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CELL ULTRASTRUCTURE IN DISEASE

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Abstract

The doctor of today must adopt the 'cellular way of thinking' in the evaluation of diseases. This ultrastructural outlook provides him with much indispensable information that also serves a practical purpose. A diseased cell organelle is at the basis of every clinical sign and any attempt of therapy must be aimed at that specific point of lesion. We intend, in the light of a long experience, to propose to clinicians a new way of thinking in which a precise correlation between symptoms and submicroscopic changes of the cell is considered. Many different examples amply justify this proposal. Electron microscopy can contribute by enabling a) identification of structural subcellular modifications suitable for the finest differential diagnosis, b) more and more complete understanding of pathogenic pathways of various diseases, c) the establishment of guidelines for precise pharmacological interventions at the molecular level.

KEY WORDS: Electron microscopy, diagnostic pathology, glomerulopathy, myopathy, lysosomal storage disease, atherosclerosis, polyarthropathy, immotile-cilia syndrome.

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Introduction

A doctor today must have a 'cellular way of thinking' when evaluating a disease. The ultrastructural outlook will provide him with much indispensable information also for practical purposes. A diseased organelle is at the basis of a clinical sign and any therapeutic attempt must aim at the specific point of lesion.

The electron microscope has been responsible for a morphological revolution during the last thirty years, opening new frontiers in pathology. Instrumental and methodological progress allows at the moment not only a structural identification of a specific lesion, but also a correlation between morphology and function.

Till now, many reviews of the application of electron microscopy to medicine have described different ultrastructural alterations in various diseases (Bronzini 1981, Burns et al. 1975, Johari et al. 1980, Ghadially 1980, 1983, Johannessen 1978-1985, 1984, Laschi 1973, 1974, 1980, McKay 1981, Mukherjee 1982, Trump and Jones 1978-1982); thus, new information that is particularly useful from a diagnostic point of view has been supplied to pathologists.

In this paper we would like to point out a new opening in research, namely a precise correlation between the patient's symptoms and the submicroscopic changes of the cell. We think that it is indispensable to examine diseases differently than in the past and to consider pathology from another angle, that is, not only as a classification and diagnosis of disease, but as an approach to abnormal biology. By this pathobiological view of disease it will be easier to explain clinical signs with the morpho-functional changes at a molecular level. Thus we can daily improve our understanding of many diseases and the electron microscope is an indispensable tool in this progress.

In front of a patient showing edema where the laboratory tests have shown proteinuria, the first thought must obviously be one of a

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lesion of the morphological basis of glomerular filtration. Electron microscopy has shown a complexity and variety of the components of this filter apparatus which may partially or totally be involved causing the same symptom whatever is the cause of the clinical condition. Renal biopsy becomes the sole test that is able to solve the questions of differential diagnostics and to direct the therapy properly (Casanova and Laschi 1985). In fact, it is possible to detect: fine changes that are otherwise invisible as limited to the foot processes of the podocytes (minimal change glomerulopathy); thickening of the basement membrane without morphological alterations (diabetic glomerulopathy); thickening of the basement membrane with inner morphological alterations (lamellation in hereditary nephritis) or by apposition of outer material (immunodeposits in membranous glomerulopathies) (Fig 1).

During acute renal failure with reduction of filtration rate and hypertension a characteristic intracellular modification can be detected in both the proximal and the distal tubules and is seen as a prominent increase in microfilament bundles. Immunoelectronmicroscopy indicates a contractile function of the bundles and a hypertrophy may well be considered as a sign of a compensatory mechanism to enhance intratubular pressure of urine.

Icterus suggests a lesion of the liver cell involving one or several of the membrane systems, both intracellular and parietal. Membrane integrity is a necessary condition of individual and social homeostasis of both hepatic cells and other cells. Microvillar changes at the bile capillary level are ultrastructural markers of cholestasis even if submicroscopic characteristics distinguishing intrahepatic and extrahepatic cholestasis are as yet lacking. Research and localization by electron microscopy of the different antigens of viral hepatitis may be particularly valuable to define the disease (Laschi and Busachi 1982). The characterization of Ito-cells has been another important discovery in electron microscopy and the phenotypic modulations they undergo, both quantitatively and qualitatively, make it possible to foresee with greater reliability the fibrotic evolution of liver diseases (Fig 2).

Cardiologists may sometimes be confronted with patients having atypical chest pain. Electrocardiography may display ischemia-like changes and conduction defects, which arouse a suspicion of ischemic cardiopathy, however coronary arteriography may be normal. Endomyocardial biopsy, under these conditions, is particularly useful and may at the ultrastructural level show changes in some of the structural features of cardiomyocytes (mitochondria, intercalated discs) (Fig 3) which will indicate the metabolic shortcoming responsible for the defects in stimulus transmission (Laschi et al. 1986).

Pediatricians are frequently confronted with babies that are affected by neuromuscular hypotonia. The diagnostic and prognostic perspectives differ in different diseases. Laboratory data and electromyography are frequently

Fig 1: Membranous glomerulonephritis in a 30 year old woman. The glomerular capillary basement membrane appears thickened by apposition of dense subepithelial deposits. Bar = 2 µm.

Fig 2: Chronic hepatitis affecting a 35 year old man. An Ito cell appears during its phenotypic transformation from lipocyte to fibroblast-like cell. Numerous collagen fibrils are present in the extracellular space. Bar = 1 µm.

Fig 3: Atypical metabolic cardiomyopathy in a 54 year old man. Intercalated discs show characteristic bullous dilatations of the gap junctions. Bar = 1 µm.

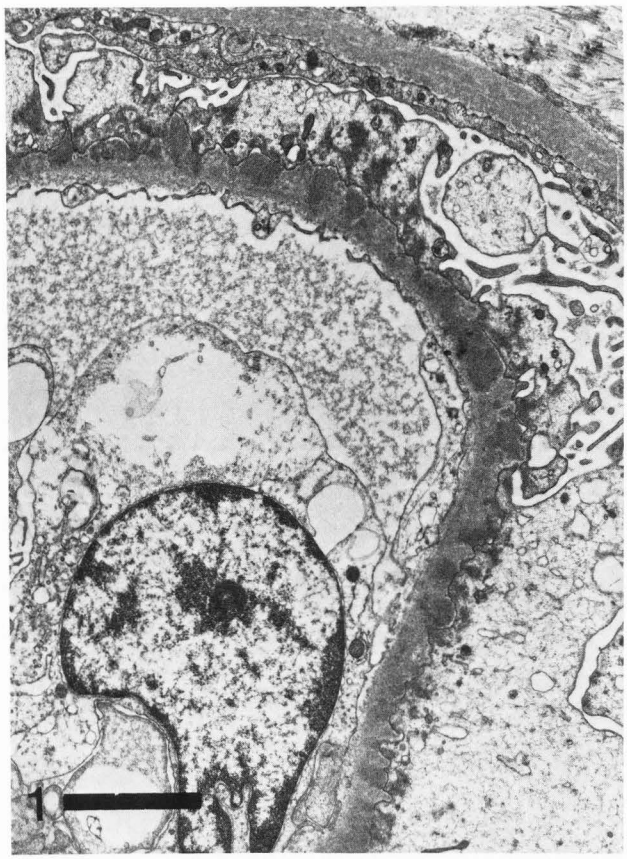
Fig 4: Nemaline myopathy in a 4 months old floppy infant. The muscle cell shows accumulation of typical electrodense rod bodies. Bar = 2 µm.

insufficient, while an ultrastructural investigation of a muscle biopsy sometimes is conclusive. Indeed, it may show specific alterations of single structures of the skeletal muscle cell (Fig 4), characteristic of a group of congenital non-progressive myopathies (centrofibrillar, centronuclear, nemaline, and others) with a prognosis that differs from that in muscle dystrophy or spinal atrophy.

Scanning electron microscopy allows a visualization of large surface areas with three-dimensional images at high resolution. It is the tool of choice in numerous patho-morphological studies and its usefulness in biomedical research becomes more and more evident. Gastrointestinal pathology offers a good example (Bonvicini et al. 1985). In peptic ulcer the study of the duodenal epithelial lining (Fig 5) and its microvilli will allow to detect very early lesions (where endoscopy would often show a normal morphology!), different phases of the disease, cicatrization modes, response time to specific drugs during the healing processes and probably even some markers in patients at major risk of relapse. Moreover, backscattered electron imaging seems to be of great potential value to discriminate between elements of different atomic numbers (Scala et al. 1985).

Many storage diseases are due to a specific congenital alteration of the organelle called lysosome (lysosomal diseases), in which one or more of the acid hydrolases may be absent or deficient. Correlation between the clinical pattern and the ultrastructural aspects of the stored material within the lysosomes of parenchymal (Fig 6) or blood cells may be immediate.

In atherosclerosis, after many years devoted to the classification of risk factors, now the ultrastructural investigation of the arterial wall (Fig 7) shows such 'views' that allow a new interpretation of the disease evolution and its possible regression (Laschi 1985). Smooth muscle cells and their transformation secreting different macromolecules of the extracellular matrix represent a particularly stimulating aspect of cell pathobiology. The present aim is to identify lesions susceptible to pharmacological treatment before the point of no return.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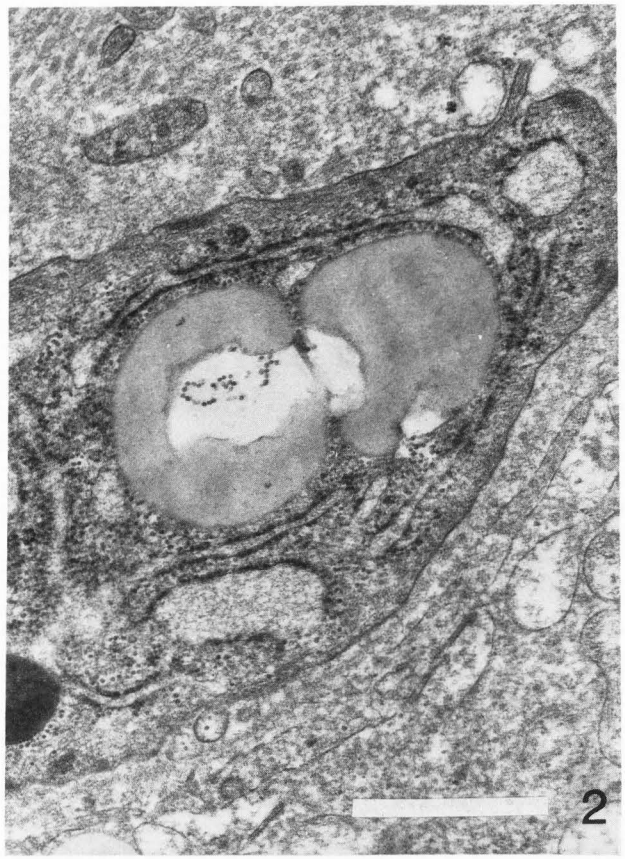
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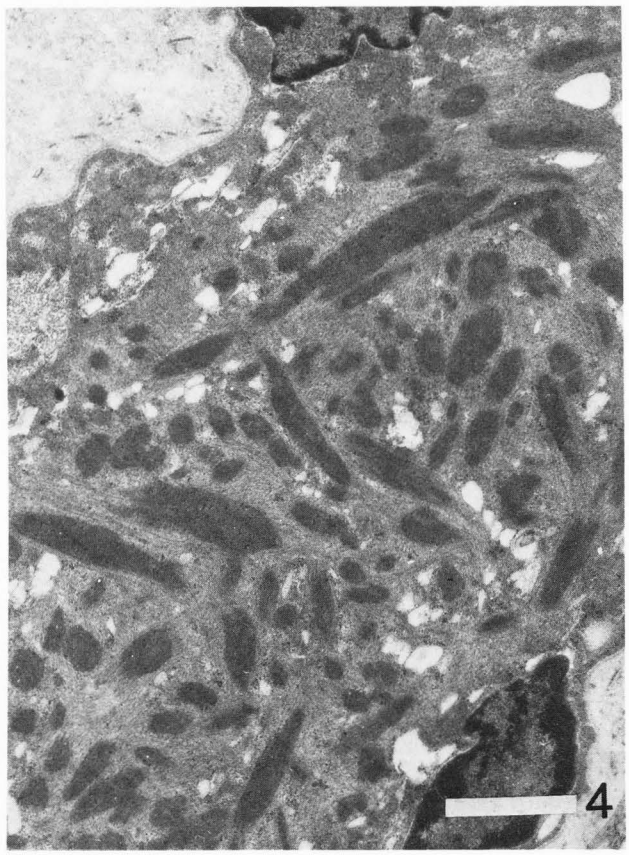
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There are some rheumatologic patients who suffer from polyarthropathy of indeterminate nature in which the detailed morphological analysis of joint effusions is the only way to understand quality and stage of the disease. The study of synovial fluid by polarized light microscopy is sometimes doubtful and of questionable value. Electron microscopy may show within the cells of the exudate (Fig 8) microcrystals of a minimal size (whose aspect differs depending on the chemico-physical structure), well explaining the clinical findings: relapsing pains and swelling evidently are due to a precipitation of microcrystals into the synovial cavity (Laschi et al. 1986). Moreover, the application of X-ray microanalysis allows the characterization of the elemental composition of the intracellular microcrystals. By this tool the pathogenesis of a new important disease, the hydroxyapatite rheumatism, has recently been elucidated (Cenacchi et al. 1985).

Another disease that today can be referred to a specific ultrastructural lesion and that is characterized by various symptoms (such as chronic infections in the respiratory tract, living but immotile spermatozoa, and in about half the cases situs viscerum inversus) has been termed the immotile-cilia syndrome because of its ultrastructural characteristics: a partial or total absence of the dynein arms in the axoneme of cilia or sperm tails (Afzelius 1976). Some variants of the lesion have been described and may characterize the disease, maybe at a different degree of severity; these variants include absence of only the outer dynein arms or only the inner ones, defective or absent spokes or spoke-heads.

Conclusion

We hope to have been able to give a sufficiently clear picture that shows how an ultrastructural feeling and knowledge can help both pathologists and clinicians in the study and understanding of many diseases. Already a new era is coming, full of further interest due to the new techniques that are being developed in ultrastructural research.

We are still unable to exploit the full potential of the modern electron microscopes, both the transmission and the scanning microscopes. Biological specimens are not analyzed optimally: whereas we can see a gold particle we cannot see a molecule of albumin because it has not been prepared in the correct way. All of us should be engaged in the study of new methods for specimen preparation and manipulation, which will involve the interaction of a wide range of scientific disciplines.

Immunocytochemistry will be one of the most important techniques in the study of diseased cells in the next few years. In this respect investigations on new fixatives and in particular new embedding media or improved cryotechniques that will adequately preserve antigenicity and ultrastructure are indispensable. Moreover, scanning electron-immuno-techniques will add further dimensions to ultrastructural immunology.

Fig 5: Duodenal epithelial cells from a 37 year old man with peptic ulcer. By SEM minimal surface changes are seen, such as microvillar blebs. A small area of disepithelialization is shown in the center. Bar = 5 µm. →

Fig 6: Neuronal cytoplasm from a 1 year old child with GM_2-gangliosidosis type I (Tay-Sach's disease). There are hypertrophic lysosomes showing characteristic concentric/ whorled pattern of lipid leaflets. Bar = 1 µm.

Fig 7: Carotid atheroma from a 60 year old man. Three-dimensional view of numerous new microvessels along the plaque thickness. Bar = 10 µm.

Fig 8: Gout arthritis from a 55 year old man. The mononuclear phagocyte of the synovial fluid contains intralysosomal rod-shaped microcrystals. Bar = 2 µm.

The freeze-fracture technique is particularly useful in its ability to demonstrate cell surface specializations and will probably gain importance in pathology. Recently different types of malignant cells were distinguished on the basis of the organization of their tight junctions (Mukherjee 1982).

In conclusion, electron microscopy has greatly contributed to an improved understanding of medicine by carrying out observation of the patient's innermost details. We think that the best is yet to come, particularly if exchange of knowledge and collaboration will continue to occur between biologists, pathologists and clinicians.

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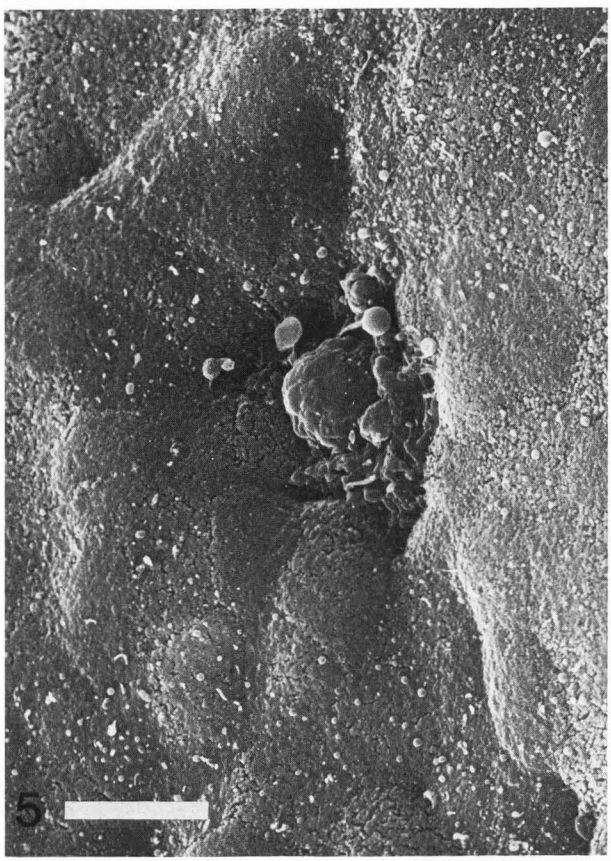
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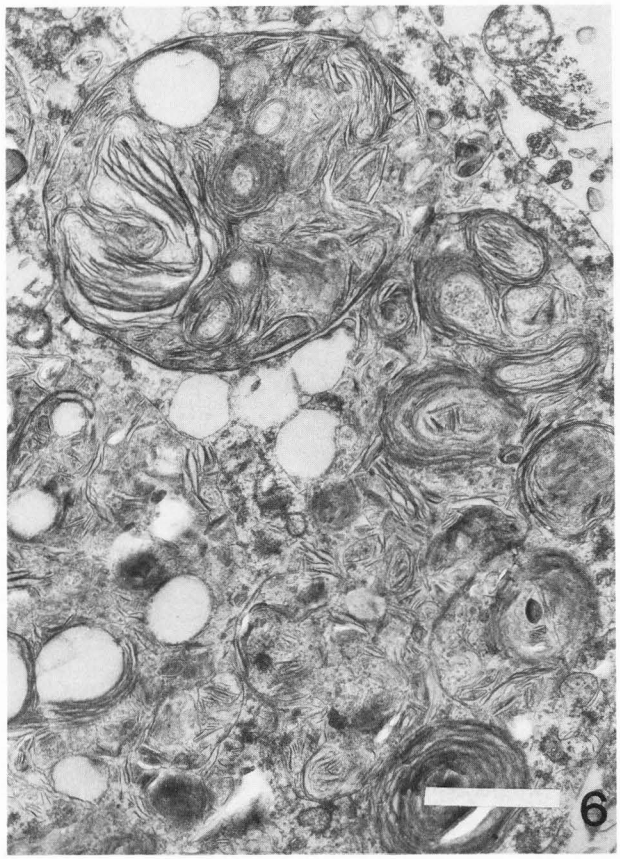
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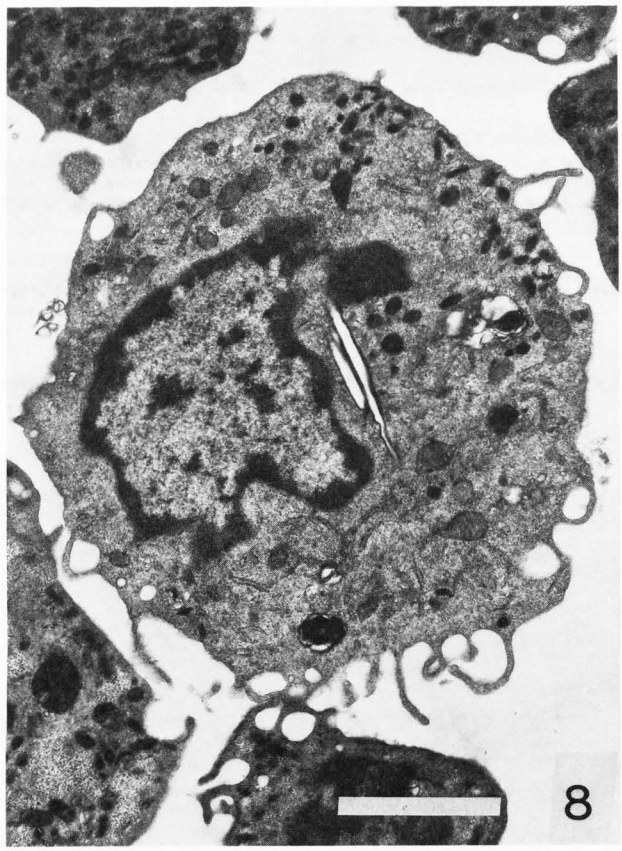
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Discussion with Reviewers

S.O. Bohman: This is a very elegant overview of ultrastructural pathology. I would like to ask you for your opinion about a practical matter in this context: How should one make sure that appropriately fixed tissue is available for EM from the relevant cases in a large material of surgical specimens? Should EM-tissue be collected from all surgicals and biopsies or do you think that the cases suitable for EM-investigation can be selected beforehand, i.e., before the paraffin sections have been studied?

Authors: It is important to distinguish between bioptical and surgical specimens. In the case of biopsies, the clinical criteria can accurately indicate whether a lesion is suitable for ultrastructural investigation or not. In the case of surgical specimens, basically from tumours, obviously not all specimens have to be prepared for EM, but only those in which one already at the macroscopical level can foresee some doubts about their histogenesis. Anyway, it is well known that both specimens fixed in formalin and those already embedded in paraffin may be recovered for specifically oriented EM analysis. Such specimens are not technically perfect but valid for diagnostic purposes.

S.O. Bohman: I would be very interested to hear about your experience with the ultrastructural diagnosis of Kartagener's syndrome (immotile-cilia syndrome). First, it involves quite a lot of work since the specimen has to be correctly oriented and the sections as well as the staining have to be of very good quality. Second, as I understand it, other processes as for example inflammation due to virus infection may, at least focally induce unspecific ultrastructural changes in the cilia of the respiratory tract. Do you have an opinion about the criteria for accepting a patient for a nasal mucosa biopsy in order to study cilia ultrastructure? In how large a proportion of these cases can one expect to reach a conclusive ultrastructural diagnosis of either normal appearance or immotile-cilia syndrome and how many are difficult to interpret?

Authors: Undoubtedly the preparation of these specimens is very critical for a precise diagnosis. This is the reason why the time necessary for these investigations is much longer than for other diseases. A nasal mucosa biopsy can only be suggested after a complete set of tests measuring the mucociliary clearance has been carried out, in addition to a study of sperm motility. Once these aspects have been considered, a conclusive ultrastructural diagnosis may be feasible. Of course, the pathologist must be able to interpret the state of the nasal mucosa as a whole, without limiting himself to the appearance of the cilia.