Electron Microscopic Study of Fingernails in the Disease of Mljet (Mal de Meleda)*

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Results are presented of the electron-microscopic examinations of fingernail clippings from 4 patients suffering from Mljet (Mal de Meleda) disease, of 3 obligate heterozygotes of this autosomal recessively inherited disease, as well as of 3 controls. In the nails of the diseased persons, significantly more osmiophilic material was found than in those of controls. In the nails of heterozygotes, the findings were intermediate between those of patients and controls. In the nails of aged persons, a strongly osmiophilic matrix keratin was found. Histochemically, lipids were demonstrated in the ventral plate of diseased nails. The probable significance and implications of these findings are discussed. *Key words: Disease of Mljet; Nail clippings; Electron microscopy; Heterozygotes.* (Received September 6, 1983.)

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In 1982, one of the present authors described the clinico-morphological alterations found in the fingernails and toenails of 11 patients with the disease of Mljet (Mal de Meleda) (1). The alterations of the fingernails were almost polymorphous, whereas those of the toenails were uniform. We wondered whether the ultrastructures of the various morphologic fingernail alterations in this disease would be alike—or would also be heterogeneous. The present investigations were undertaken to elucidate this question. At the same time, we wished to evaluate the possibility of utilizing electron-microscopic examination of the free margin of fingernails as an appropriate test for heterozygotes with the disease of Mljet.

MATERIAL AND METHODS

Fingernail clippings from 4 affected persons (all females) with the disease of Mljet, aged 28, 33, 44 and 76 years respectively, were examined. The normal fingernails of a 19-year-old son of an affected male, of a 42-year-old brother of a patient, as well as of a 70-year-old man, the father of an unmarried affected female—all obligate heterozygotes for the disease—were also examined. Clippings of the normal fingernails of a 9-year-old girl, of a 29-year-old woman, and of a 68-year-old man, were taken as controls.

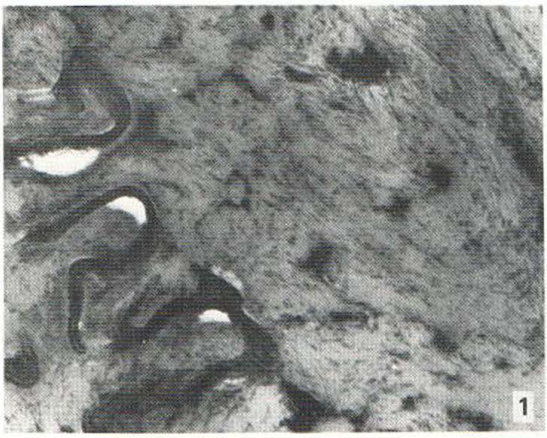
The specimens were treated according to the method described by Orfanos et al. (2). After 10 days of fixation in 1% OsO_4, the samples were dehydrated in a graded ethanol series and embedded in Epon 812. Thin sections were cut with a diamond knife and stained with uranyl magnesium acetate and lead citrate (3). Stained sections were examined in an electron microscope (JEM 100 B). Specimens of 2 patients with Mljet disease and of 2 controls, were not stained, but only fixed in OsO_4. The histochemistry of lipids of the nail of one patient and of 2 controls was also studied.

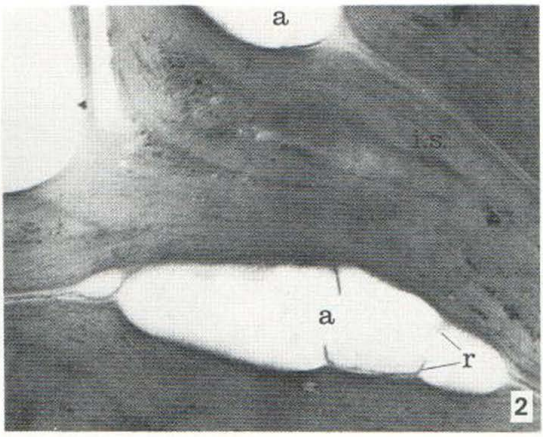
RESULTS

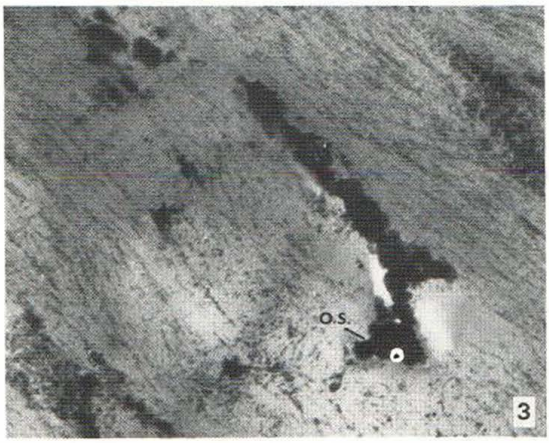
A) *Fingernails of persons with Mljet disease*

Since the electron-optic findings were nearly identical, they will be summarized.

* This work is dedicated to Prof. Dr Dr hc U. W. Schnyder, Zürich, on the occasion of his 60th birthday.







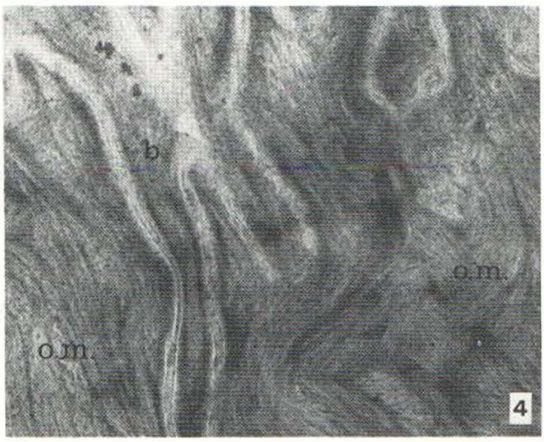


Fig. 1. Large quantity of irregularly shaped, homogeneous, osmiophilic material of varying size, in the nail keratin of a 28-year-old affected female.

Fig. 2. Ampullaceous dilatations of the intercellular space. The matrix-keratin is osmiophilic. Nail of a 76-year-old affected female.

Fig. 3. Osmiophilic material in a section of non-stained nail of a 28-year-old patient. ×120 000.

Fig. 4. The cell border is very curvilinear. Strongly osmiophilic matrix-keratin in the nail of a 70-year-old heterozygote. ×75 000.

Key to symbols
a, ampullaceous dilatation of the intercellular or paramembranous spaces; *b*, cell border; *i.s.*, intercellular space; *o.m.*, osmiophilic matrix; *o.s.*, osmiophilic substance; *v*, vacuole partly filled with o.s.; *r*, remnants of cell membrane or/and intercellular substance.

Numerous, irregularly shaped homogeneous osmiophilic materials of varying size, were found in the keratinized cells of the nails (Fig. 1). The borders of the cells had a much more meandering outline; in cross-section they were sometimes indented. In places, optically empty spaces, sometimes in the form of ampullaceous dilatation with remnants of cell membrane were found between the cells (Fig. 2). Frequently no intercellular substance was found in the dilatations. Some keratinized cells contained nuclear remnants. In the cells of our 76-year-old patient, an expressive osmiophilic matrix-keratin was found. In the nails of the 3 younger patients, the osmiophily of the matrix-keratin was scanty. Part of the

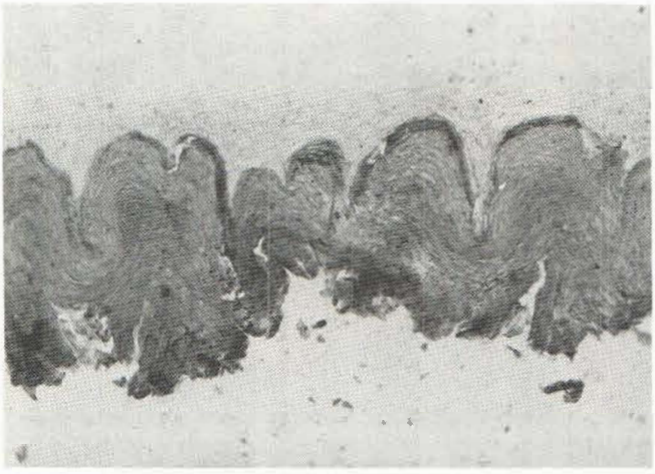


Fig. 5. Undulating layers of keratinized cells of the ventral nail plate of a patient with the disease of Mljet. Phospholipids on the upper border (Baker, ×250).

sample of one patient was not stained, but the osmiophily in the nail was nevertheless quite evident, a sign that the distinct contrast was not the result of the binding of lead ions to proteinaceous matrix substance during the staining procedure (Fig. 3).

In the nails of affected persons, no morphologically normal appearing cells were found.

B) *Fingernails of obligate heterozygotes*

(*a*) In the nail of the 19-year-old son of an affected subject, and of the 42-year-old brother fo a patient, there was less osmiophilic material in the cells than in those of the affected persons. Optically empty spaces were found between the membrane and the keratinized cell. The cell borders were meandering.

(*b*) In nails of the 70-year-old heterozygote, the cell borders were very curvilinear. The electron-dense intercellular substance was not invariably present. Large dilatations between the cells were seen. The osmiophilic material was smaller and scanty; vacuoles were partly filled with osmiophilic material. The osmiophilic matrix was conspicuous (Fig. 4).

In the nails of the 3 heterozygotes, morphologically normal appearing cells, with normal intercellular relations, were found.

C) *Fingernails of controls*

(*a*) In nails of the 9-year-old girl, the osmiophilic material in the cells was scanty. The borders of the cells appeared meandering, even indented. The intercellular spaces were filled with osmiophilic substance. No electron-dense osmiophilic matrix-keratin was seen in the cells.

(*b*) In nails of the 29-year-old woman, the findings corresponded to those of the previously described child.

(*c*) In nails of the 68-year-old man, a few homogeneous granules of varying size, and many dilated intercellular spaces devoid of osmiophilic material, were found. The borders of the cells were meandering. Electron-dense osmiophilic matrix in the keratinized cells was abundant.

D) To ascertain whether the nails in Mljet disease contain histochemically detectable amounts of lipids, and to establish whether the osmiophilic material seen electron-

Fig. 6. Deposits of Sudan IV—positive material deeper in the intermediate nail plate of a pateint with the same disease. Numerous slits can be seen (Sudan IV, ×400).

microscopically could be derived from the nail lipids, we studied the histochemistry of nail clippings of a patient with the disease of Mljet, as well as of 2 controls. Part of each sample of nail clippings was fixed in formol–calcium chloride and processed according to Baker, for demonstration of phospholipids. Another part of each sample was frozen and stained with Sudan IV. We describe here the results of these investigations:

In the altered nail of a Mljet patient, the ventral surface of the nail plate was uneven. An undulat layer of about 10–40 rows of keratinized cells could be seen (Fig. 5). At the upper border of this layer and deeper, numerous slits could be discerned. Some were completely or partly filled with a homogeneous, thin material, which positively reacted to phospholipids. The Sudan IV also demonstrated deposits of lipids between the dorsal and ventral nail plate, but also deeper in the slits in the ventral nail plate (Fig. 6).

The nails of controls also contained lipids, though in smaller quantity than in the diseased nails. The above-mentioned slits were missing in the nails of a younger control, whereas they were present in the nail of a 68-year-old man without the disease.

The undulating layer of keratinized cells on the ventral side of the ventral plate stains more intensely with Baker than with hematoxylin-eosin and Sudan IV. It seems that the ventral nail plate contains more phospholipids in the altered, than in the control nails.

DISCUSSION

Since the ultrastructural alterations found in the fingernail clippings of all 4 patients affected by the disease of Mljet were almost identical, it seems highly probable that they are inherited, i.e. determined by the actions of the pathologic gene. This claim does not exclude the role of extrinsic factors in shaping the phenotype. On the contrary, it is probable that the clinical polymorphism of the nails in our cases of Mljet disease, in contrast to their ultrastructural monomorphism, is the consequence of influences of various environmental factors on the genetically determined particular structure of these nails. From the comparison of our electron-microscopical findings it follows that:

1. In the keratinized cells of the free margin of nails of 4 patients, there was significantly more osmiophilic material of varying size, than in the free margin of nails of controls and of obligate heterozygotes.

2. There was little osmiophilic material in the keratinized cells of the nails of controls.

3. In the nails of the three obligate heterozygotes, the findings were intermediate, as here there were more alterations than in the control samples, but fewer than in the samples of homozygotes. However, to answer the question whether one can use the electron-microscopic findings in nails to confirm the heterozygous state of the disease of Mljet, further investigations are required.

4. Histochemically the altered nail contains amorphous substances that react positively to Baker stain, as well as to Sudan IV. These substances are almost certainly lipids. The nails of controls also contain Baker-positive and Sudan IV-positive substances, but in smaller quantity. Light-optically, many slits were found in the altered nail of the disease of Mljet. There were also a few in the sample from a 68-year-old healthy control.

5. In the nail keratin of aged persons (of a control, a heterozygote and one person affected by the disease of Mljet) strongly osmiophilic matrix was found. It is possible that its presence is an expression of the ageing of keratin.

It is perhaps probable that the morphologic alterations found in the nails of patients affected by Mljet disease are caused by some genetically determined disorder of enzyme activities, responsible for the regulation of normal turnover of lipids in the keratin fibrils and cell membrane. Lipids are considered to be essential constituents of keratin fibrils (4) and of cell membranes (5, 6). Although the investigations of Elias et al. (7, 8, 9) demonstrated that there are higher concentrations of hydrophilic materials in the membranes of the stratum corneum than in the cytoplasm of keratinocytes, this author (9) concludes with the following assertion: "An ever increasing number of acquired and genetic disorders of keratinisation appear to be accompanied by lipid abnormalities ... It seems evident that skin scaling abnormalities are related to aberrant skin lipid metabolism. We would predict that over the next few years links will be firmly established between several keratinizing skin diseases and altered skin lipids."

The structure of the nail differs from that of stratum corneum. However, the nail consists of peculiarly keratinized cells. The disease of Mljet is an autosomal recessively inherited anomaly of keratinization. The results of our work demonstrate that that there are links between the faulty keratinization of the nails in this disease and the alteration of skin lipids.

It seems that the electron-microscopic examination of the free margin of the nails is an interesting, though as yet neglected method of investigation which could enable us to gain knowledge of unknown pathogenetic mechanisms of some skin disorders.

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