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Eccrine gland involvement in Krabbe's disease

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Summary. Lysosomal storage inclusions were observed in skin eccrine gland secretory and myoepithelial cells in three cases of Krabbe's disease. In addition to storage there were numerous degenerative changes, occasionally resulting in cell necrosis. These findings suggest a generalized nature of the storage process in this lysosomal enzymopathy and point to high galactocerebroside turnover in eccrine gland epithelium. This knowledge may be of value in the biopsy diagnosis of Krabbe's disease.

Key words: Krabbe's disease – Eccrine gland storage – Ultrastructure

Introduction

Krabbe's disease is defined biochemically as a deficiency of galactocerebrosidase (E.C. 3.2.1.46) with consequent storage of galactocerebroside in the cell lysosomal compartment. The tissue most affected is the white matter, predominantly in the brain, where phagocytes, the so-called Krabbe cells (Suzuki and Suzuki 1989) are found. Non-neural tissues are generally considered to be free of galactocerebroside but we describe a new storage site, the eccrine gland epithelium. Knowledge of this may have both practical and theoretical implications.

Materials and methods

Three cases of biochemically verified Krabbe's disease were examined. Two were of the early onset type with a typical clinico-pathological phenotype, the patients dying at the age of 18 months and 15 months, respectively. The third, a boy, succumbed to the disease at the age of 6 years. In the first two cases neuropathological findings were typical of florid globoid cell leukodystrophy. In the third case, the disease was of the diffuse sclerosis type with burnt-out storage phenomena (McKelvie et al. 1990). Skin biopsies were taken from the inner part of the upper arm of each patient, fixed in 10% phosphate buffered paraformaldehyde and post-fixed in 1% osmium tetroxide, dehydrated in ethanol and embedded in araldite-epon mixture. Semi-thin sections were cut with a glass knife and stained with alkaline toluidine blue. The ultra-thin sec-

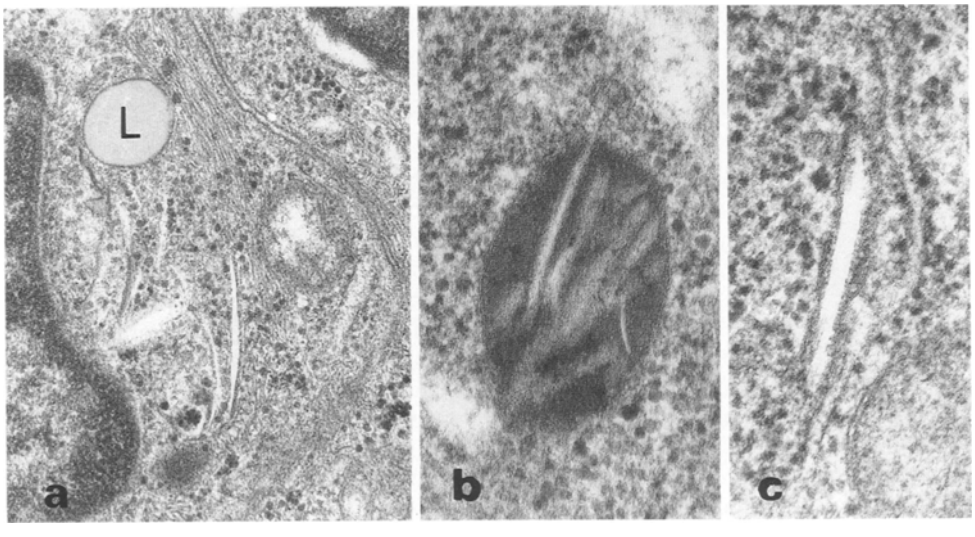

Fig. 1a–c. Eccrine gland epithelium with discrete crystalline deposits. The dense body in **b** represents initial lipofuscin formation with simultaneous lipid crystalline deposition. *L*, Lipid droplet. **a** × 16,000; **b, c** × 52,000

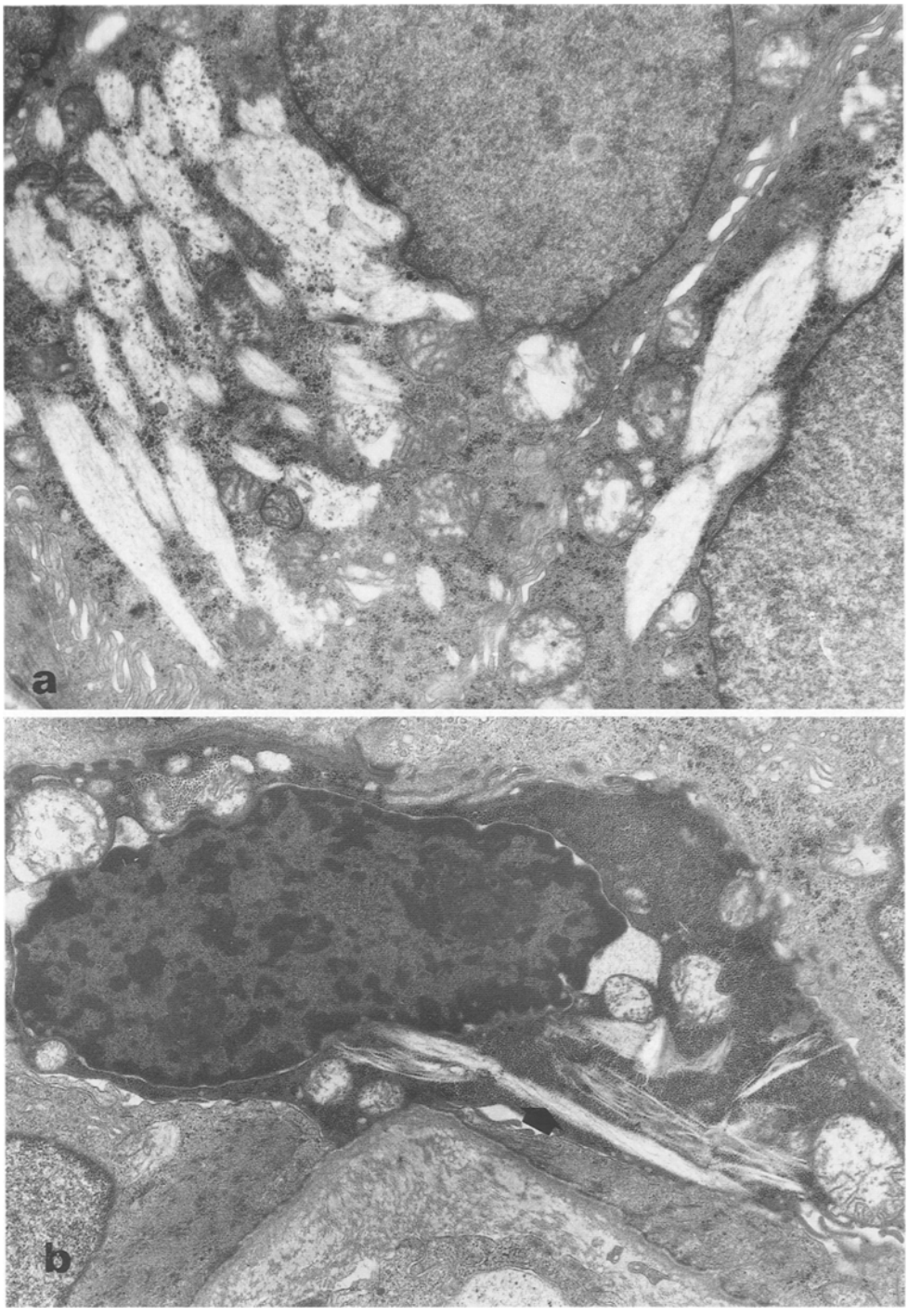

Fig. 2a, b. Typical fusiform storage lysosomes with predominantly tubular inclusions in a sweat gland epithelial cell (**a**) and in a regressively changed cell situated close to the peripheral part of the gland tubule (**b**). Note the twisted tulue in **b** (*arrow*) and mitochondrial degeneration. **a** × 6,000; **b** × 4,000

tions were cut with a diamond knife and doubly contrasted with uranyl acetate and lead citrate.

Results

The skin eccrine glands were essentially unremarkable in semi-thin toluidine-blue-stained sections, except for occasional regressive changes in epithelial cells showing hydropic degeneration.

Ultrastructural alterations were essentially identical in all three cases. There was an extremely variable number of vacuolar, crescent-shaped, slit-like, fusiform inclusions or larger irregular vacuolar fusiform inclusions ranging in length from 1 to 8 μm (Figs. 1, 2). The limiting single membrane was present, but occasionally it could not be demonstrated unequivocally. The inclusions contained fibrillary and straight hollow tubular deposits with contours of lower density compared to analogous deposits in storage cells in the brain lesions of the respective case. The hollow tubular structures were often slightly but definitely angulated, but the polygonal profiles were not seen. Twisted tubules (Fig. 2b) were exceptional. Dense lysosomal structures corresponding to the initial phase of lipofuscin development, best seen in the oldest patient (case 3) were often intermingled with narrow tubular structures.

The storage changes were found in both epithelial

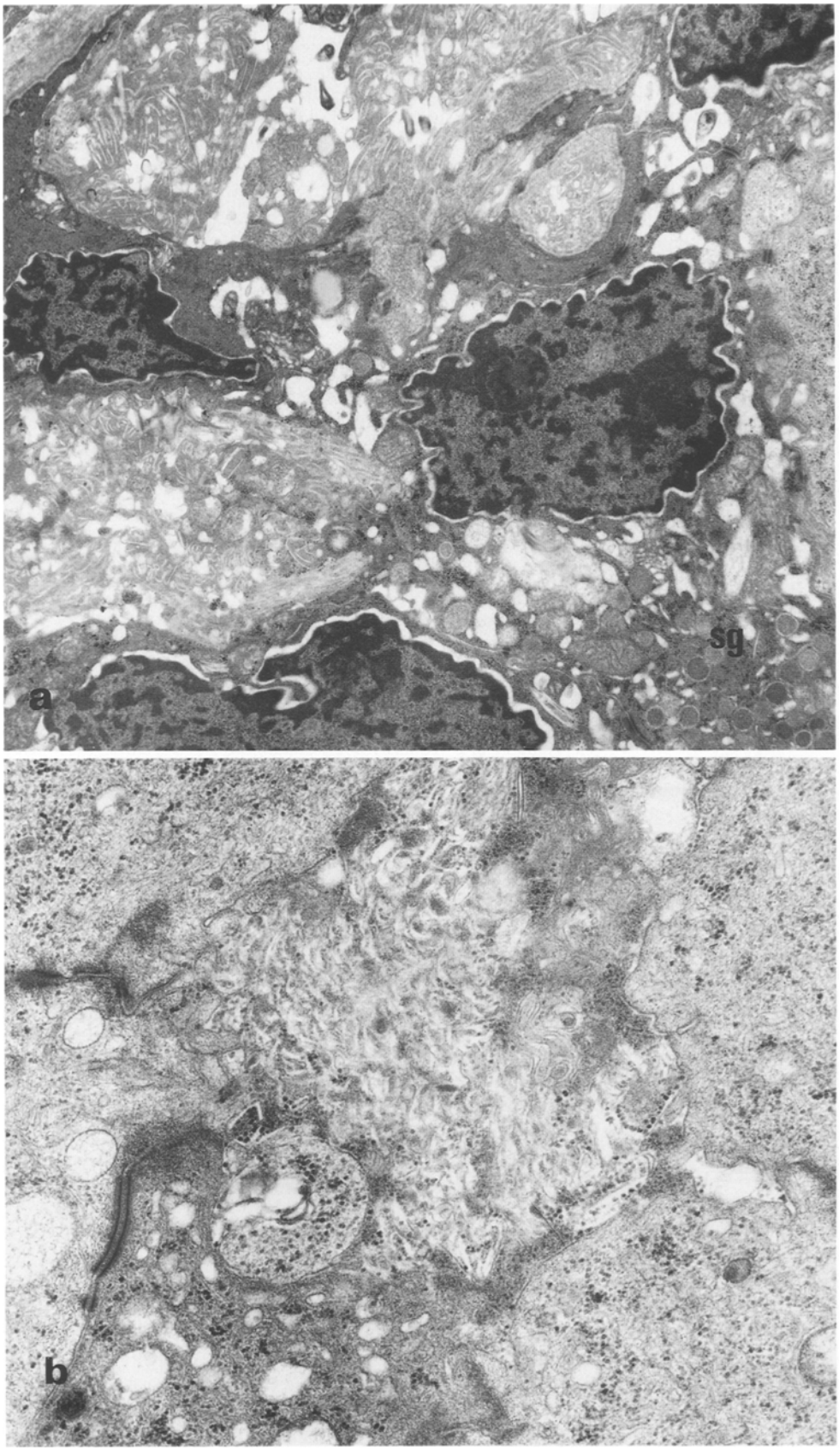

Fig. 3. **a** Extensive degeneration in the sweat gland. The cell debris contains multitude of fine crystalline deposits (*sg*, remnants of secretory granules). **b** The same material as in **a** localized in the gland lumen. **a** × 5,600; **b** × 17,000

(secretory) and myoepithelial cells with roughly equal intensity. The appearance and density of the eccrine gland deposits corresponded exactly to those in the occasional Krabbe cells seen in the myelinated nerves in the skin.

Along with the storage there were, in both epithelial and myoepithelial cells, severe alterations of the cell structure, especially of mitochondria. These varied considerably in size, many of them contained intracristal alpha and beta glycogen particles or displayed focal lytic

changes with concentric membranous deposits. Some of them, especially those found in the most severely altered cells, were transformed into optically empty vacuoles. Occasional cells were necrotic, the cell debris being largely intermingled with slender crystals resembling those in the cytoplasmic inclusions. Similar deposits were occasionally seen in the gland lumina (Fig. 3). The epithelial cells also contained frequent focal accumulations of glycogen alpha particles with small included lipid droplets or with irregular lucent cytoplasmic areas sometimes containing loose membranes. The secretory granules were normal. The ductal portions of the glands were free of storage. Their mitochondria often showed circular cristae, a change regarded as a non-specific finding (unpublished personal observation).

Other changes included occasional dystrophic axons distended by mitochondrial and dense pleomorphic body accumulation, and wavy redundant basement membrane around some Schwann cells of myelinated nerve fibres. Some myelinated nerves contained storage histiocytes of the globoid type.

Discussion

This evidence of storage in eccrine gland epithelium extends the list of extraneural tissues affected in Krabbe's disease. Thus far, storage has been described in kidney epithelium and lymph node cortical macrophages in the twitcher mouse model (Takahashi et al. 1983) and in renal tubules in the canine form of the disease (Suzuki 1986). However, in human cases these and other locations were found to be free of ultrastructurally detectable deposits (Clarke et al. 1981; Takahashi et al. 1983; Yunis and Lee 1972). All the other possible extraneural storage sites in human cases showed normal concentration of galactosylceramide but a slightly increased concentration of galactosylsphingosin (psychosin) (Kobayashi et al. 1987, 1988). The only exception is an increase of galactosylceramide in human liver in Krabbe's disease, reported by Dawson (1973). The skin eccrine glands are thus the first extraneural structures displaying ultrastructurally detectable storage in the human form of Krabbe's disease (Kimura 1991).

The are several implications. First, the presence of storage in all three cases suggests that eccrine gland involvement may be a regular feature of human Krabbe's disease which is important in evaluating skin biopsies from suspicious cases. It emphasizes the value of the evaluation of skin eccrine glands, which are valuable structures in the biopsy diagnosis of lysosomal storage diseases. However, it should be borne in mind that some of the storage inclusions (the slit-like ones) may resemble the "leaflets" frequently seen in peroxisomal disorders associated with defects in very long chain fatty acid oxidation (Powers 1985). This was also the initial diagnostic suggestion in one of our cases.

Second, the findings provide morphological evidence of a relatively high lysosomal turnover of galactocerebroside in the eccrine gland epithelium. This is of interest in view of the high concentration of galactosylceramide found in pig epidermal cells (Hamanaka et al. 1988) which also suggests that the skin epithelium and its derivatives may be an important extraneural domain for galactocerebroside turnover.

The relatively severe, regressive changes occascionally resulting in necrosis have never been seen before in many skin biopsies in this disease (unpublished observations) and represent a strong argument in favour of the psychosine toxicity theory (reviewed by Suzuki and Suzuki 1989). The effect of the eccrine gland storage on sweat gland secretory function is not known. So far, there has been no report concerning either dyshidrosis or glandular dysfunction in Krabbe's disease.

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