneys, which are the two typical organs with abundant peroxisomes (19).

It has been reported that the epidermal SOD activity is lower than that of other aerobic tissues (20). Our recent study suggests that the distribution of the epidermal SOD might not be homogeneous (6). Ogura et al. (21) also reported that SOD activity is higher in the lower layer of cow snout epidermis. In this study, immunogold labelling was observed strongly in basal cells when compared with the upper keratinocytes.

Deranged SOD activity as well as immunohistochemical

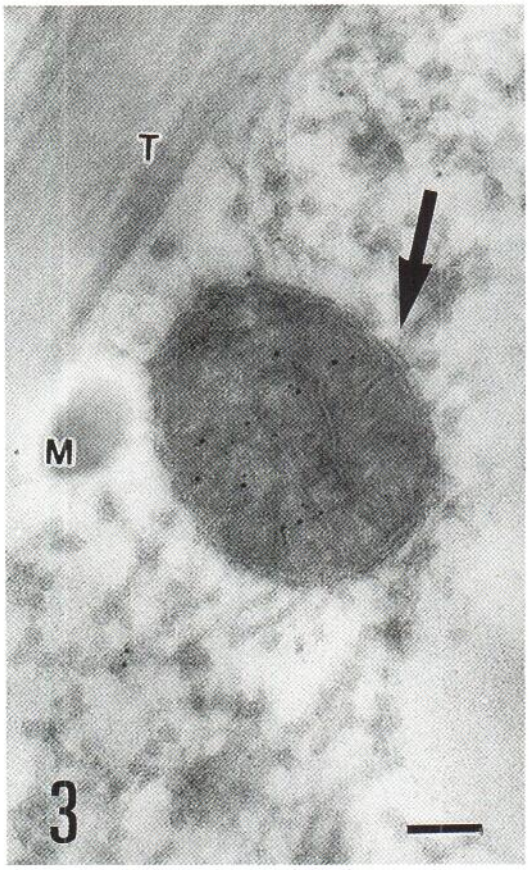


Fig. 3. Immunogold labelling (5 nm) for Mn-SOD in epidermal basal cells. Higher magnification (Bar 0.1 μm). A mitochondrion is labelled by gold particles (*arrow*). No labelling on tonofibrils (*T*) or on melanosomes (*M*).

staining patterns have been described in various pathologic conditions of the skin (6, 7, 22–25). Because of the significant role of SOD in protecting cells against toxic reactive oxygen species, the alteration of the SOD should have profound effects on cell biology. Whether this alteration is accompanied by the deranged subcellular localization of Cu,Zn-SOD and Mn-SOD requires further investigation.

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REFERENCES

1. Fridovich I. The biology of oxygen radicals. Science 1978; 201: 875–880.
2. Sherman L, Dafni N, Lieman-Hurwitz J, Groner Y. Nucleotide sequence and expression of human chromosomal 21-encoded superoxide dismutase mRNA. Proc Natl Acad Sci USA 1983; 80: 5465–5469.
3. Hjarmarsson K, Marklund SL, Engstrom A, Edlund T. Isolation and sequence of complementary DNA encoding human extracellular superoxide dismutase. Proc Natl Acad Sci USA 1987; 84: 6340–6344.
4. Ho YS, Crapo JD. Isolation and characterization of complementary DNAs encoding human manganese containing superoxide dismutase. FEBS Lett 1988; 229: 256–260.
5. Ohkuma N, Matsuo S, Iizuka H, Ohkawara A. Superoxide dismutase in epidermis. J Dermatol (Tokyo) 1987; 14: 218–223.
6. Kobayashi T, Matsumoto M, Iizuka H, Suzuki K, Taniguchi N. Superoxide dismutase in psoriasis, squamous cell carcinoma and basal cell epithelioma: an immunohistochemical study. Br J Dermatol 1991; 124: 555–559.
7. Ohkuma N, Kajita S, Iizuka H. Superoxide dismutase in epidermis: its relation to keratinocyte proliferation. J Dermatol (Tokyo) 1987; 14: 562–568.
8. Marklund SL. Extracellular superoxide dismutase in human tissues and human cell lines. J Clin Invest 1984; 74: 1398–1403.
9. Keller G-A, Warner TG, Steimer KS, Hallewell RA. Cu,Zn-

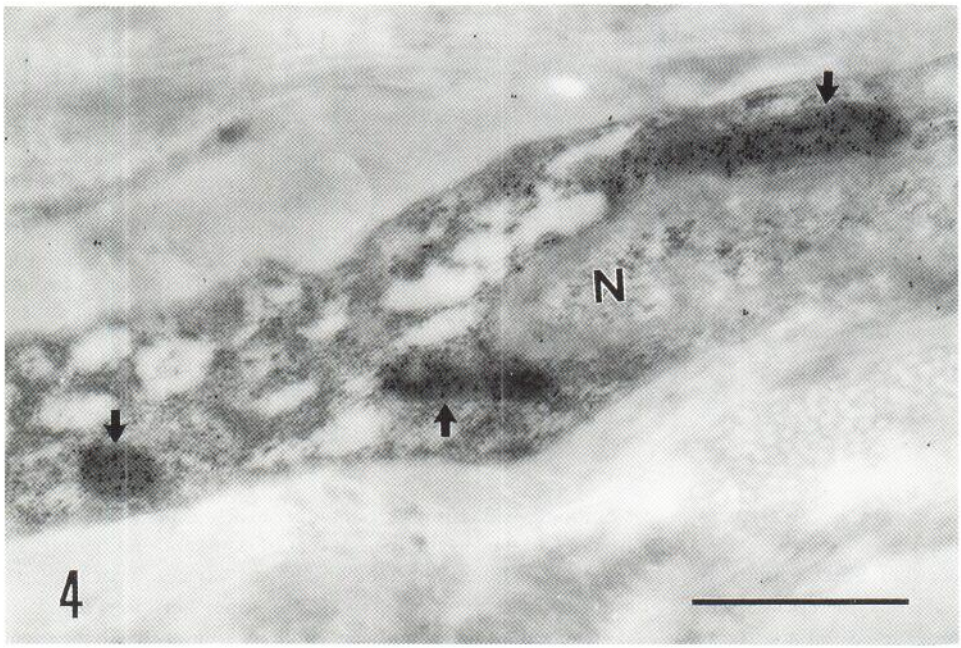

Fig. 4. Immunogold labelling (10 nm) for Mn-SOD in dermal fibroblast. Mitochondria (*arrows*) are labelled by gold particles. The cytoplasmic labelling is not above the background (observed after incubation with preimmune serum) (Bar = 1 μm).

superoxide dismutase is a peroxisomal enzyme in human fibroblasts and hepatoma cells. Proc Natl Acad Sci USA 1991; 88: 7381–7385.

10. Tolbert NE, Essner E. Microbodies: peroxisomes and glyoxysomes. J Cell Biol 1981; 91: 271s–283s.
11. Oka S, Ogino K, Matsuura S, Yoshimura S, Yamamoto K, Okazaki Y, et al. Human serum immunoreactive copper, zinc-superoxide dismutase assayed with an enzymemonoclonal immunosorbent in patients with digestive cancer. Clin Chim Acta 1989; 182: 209–220.
12. Kawaguchi T, Noji S, Uda T, Nakashima Y, Takeyasu A, Kawai Y, et al. A monoclonal antibody against COOH-terminal peptide of human liver manganese superoxide dismutase. J Biol Chem 1989; 264: 5762–5767.
13. Kellenberger E, Carlemalm E, Villiger W, Roth J, Garavito M. In: Low denaturation embedding for electron microscopy of thin sections. Waldkraiburg: Chemische Werke Lowi G.m.b.H., 1980: 29–48.
14. Roth J. The colloidal gold marker system for light and electron microscopic cytochemistry. In: Bullock GA, Petrusz P, eds. Techniques in immunocytochemistry. Vol 2. London, New York, Paris, San Francisco, San Paulo, Sydney, Tokyo, Toronto: Academic Press, 1983: 217–284.
15. Chang L-Y, Slot JW, Geuze HJ, Crapo JD. Molecular immunocytochemistry of the Cu, Zn-superoxide dismutase in rat hepatocytes. J Cell Biol 1988; 107: 2169–2179.
16. Slot JW, Geuze HJ, Freeman BA, Crapo JD. Intracellular localization of the copper-zinc and manganese superoxide dismutases in rat liver parenchymal cells. Lab Invest 1985; 55: 363–371.
17. Leighton F, Coloma L, Koenig C. Structure, composition, physical properties, and turnover of proliferated peroxisomes. J Cell Biol 1975; 67: 281–309.
18. Yokota S, Fahimi HD. The peroxisome (microbody) membrane: effects of detergents and lipid solvents on its ultrastructure and permeability to catalase. Histochem J 1978; 10: 469–487.
19. Yamamoto K, Voelkl A, Fahimi HD. Biogenesis of peroxisomes in regenerating rat liver. II. Immunoelectron microscopical investigation of catalase and acyl-CoA oxidase. Acta Histochem Cytochem 1989; 22: 443–457.
20. Carraro C, Pathak MA. Characterization of superoxide dismutase from mammalian skin epidermis. J Invest Dermatol 1988; 90: 31–36.
21. Ogura R, Ueta H, Sugiyama M, Hidaka T. Distribution of superoxide dismutase activity on the epidermis: measurement with electron spin resonance spin trapping. J Invest Dermatol 1990; 94: 227–229.
22. Hashimoto Y, Ohkuma N, Iizuka H. Reduced superoxide dismutase activity in UVB-induced hyperproliferative pig epidermis. Arch Dermatol Res 1991; 283: 317–320.
23. Hamanaka H, Miyachi Y, Tachibana T, Imamura S. Lowered Cu, Zn-superoxide dismutase activity in human malignant skin tumors. J Dermatol (Tokyo) 1991; 18: 258–261.
24. Iizawa O, Kato T, Tagami H, Niwa Y. Lipid peroxides and superoxide dismutase activity in trichophyte lesions. Arch Dermatol 1991; 127: 1241–1243.
25. Iizuka H, Asaga H, Koike K, Iizuka S: Decreased Cu,Zn-superoxide dismutase activity in psoriatic hyperproliferative epidermis. Eur J Dermatol (in press).