By the same author:

Fine Structure of Synovial Joints
Diagnostic Ultrastructural Pathology
Diagnostic Electron Microscopy of Tumours, 2nd edn

To Edna
My wife and grand companion
With unabated love and esteem

Ultrastructural Pathology of the Cell and Matrix

A Text and Atlas of Physiological and Pathological Alterations in the Fine Structure of Cellular and Extracellular Components

Third Edition

Volume II

Feroze N. Ghadially

MB BS (Bom.), MB BS (Lond.), MD, PhD, DSc (Lond.), Hon. DSc (Guelph), FRCPath., FRCP(C), FRSA
Isaak Walton Killam Laureate of the Canada Council, W.S. Lindsay Professor of the College of Medicine and Professor of Pathology, University of Saskatchewan, Saskatoon, Canada

Formerly Reader in Neoplastic Diseases and Senior Lecturer in Experimental Pathology, University of Sheffield, England

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Preface to the third edition

The laudatory reviews and warm reception accorded to the second edition of this book have encouraged me to produce a larger third edition which deals with many more ultrastructural changes and lesions. The task of cataloguing and classifying them is endless as David Lagunoff surmises in his review (*Journal of the American Medical Association* 1982, **248**, 1246). He states: 'For his inhospitality to Perseus, Atlas was forced to bear the burden of the heavens through eternity. Sisyphus, for his greater transgression, became responsible for repeatedly pushing a large stone up a hill and on nearing the summit having it escape his grasp and roll down. I don't know if Ghadially's task in producing his book was freely assumed or thrust upon him, but his labors seem closer to those of Sisyphus than Atlas. He has (in his own words) directed his efforts to "cataloguing, classifying, describing and illustrating virtually every intracellular lesion." Each time he must think he is about to roll the last abnormality up his mountainous catalog, a new group of changes comes rolling out of the journals.'

The task of producing this work was self-imposed in the mistaken belief that it would be quick and easy: by the time I discovered otherwise it was too late to turn back. I have, however, learnt that there is no better way of learning a subject than writing a book about it!

A substantial number of new ultrastructural changes and lesions have come 'rolling out of the journals'. Incorporation of this new knowledge has been accomplished by adding 43 new sections and enlarging and rewriting several old ones. The remaining sections have been revised and updated. The number of: (1) pages has increased from about 950 to over 1300; (2) sections from 186 to 229; (3) electron micrographs from 885 to 1227; (4) line drawings from one to 26; and (5) references from about 3500 to a little over 5800. I trust these additions and changes will enhance the value of this book to those who examine pathological tissues with the electron microscope.

F. N. Ghadially

Preface to the second edition

Ultrastructural Pathology of the Cell and Matrix is a revised and expanded second edition of *Ultrastructural Pathology of the Cell*. The change in title is necessitated by the addition of a chapter on the extracellular matrix (extracellular components). This was done on the advice of colleagues who felt that the usefulness of the book was marred by the omission of such common structures as collagen and elastic fibres.

I wrote the first edition of this book with the aim of cataloguing, classifying, describing and illustrating virtually every intracellular lesion within the covers of a modest-sized volume. It seemed to me that although this goal was clearly impossible to attain, striving to attain it might produce a useful book. The net result was that the first edition was never 'finished', it had to go to press when it had grown to a size and price apparently incompatible with economic viability.

The unexpected demand for the book, leading to two reprintings of the first edition have allayed our (publisher's and author's) fears, and encouraged the production of a larger work that comes closer to the original goal or, to be more accurate, the expanded goal which now includes various extracellular components.

Altogether 56 completely new sections have been added. Many old sections have been enlarged and others rewritten or revised, and the book has grown from 560 pages to nearly 1000 pages. The number of illustrations has increased from 520 to 885 and the references from about 1800 to a little over 3500. I trust that this has enhanced the value and utility of this book to those who use the electron microscope to examine pathological tissues.

F. N. Ghadially

Preface to the first edition

There can hardly be a disease or pathological process where electron microscopy has not added new details and dimensions to existing knowledge. The innumerable published papers and books on the ultrastructure of tissues altered by disease or experimental procedures bear eloquent testimony to the many major contributions made by this technique.

Although the student interested in the pathology of certain systems, organs and tissues such as liver (David, 1964), muscle (Mair and Tomé, 1972), synovial joints (Ghadially and Roy, 1969), kidney (Dalton and Haguenau, 1967) and peripheral nervous system (Babel *et al.*, 1970) is now catered for and excellent books dealing with the ultrastructure of normal cells and tissues are available (e.g. Fawcett, 1966; Porter and Bonneville, 1973); Lentz, 1971; Rhodin, 1974), there is as yet no book from which one may learn in a systematic fashion about the numerous changes that occur in cellular organelles and inclusions as a result of disease or experimental procedures. On confronting an unfamiliar or unknown morphological alteration in some particular cellular structure the questions that arise are: (1) has this been seen before? (2) if so, in what situations has such a change been seen? (3) what is the significance of the change? and (4) how can one retrieve information on this point from the formidable, scattered literature on the ultrastructure of normal and pathological tissues?

It is my hope that this book will help to answer such questions and serve as a brief textbook and atlas of cellular pathology at the ultrastructural level. Within its covers I have collected, classified, described and illustrated various alterations that are known to occur in cellular organelles and inclusions as a result of changing physiological states, diseases and experimental situations.

In keeping with the traditional practice adopted in many past pathology texts, each chapter commences with a discourse on normal structure and function. This is followed by essays devoted to various morphological alterations that have hitherto been witnessed. The introduction and preliminary essays on well known normal structures (e.g. nucleus and mitochondria) are, of necessity, brief. They do little more than set the scene and outline the classification and nomenclature employed. The advanced electron microscopist may find that some of the passages in these essays are of a rather elementary nature, but this material is included on the assumption that some readers may not be too familiar with current electron microscopic concepts and topics. Less well known normal structures (e.g. rod-shaped tubular bodies and nuclear bodies) are dealt with more fully, for information on such structures is often

not easy to find and unfamiliarity with such structures is likely to lead to errors of interpretation.

The sections dealing with morphological alterations and lesions follow a fairly standard pattern in most instances. A brief introduction dealing with matters such as definition, nomenclature, correlations with light microscopy, and historical aspects of the subject is followed by a morphological description supported by accompanying illustrations. After this comes a section where I have listed the sites and situations in which the particular morphological alteration has been seen and the authors who have reported its occurrence. This section often contains numerous references. (Some readers may find these lists irksome, but they are essential for the research worker and student seeking further information.) Then follows a discussion and interpretation of the morphological change under survey. The principle I have followed here is to present as many known theories and ideas as possible even though I may not be in sympathy with some of them. I have also often indicated what I have come to think about the matter as a result of my own studies and reading of the literature. However, I do not feel that an author should judge every issue, and I have, at times, done little more than report as faithfully as I can the views propounded by others. Not all essays follow the above-mentioned pattern for there are instances where the story is told more profitably within a different format. For example, instead of devoting a section to every change in mitochondrial morphology which has been suspected as representing an involuting or degenerating mitochondrion, I have collected these changes into a section entitled 'mitochondrial involution and elimination'.

One of the functions I would like this book to serve is as a gateway to the relevant literature. Since this is not a book primarily devoted to normal structure and function (even though a substantial number of pages are devoted to such matters), the references in sections dealing with well known normal structures are somewhat sparse. When dealing with little known normal structures or with alterations and lesions, I have tried to include virtually every reference on the subject that I am aware of. When such citations are few they are all presented in the text; when too many, I have included review articles and also tried to include the earliest and latest paper on the topic.

Although the format is designed primarily for those who examine pathological tissues with the electron microscope, I hope that this book will also be of interest to the teacher of pathology and the practising pathologist. This book is not for the individual who has no knowledge of cell fine structure at all (there can be few who fall into this category today!) nor is it for the expert ultrastructural pathologist. It is addressed to the much larger intermediate group of workers who may wish to acquire a basic knowledge of general ultrastructural pathology on which they may pursue their own special interests with greater confidence and a wider understanding.

The teacher of pathology attempting to relate classic pathology with the now familiar concepts of cell ultrastructure has had to search through a wide variety of books and journals and at best may only find patchy information. The hospital pathologist, similarly, has up to now had little reason to embark on the exhausting pursuit through the published literature, often in journals which are not on his usual reading list, in search of clues which might lead to a better understanding, or an earlier or more precise diagnosis of human disease. However, in some fields such as the interpretation of the liver, renal and muscle biopsy and the diagnosis of viral and storage diseases and certain tumours the electron microscope is already proving its worth. The scope of electron microscopy will undoubtedly extend into wider areas of diagnostic histopathology. Further, more and more papers in journals of pathology now incorporate the results of ultrastructural studies, and the reader unfamiliar with the range and limitations of electron microscope technology may well be at a loss in attempting to interpret published work.

Today it is not possible to present to the student an up-to-date account of many pathological processes and disease states without discussing ultrastructural changes. Cell injury, cloudy

swelling, necrosis, fatty degeneration of the liver, the detoxification of many drugs by the liver, brown atrophy of the heart and lipofuscin, pigmentary disorders of the skin and melanomas, haemorrhage, haemosiderin, erythrophagocytosis and siderosomes, glycogen storage diseases, lipoidoses, Wilson's disease, silicosis, rheumatoid arthritis and melanosis coli are all better understood by virtue of a knowledge of the underlying fine structural changes.

Correlations between light and electron microscopic findings are singularly interesting and satisfying and I have lost no opportunity of dwelling upon such matters. However, the function of electron microscopy is not simply the resolving of old controversies bequeathed by light microscopists. Electron microscopy is a science in its own right with its practitioners, problems and preoccupations. Many structures such as the endoplasmic reticulum and polyribosomes were unheard of in the light microscopic era, while the structural details of others such as the nucleolus, centrioles and cilia were but poorly resolved. The presence of the nuclear envelope and the cell membrane were suspected and the existence of the Golgi complex doubted. Clearly, alterations in such structures belong to the realms of ultrastructual pathology, and correlations with light microscopy are often tenuous and at times non-existent. Such findings may not have a direct appeal to the light microscopist but such matters cannot be ignored for they have materially altered our thinking about cellular physiology and pathology. Indeed, the main preoccupation of this book is with such matters, but I hope that I have presented this material in a manner which will make interesting reading for both light and electron microscopists.

F.N.G.

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The task of searching the massive literature on the ultrastructure of normal and pathological tissues, classifying and filing reprints and xeroxed copies of papers (in well over a thousand files), preparing reference lists and correcting and organizing the text into publishable format was executed by my wife, Mrs E.M. Ghadially. The expert assistance of our library staff, Dr Wilma P. Sweaney and Mrs Eva Wong in running Medline and Medlar searches on innumerable subjects is gratefully acknowledged. The text was typed by Mrs E.M. Ghadially. Her skill at promptly converting an almost illegible manuscript into well-laid out error-free sheets was a major factor in accelerating the production of this work.

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7

Lysosomes

Introduction (history, classification and nomenclature)

The term 'lysosome' is used to describe a group of membrane-bound particles which contain acid hydrolases. These organelles are now considered to be the main part of an intracellular digestive system (sometimes referred to as the 'vacuome' or 'vacuolar apparatus') capable of digesting or degrading a variety of endogenous and exogenous substances (de Duve and Wattiaux, 1966). The other parts of the system include structures such as pinocytotic and micropinocytotic vesicles and phagocytic vacuoles which transport exogenous substances into the cell.

The classic cell organelles were discovered by morphologists long before they could be isolated and studied by biochemists; but the existence of lyosomes was predicted from biochemical studies on isolated cell fractions (rat liver) before they were identified with the electron microscope (for a historical review, *see* de Duve, 1969). This simple historical fact is of more than academic interest, for while most organelles are characterized by their morphological features, the identification of a structure as a lysosome rests on the demonstration of acid hydrolases within it. It thus becomes necessary to review briefly the historical aspects of this subject.

The lysosome concept began with the work of Christian de Duve and his colleagues in Louvain. During the course of certain unrelated experiments they observed that the acid phosphatase activity of rat liver homogenates and certain fractions thereof (particularly the mitochondrial fraction) was about seven to nine times lower in freshly prepared fractions than in those stored for a few days in the frozen state.

This led to the suspicion that in freshly prepared fractions the enzyme might be located in a membrane-bound structure and was hence not available for reaction with the substrate (structure-linked latency), but, due to freezing and thawing, rupture of the membrane occurred, releasing the enzyme into the medium. Further fractionation of the 'mitochondrial fraction' into light and heavy components showed that most of the acid phosphatase activity resided in the light fraction, while the cytochrome oxidase activity occurred predominantly in the heavy fraction. Thus it became evident that the enzyme (acid phosphatase) did not reside in mitochondria but in a hitherto unidentified membrane-bound structure or organelle smaller than the mitochondrion.

Biochemical studies next revealed that at least four other acid hydrolases showed the same kind of structure-linked latency and sedimented out in company with the particles believed to contain acid phosphatase. Thus the idea of a membrane-bound organelle containing a battery of acid hydrolases was born and the term 'lysosome' (meaning 'lytic body') was coined by de Duve *et al.* (1955) to describe it.

The morphological identification of these particles was accomplished by Novikoff who examined with the electron microscope the lysosome-rich fraction from rat liver prepared by de Duve and his co-workers (Novikoff *et al.*, 1956). From this it became apparent that lysosomes of rat liver were single-membrane-bound bodies with electron-dense contents morphologically identical to the peribiliary dense bodies described earlier by Rouiller (1954) *(Plate 252, Fig. 1)*.

The development and application of cytochemical methods for the *in situ* demonstration of acid phosphatase activity in ultrathin sections of tissues (for references, *see* the review by Novikoff, 1961) not only confirmed the presence of acid phosphatase in the peribiliary dense bodies but also led to the discovery of lysosomes in a variety of cells. Despite criticisms from de Duve, who considered that a battery of acid hydrolases should be demonstrated before a structure was labelled a lysosome, acid phosphatase came to be accepted as a marker for identifying lysosomes *(Plate 253)*. It also soon became evident that these versatile organelles were involved in a variety of cellular functions.

Acid phosphatase activity has now been demonstrated in a variety of diverse structures long known to morphologists. Besides the peribiliary dense bodies, these include various others such as some of the granules in neutrophil leucocytes, the granules in eosinophil leucocytes, hyaline droplets in kidney tubules, haemosiderin granules in macrophages and other cells, and lipofuscin granules in heart, brain and various other tissues. Indeed, lysosomes have been found in virtually all animal cells examined to date, including even mature erythrocytes (page 646). They are also known to occur in many plant cells. Lysosomes are frequently encountered in pathological tissues, at times in greatly increased numbers as in the case of the lupus kidney illustrated in *Plate 252, Fig. 2* and in other situations described later in the chapter.

The enzyme content of lysosomes has been the subject of numerous investigations. Variations in enzyme content have been noted between lysosomes obtained from different tissues, but all contain a battery of hydrolases. About 40 enzymes capable of acting on almost every type of chemical bond have now been descibed (de Duve, 1970). Tappel (1969) gives a detailed list of enzymes discovered in lysosomes. He states that 'data on the enzyme content of lysosomes and other information support the present concept that the following polymeric or complex compounds are hydrolysed in the lysosome; proteins and peptides, DNA, RNA, polysaccharides, the oligosaccharide portion of glycoproteins and glyocolipids, lipids and phosphates'. However, many workers have pointed out (Wattiaux, 1969; *see also* page 612 for further discussion on this point) that lysosomes as a class are somewhat deficient in lipases, and this is one of the reasons why they turn into residual bodies containing lipofuscin.

Many functional and morphological forms of lysosomes have been indentified and named (de Duve 1963; de Duve and Wattiaux, 1966). One such is the primary lysosome (also called 'virgin', 'true', 'pure' or 'original lysosome' and 'protolysosome') which is, in essence, a vesicle containing a battery of acid hydrolases. Here, the contents are usually of a medium or low electron density so that it cannot be unequivocally distinguished by morphological features alone from other vesicular structures in the cell and cytochemical methods have to be employed for its identification. However, certain primary lysosomes such as some of the granules in the neutrophil leucocyte (which are in fact storage granules containing acid hydrolases) are recognizable by their morphological features. Primary lysosomes are thought to be produced in a manner akin to zymogen granules and other secretory granules. The hydrolytic enzymes,

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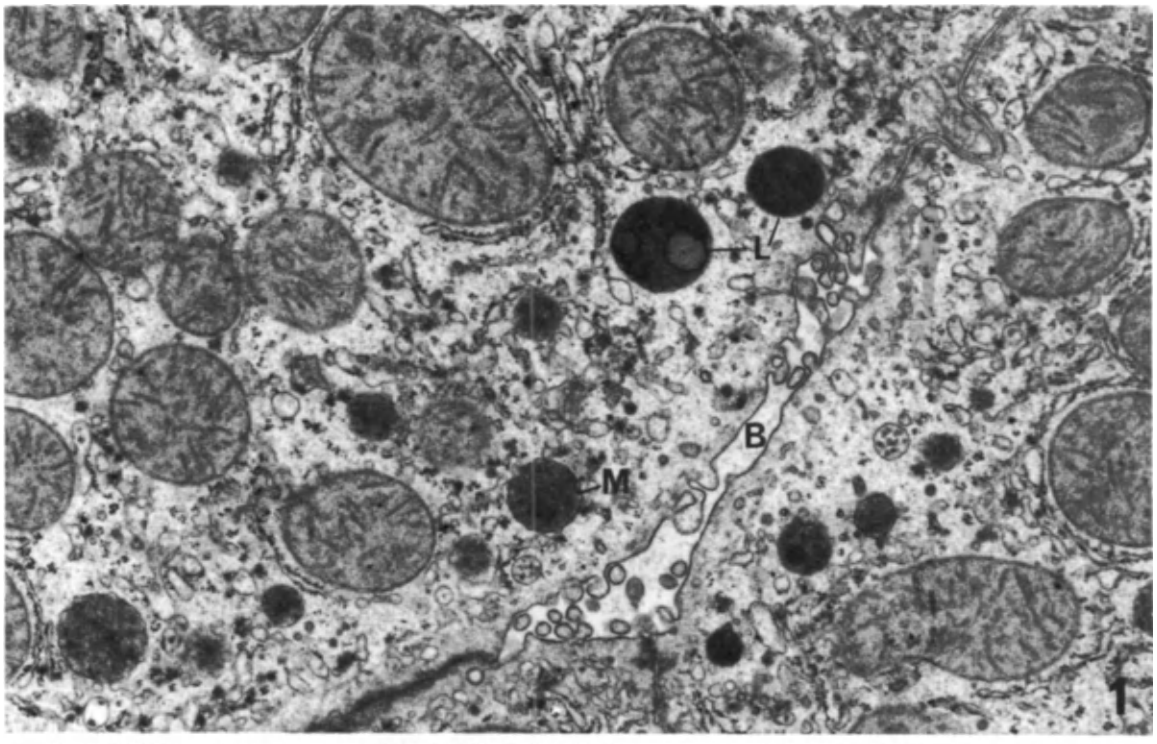
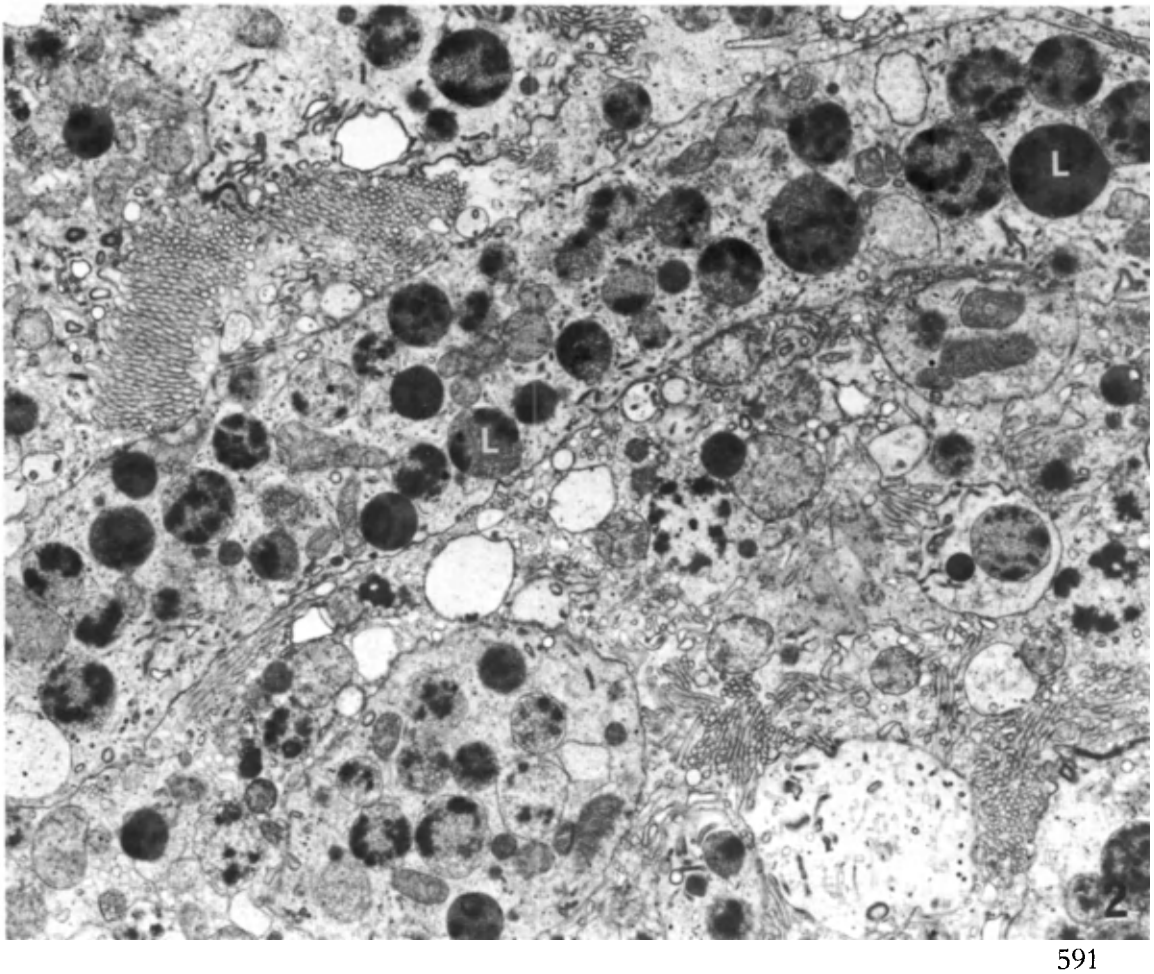
Plate 252

Fig. 1. Normal rat hepatocytes, showing a bile canaliculus (B), some microbodies (M) and peribiliary dense bodies now known to be lysosomes (L). × 21 000

Fig. 2. Disorganized tubular epithelial cells from the kidney of a patient with systemic lupus erythematosus, showing a markedly increased number of lysosomes (L). Similar but far fewer lysosomes are also found in the normal kidney. × 8000


L
B
M
1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2

being proteins or glycoproteins are believed to be elaborated by the polyribosomes on the rough endoplasmic reticulum. Subsequent stages of primary lysosome formation are, however, debatable. In some sites the classic route from rough endoplasmic reticulum to Golgi complex is probably taken, followed by packaging of enzymes into smooth membrane-lined vesicles. In others, it is suggested that the primary lysosomes are formed by a pinching-off process directly from the rough endoplasmic reticulum or that the hydrolases are first transported to the smooth endoplasmic reticulum and the primary lysosomes originate from this structure. The role of the endoplasmic reticulum and Golgi-associated vesicles in the formation of primary lysosomes is stressed in the GERL (Golgi, endoplasmic reticulum, lysosomes) concept of Novikoff *et al.* (1964).

The primary lysosomes, however derived, contain hydrolytic enzymes but no substrate to act upon. Structures in which the enzymes confront substrates and digestion ensues are called secondary lysosomes. Since the substrate may be derived exogenously or endogenously, two main types of secondary lysosomes are possible, namely heterolysosomes (phagolysosomes) and autolysosomes (cytolysosomes, autophagic vacuoles, cytosegrosomes, sites of focal cytoplasmic degeneration or degradation). (Heterolysosomes and autolysosomes are discussed in greater detail on page 594.) At times exogenous and endogenous material may occur in the same secondary lysosome, when the term 'ambilysosome' may be employed.

The term 'residual body' (telolysosome) is employed to describe late forms of secondary lysosomes, however derived, where digestion is nearing completion or is completed and enzymic activity is scant or absent. Such bodies are loaded with undigested electron-dense residues of various kinds. Lipofuscin granules and haemosiderin granules (siderosomes) of light microscopy are now regarded as examples of residual bodies.

Certain enzyme-less members of the vacuolar system are called prelysosomes. These contain sequestrated material destined for lysosomal digestion. The only clear-cut example of this is the phagosome renamed by de Duve and Wattiaux (1966) as a heterophagosome. This is a single-membrane-bound structure containing material taken up by a process of pinocytosis, micropinocytosis or phagocytosis. Digestion ensues when fusion with a primary lysosome converts a heterophagosome into a heterolysosome. To allow for the possible existence of prelysosomes of the autophagic line the term 'autophagosome' has been coined, and to allow for the possible existence of prelysosomes containing exogenous and endogenous material the term 'ambiphagosome' has been suggested.

In concluding, it is worth noting that the lysosome concept has played an important role in our understanding of many physiological and pathological processes. An understanding of lysosome function and dysfunction is now essential to the understanding of various topics of interest to pathologists, as will become evident on perusal of this chapter.

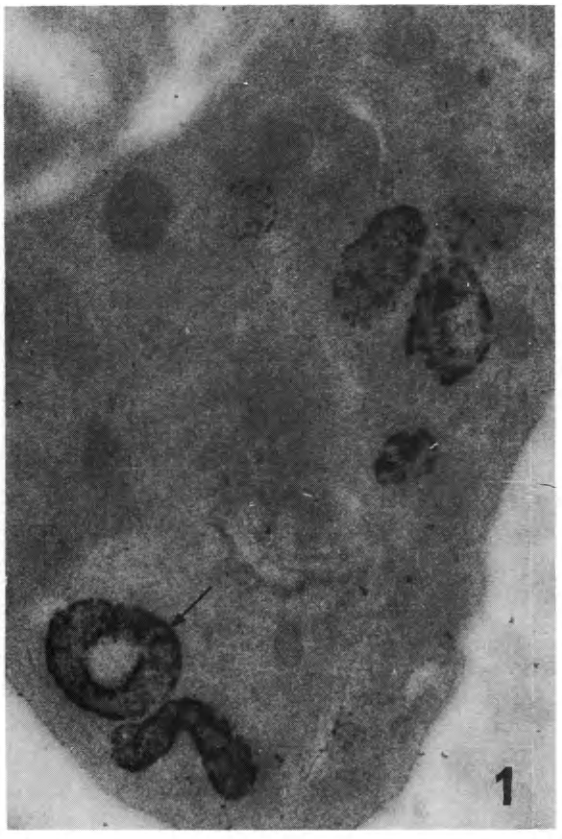
Plate 253

The manner in which acid phosphatase activity is demonstrated in lysosomes is illustrated here with the aid of material from rheumatoid joints. *See also Plate 304.*

For the demonstration of acid phosphatase activity, thin slices of tissue or cells are incubated with Gomori medium which contains sodium-β-glycerophosphate and lead nitrate. At sites of acid phosphatase activity the glycerophosphate is hydrolysed and the phosphate combines with the lead ions, to produce a precipitate of electron-opaque lead phosphate (arrows). Thus the coarse black granular deposits seen in these electron micrographs represent sites of acid phosphatase activity.

Fig. 1. Type A synovial cells from a case of rheumatoid arthritis, showing acid phosphatase activity localized in organelles interpreted as lysosomes. × 21 000 *(From Barland, Novikoff and Hamerman, 1964)*

Figs. 2–5. Synovial fluid neutrophils from patients with rheumatoid arthritis, showing acid phosphatase activity in a variety of lysosomal bodies. × 52 000; × 41 000; × 32 000; × 34 000 *(From Coimbra and Lopez-Vaz, 1967)*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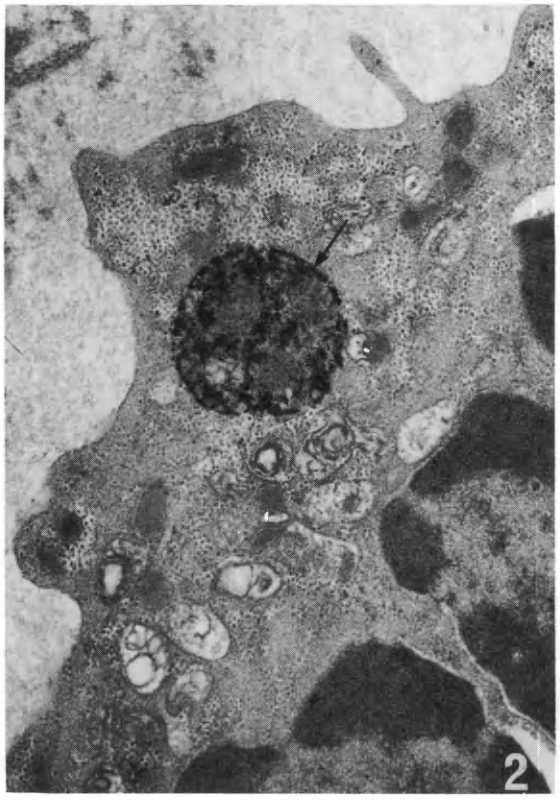
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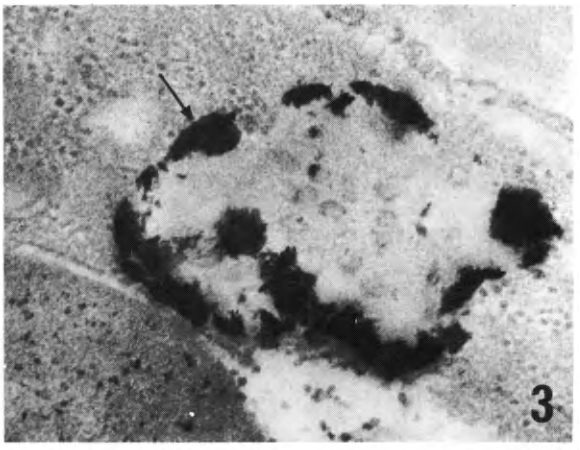
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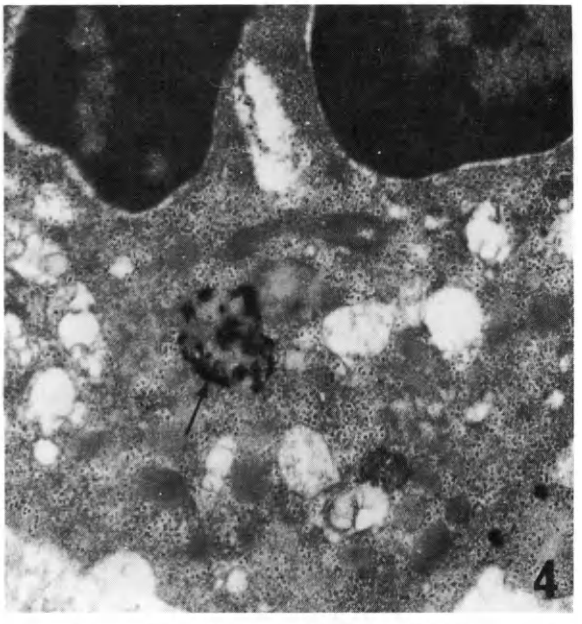
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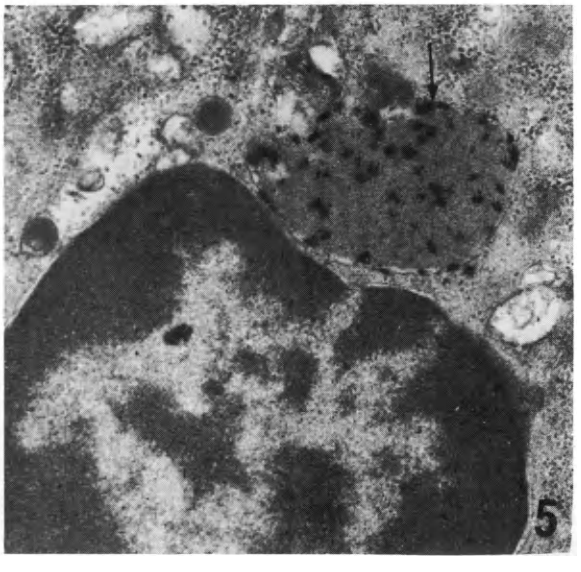

5

Heterolysosomes and autolysosomes

One of the important features of the masterly classification of lysosomes by de Duve and Wattiaux (1966) is that it clearly divides secondary lysosomes into two main classes: (1) heterolysosomes where exogenous material is digested; and (2) autolysosomes where endogenous material suffers digestion. Although the concept and nomenclature are admired and accepted, alternative terms such as 'phagolysosome' instead of heterolysosome, 'cytolysosome' or 'autophagic vacuole' instead of autolysosome and 'residual body' instead of telolysosome are also used at times.

In practice it is difficult to classify all secondary lysosomes as heterolysosomes or autolysosomes. For example, the multivesicular body (page 602), which is a special type of secondary lysosome of characteristic morphology, can be involved in the digestion of either exogenous or endogenous material. To divide these into two varieties, to call them heterolysosomes and autolysosomes and to group them with the others mentioned above seems hardly feasible or desirable.

In practice the distinction between heterolysosomes *(Plate 254)* and autolysosomes *(Plates 255* and *256)* may be simple, difficult or impossible. The heterolysosome is characterized chiefly by sequestrated cell organelles and cytomembranes within its substance, yet such membranes may at times be exogenously derived by phagocytosis of a fragment of another cell. Further partial breakdown of ingested mitochondria or other membranous organelles may render their identification within the lysosome difficult. Despite such problems, in most instances there is little difficulty in distinguishing between these two varieties of secondary lysosomes. More details on this point and on the genesis and evolution of heterolysosomes and autolysosomes form the subject of this section.

Heterolysosome formation has been studied in a variety of cell types. It is now clear that as a result of phagocytosis, pinocytosis or micropinocytosis (collectively referred to as cytosis or endocytosis), ingested material comes to lie within a single-membrane-bound structure called a heterophagosome. In each instance, the limiting membrane of the heterophagosome is derived from the cell membrane. Fusion of primary lysosomes (containing hydrolytic enzymes) with a heterophagosome leads to the formation of a heterolysosome where enzyme meets substrate and digestion of the ingested material ensues in most instances★.

A clear demonstration of this phenomenon was provided by the studies of Straus (1958, 1964) on kidney and liver tissue. He showed that ingested, cytochemically detectable foreign protein (horseradish peroxidase) first accumulates in cytoplasmic vacuoles (heterophagosomes) which stain negatively for acid phosphatase but positively for peroxidase. Later these heterophagosomes fuse with acid phosphatase containing primary lysosomes to form heterolysosomes which stain positively for both acid phosphatase and peroxidase.

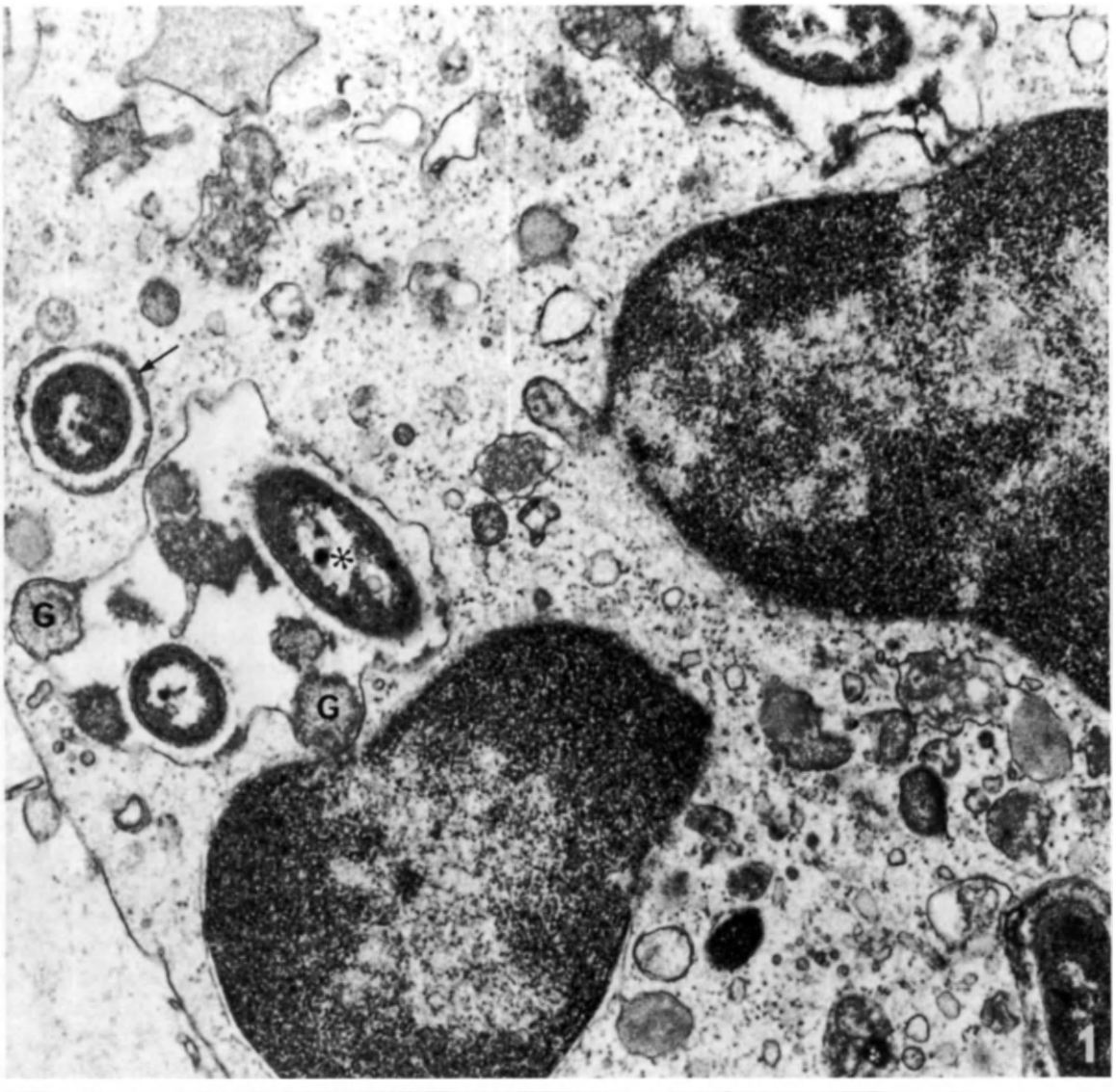
★ Enzymic digestion may be impeded for a variety of reasons, including a relative or absolute deficiency of enzymes, and ingestion of indigestible material or of a substance that inactivates lysosomal enzymes.

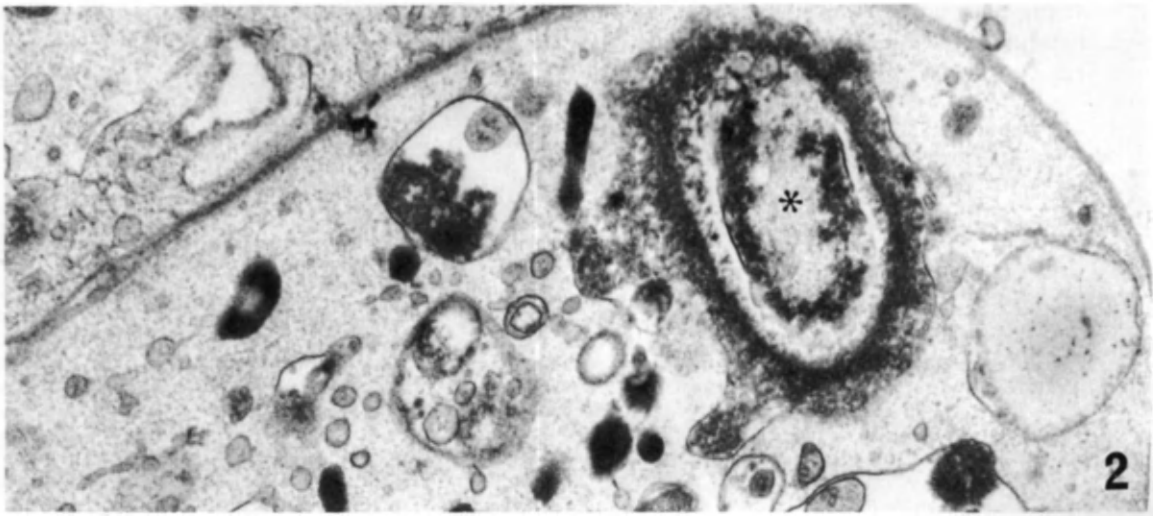
Plate 254

Neutrophil leucocytes containing phagocytosed bacteria (probably *E. coli*) found in the ascitic fluid of a patient with a perforation of the large bowel.

Fig. 1. Many heterolysosomes containing bacteria (★) are seen in this neutrophil. One of these contains recognizable neutrophil granules (G); others contain granule-derived material in a dispersed form at times aligned against (arrow) the limiting membrane of the heterolysosome. × 27 000

Fig. 2. The bacteria in the neutrophil shown in *Fig. 1* do not appear markedly altered but the one (★) seen in the heterolysosome of this neutrophil seems to be breaking down. × 30 000


G
G
*
1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Later studies have shown that when various other substances such as inert colloids, enzymes, haemoglobin and ferritin are taken up by kidney tubule cells, a similar mechanism of heterophagosome and heterolysosome formation operates. The same mechanism operates when neutrophils ingest bacteria *(Plate 254)*, and macrophages ingest red blood cells (page 628). Synovial intimal cells have a remarkable capacity for endocytosis. They can take up small particulate substances such as ferritin, gold, Thorotrast, iron dextran and carbon, or larger particulate matter such as entire erythrocytes, cell fragments and masses of fibrin and joint detritus (for references, *see* Ghadially and Roy, 1967b and Ghadially, 1980b, 1983). Here also, heterophagosome and heterolysosome formation is evident. Protozoa rely on the same method for dealing with ingested material in their food vacuoles (for more references to the above-mentioned studies, *see* de Duve and Wattiaux, 1966; Cohn and Fedorko, 1969: Daems *et al.*, 1969).

Autolysosomes (also called 'autophagic vacuoles'*) usually present as single-membrane-bound bodies containing a portion of cytoplasm bearing organelles such as mitochondria and endoplasmic reticulum and also inclusions such as glycogen and lipid *(Plate 255, Figs. 1 and 2)*. The sequestrated material may be well preserved and easily identifiable or in various states of breakdown and degradation, until a point is reached when one cannot confidently assert whether a given lysosome started out as an autolysosome or heterolysosome. Finally, a residual body containing undigested electron-dense lipidic residues (lipofuscin) is produced *(Plate 255, Figs. 3 and 4)*.

Although autolysosomes are usually bounded by a single membrane, at times a double-membrane-bound autolysosome or a structure mimicking an autolysosome may be encountered. Thus, a double-membrane-bound structure may result from a sequestrated membranous structure (e.g. endoplasmic reticulum) lining up against the wall of the autolysosome. Another possibility is that one may be witnessing an early stage in the formation of an autolysosome where a lamellar extension of the endoplasmic reticulum or a double-membraned cup-shaped structure derived from the endoplasmic reticulum or Golgi complex may wrap around organelles to form autolysosomes *(see below* for more details, and *Plates 256* and *257)*. At least two further possibilities must be considered when such double-membrane-bound structures are sighted: (1) that this is a phagocytosed cell fragment; or (2) that it is a transverse section of a finger-like projection of one cell into another. Obviously, in such instances one would expect the structure to be lined by two membranes, one derived from the cell in which the structure lies and the other from the phagocytosed fragment or the invaginating cell process.

A problem that has vexed many workers regarding the genesis of autolysosomes is how sequestration occurs and how the acid hydrolases gain access into autolysosomes. In the case of heterophagosomes, fusion with primary lysosomes has frequently been observed but in many cases of autolysosome formation the enzymes often seem to be present from the very beginning

* The term 'autophagic vacuole' is used to describe any body of an autophagic nature. Hence this term covers both autophagosomes and autolysosomes.

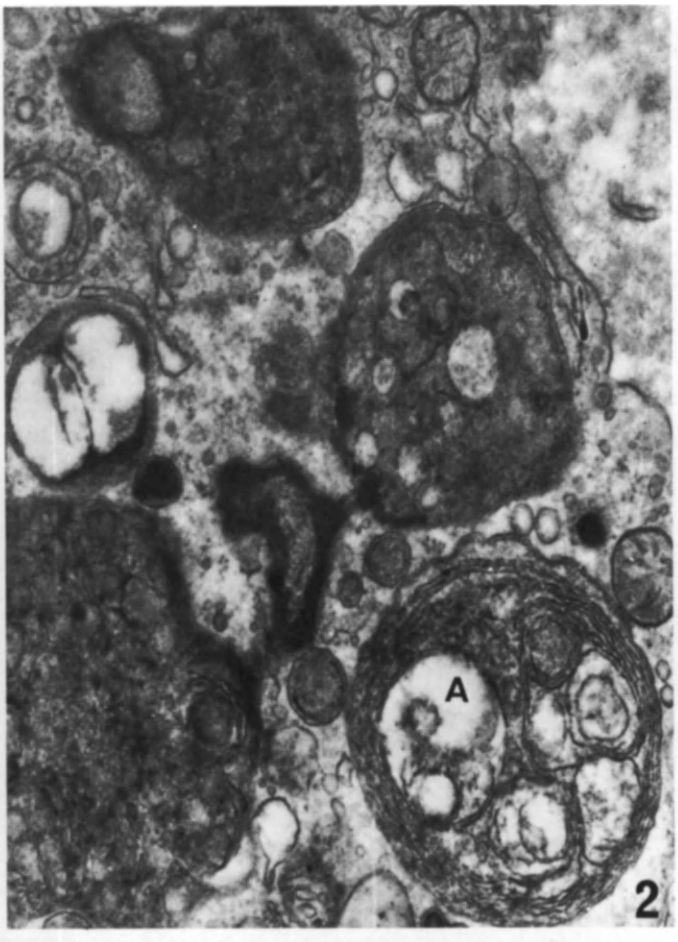
Plate 255

From a carcinogen-induced subcutaneous sarcoma of the rat.

Fig. 1. Autolysosomes with included mitochondria found in a tumour cell. × 36 000 *(Parry and Ghadially, unpublished electron micrograph)*

Fig. 2. This tumour cell contains numerous bodies interpreted as autolysosomes. In one of these (A) the cytomembranes are easily discerned, but in others where lysosomal digestion is more advanced cytomembrane remnants are barely discernible. × 32 000 *(From Parry and Ghadially, 1965)*

Figs. 3 and 4. Lipofuscin-containing residual bodies thought to be produced from autolysosomes of the type shown in *Figs 1 and 2*. Note the basic similarity between these lipofuscin granules and those illustrated in *Plates 261–263*. × 45 000; × 93 000 *(Fig. 3 from Parry and Ghadially, 1965; Fig. 4 Parry and Ghadially, unpublished electron micrographs)*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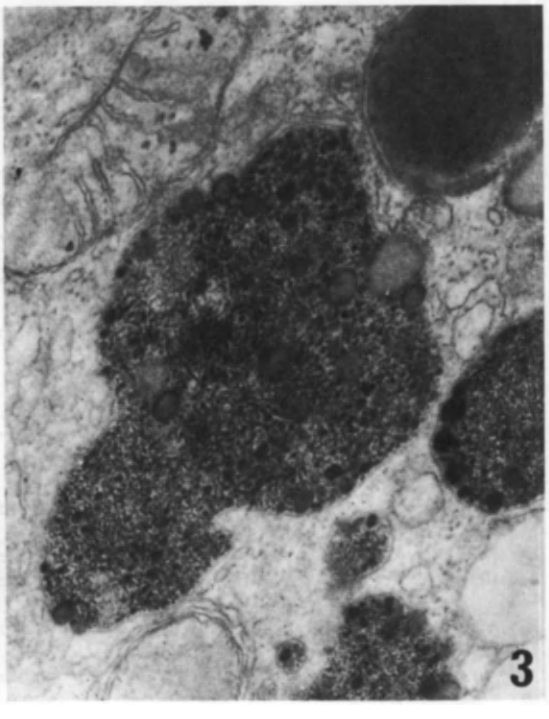
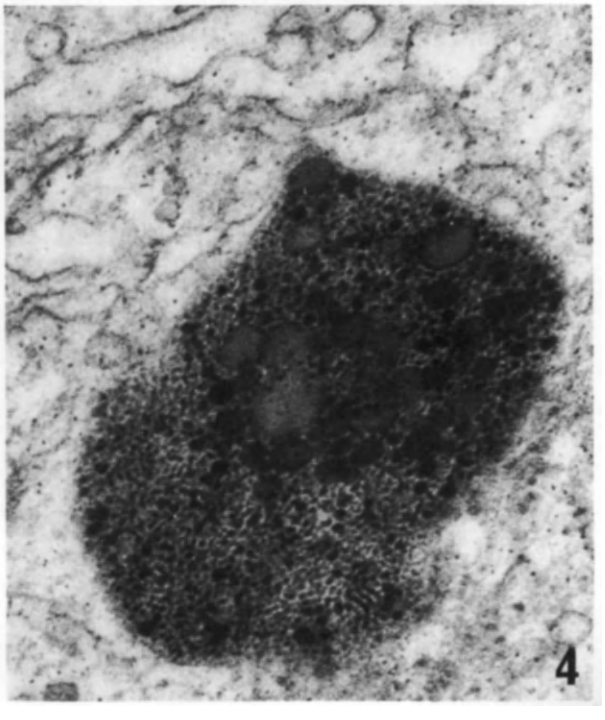




of autolysosome formation. In such cases a clear-cut stage which could be called an autophagosome (i.e. a vacuole containing sequestrated cellular material but no acid hydrolases) is either lacking or difficult to demonstrate*.

Of the many possibilities, the one that is most acceptable is that a lamellar cup-shaped structure derived from the endoplasmic reticulum or Golgi region wraps around the organelle to be sequestrated (page 600). It is likely that the hydrolytic enzymes would already be present in such a structure and either dissolution of the inner membrane or fusion of membranes would release the hydrolytic enzymes to form the autolysosome. Certainly images which could be interpreted in this fashion *(see* Ericsson, 1969, for more details and references) have been seen in situations where autolysosome formation is in progress *(Plates 256* and *257)*. Only slightly different from this is the concept of intracisternal sequestration where organelles 'drop in' dilated cisternae of the rough endoplasmic reticulum. Such a process could be looked upon as one of the ways in which an autolysosome might be formed (page 544), the required enzymes being produced by the polyribosomes on the ensheathing membrane.

However, the possibility that structures containing sequestrated cell organelles may obtain the necessary hydrolytic enzymes by fusion with primary or secondary lysosomes in the cell can by no means be ruled out. For example, it has been reported (for references, *see* Ericsson, 1969) that in Thorotrast- or iron-treated rats the peribiliary-dense bodies become loaded with these electron-dense markers. If now autolysosome formation is induced by partial hepatectomy or glucagon administration, then lysosomal bodies containing both mitochondria and the electron-dense marker are produced, and images are seen which suggest that structures containing sequestrated organelles are fusing with pre-existing secondary lysosomes containing the electron-dense marker.

Occasional autolysosomes may be found in a variety of normal tissues; they are now regarded as a normal mechanism by which old and effete organelles are disposed of by the cell. An increased number of autolysosomes generally indicates a sublethal intracellular focal injury caused by some noxious agent. A very large number of agents can induce autolysosome formation in various tissues. These include mechanical trauma, x-rays, ultraviolet radiation, innumerable chemical agents including carcinogens and various antimetabolites, hypoxia, starvation, endotoxin shock and virus infections (Hruban *et al.*, 1963†; Swift and Hruban, 1964; de Duve and Wattiaux, 1966; Ericsson, 1969).

The lysosome content of tumours is very variable, but both autolysosomes and heterolysosomes have been seen in neoplastic cells (*see* pages 644–645 for details).

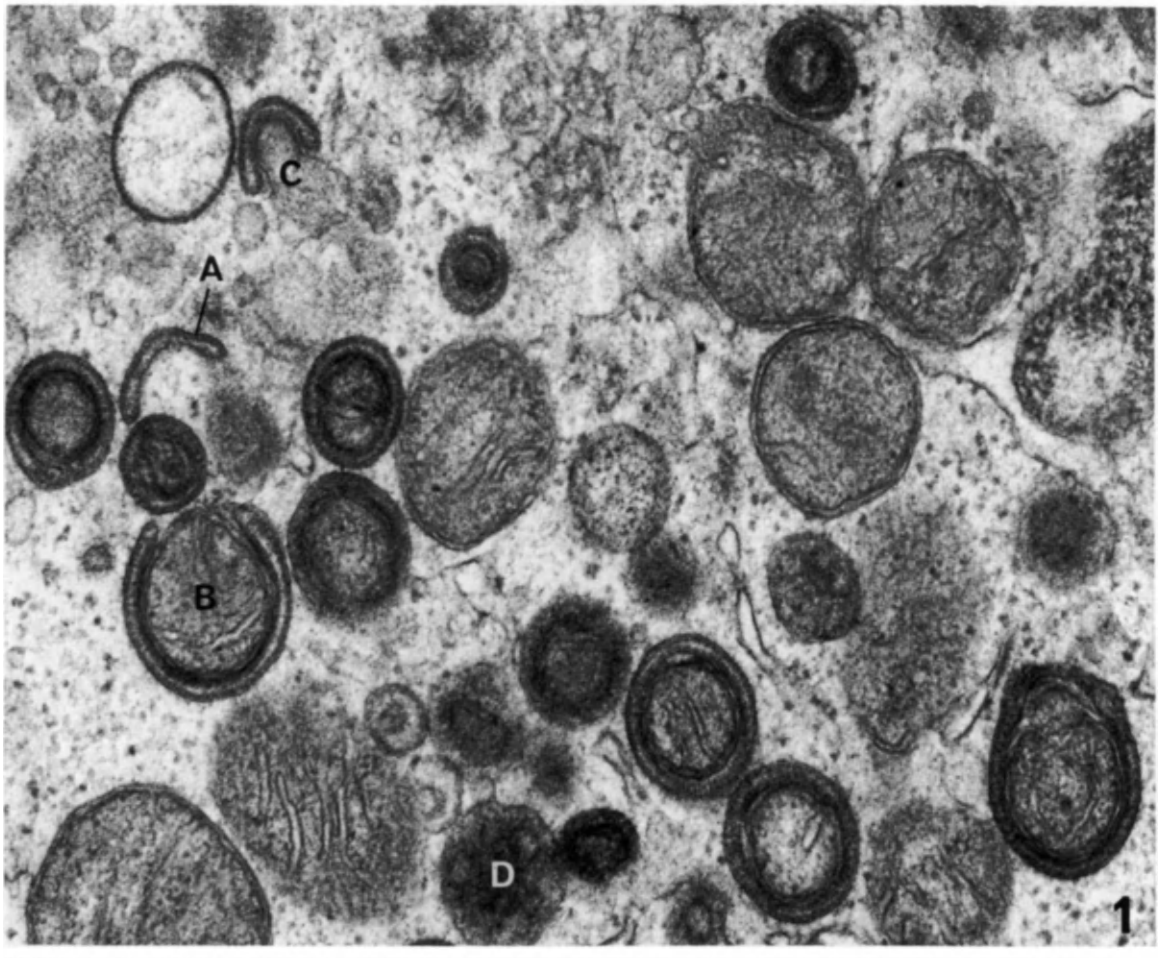
*In newly formed autophagic vacuoles acid phosphatase activity may be demonstrable in: (1) virtually all such structures (e.g. neurons, interstitial cells of the testis and cells of the corpus luteum); (2) a fraction of these structures (e.g. kidney cells and hepatocytes); or (3) almost none of the structures (e.g. cells of pancreas, prostate and seminal vesicles). Those that lack acid phosphatase activity acquire it later by fusion with primary lysosomes, and such events (i.e. fusion with primary lysosomes) may also bring additional quantities of enzymes to others. (For references *see* Réz and Meldolesi, 1980.)

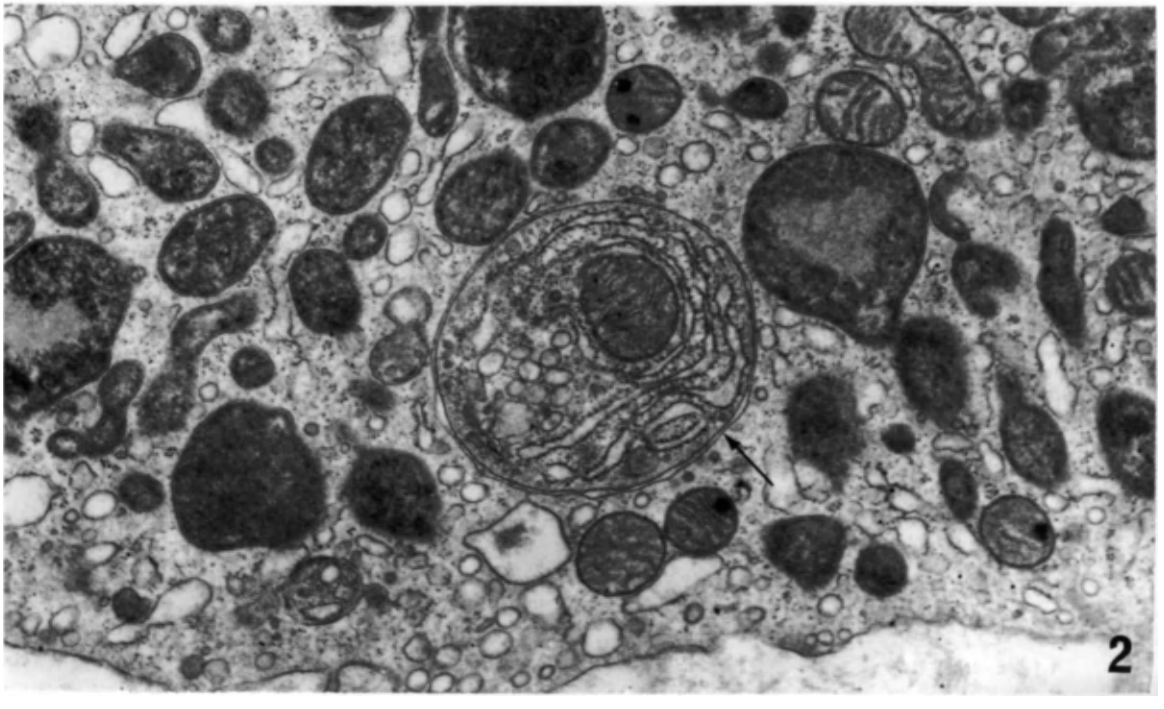
† The study of Hruban *et al.* (1963) on autophagy and degradation of sequestrated organelles (which they called 'focal cytoplasmic degradation') changed the static biochemical concept of the lysosome as a particle containing acid hydrolases into the dynamic morphologic concept of a degradative process and a common reaction to cell injury. In passing, one may note that this paper is a citation classic (Hruban, 1979). It was quoted in the literature 274 times between 1963 and 1979. Citation ratings measure the quality or impact of a work and the worker. Certainly they provide the qualitative assessment that is so glaringly absent when workers are evaluated by the number of papers produced (Garfield, 1979)

Plate 256

Fig. 1. A probable mode of autolysosome formation is depicted here. Cup-shaped bodies (A) are seen lying free in the cytoplasm or wrapping around mitochondria (B) and other structures (C). Various images suggesting the breakdown of enclosed mitochondria and ultimate formation of single-membrane-bound dense bodies (D) are seen. From the megakaryocyte of a six-month-old infant. (Same case as *Plate 286*) × 72000 *(Ghadially and Skinnider, unpublished electron micrograph)*

Fig. 2. A double-membrane-bound body (arrow) which may be interpreted as (1) an early autolysosome, (2) a phagocytosed cell fragment or (3) a finger-like evagination from an adjacent cell. From a case of melanosis coli (page 672). × 29000 *(Ghadially and Parry, unpublished electron micrograph)*


C
A
B
D
1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Lamellar cup-shaped lysosomes

Primary lysosomes are usually vesicular structures (of the coated or uncoated variety) containing hydrolytic enzymes but there occurs a lysosome which is essentially a flattened or lamellar cisterna containing hydrolytic enzymes *(Plates 256* and *257)*. Such primary lysosomes provide not only the enzymes for digestion but also the limiting membrane of some autolysosomes.

As indicated earlier (page 598) some doubt exists as to the source of the membrane which sequestrates a portion of cytoplasm to form an autophagic vacuole. There is little or no evidence supporting the thesis of *de novo* synthesis of enveloping membrane (Rumpelt and Weisbach, 1978) or the idea that the sequestrating membrane derives from the cell membrane, but appearances have been seen which suggest that a lamellar cisterna derived from the endoplasmic reticulum or the Golgi complex wraps around a portion of cytoplasm or an organelle such as a mitochondrion and sequestrates it from the rest of the cell. In other instances lamellar cisternae appear to join together and sequestrate a portion of cytoplasm *(Plate 257)*. In sectioned material such lamellar lysosomes present straight elongated profiles or curved (i.e. C-shaped and circular), profiles suggesting that these are sections through cup-shaped structures. On rare occasions these lysosomes show a more complex branched form.

It will be apparent that sequestration by a cup-shaped lysosome will initially produce a double-membrane-bound vacuole containing sequestrated material (cytoplasm and organelles) in its interior and hydrolytic enzymes between the two limiting membranes. Later dissolution of the inner membrane presumably occurs and releases the enzymes to react with substrates.

Such lamellar, cup-shaped lysosomes have been seen in: (1) normal intestinal epithelium of the rat (Moe *et al.*, 1965); (2) rat liver after ligation of portal vein (Vorbrodt and Bartoszewicz, 1968); (3) denervated and diabetic skeletal muscle of rats (Spicer *et al.*, 1980); (4) dystrophic hamster muscle (Christie and Stoward, 1977); and (5) epidermis of the external gills of the larval frog, *Rana pipiens*, during the period of gill degeneration (Michaels *et al.*, 1971) *(Plate 257)*. In an excellent review, Ericsson (1969) deals with the manner in which autophagic vacuoles develop and he cites many examples where double-membrane-bound autophagic vacuoles have been seen. Some excellent examples of cup-shaped lysosomes occurring in granuloma macrophages are depicted in *Figs. 1* and *2* in a paper by Carr and Wright (1979) but the possibility that these may be lysosomes about to engage or engaging in autophagy is neither considered nor explored. For reasons difficult to understand these authors regard them as 'the secretory granules of the macrophage'. What purpose would be served by secretory granules acquiring so peculiar a shape is difficult to fathom.

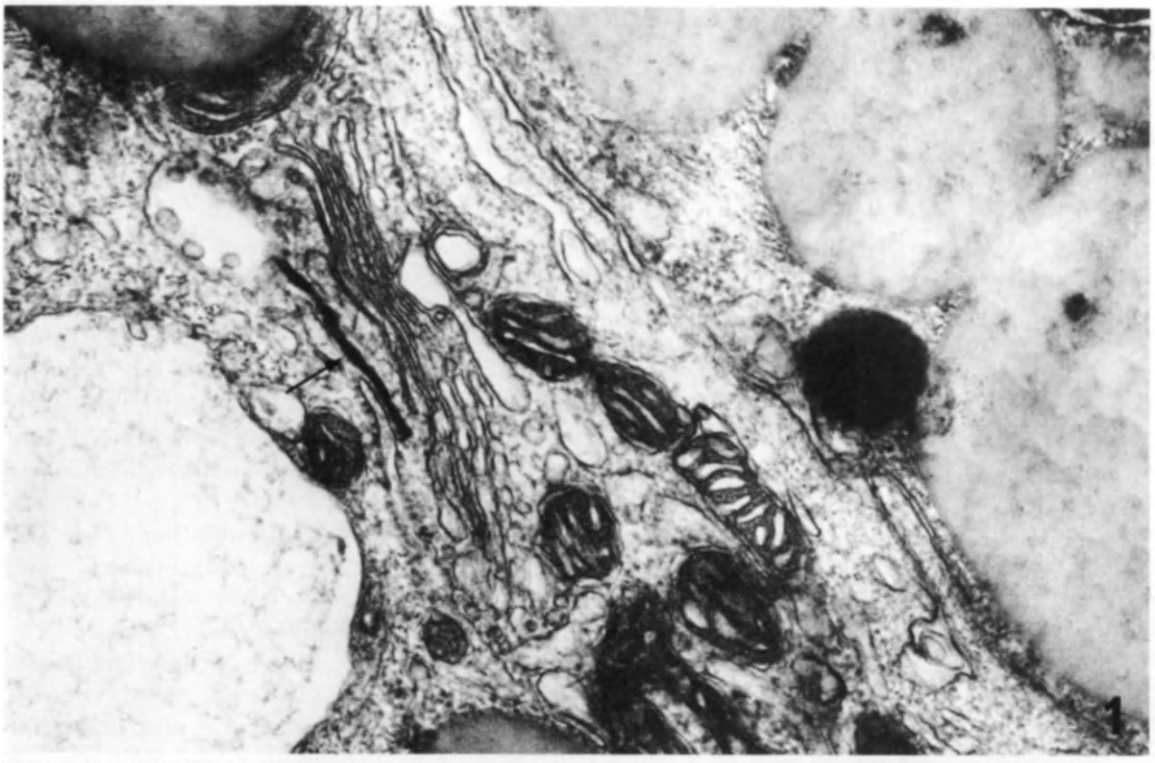
Plate 257

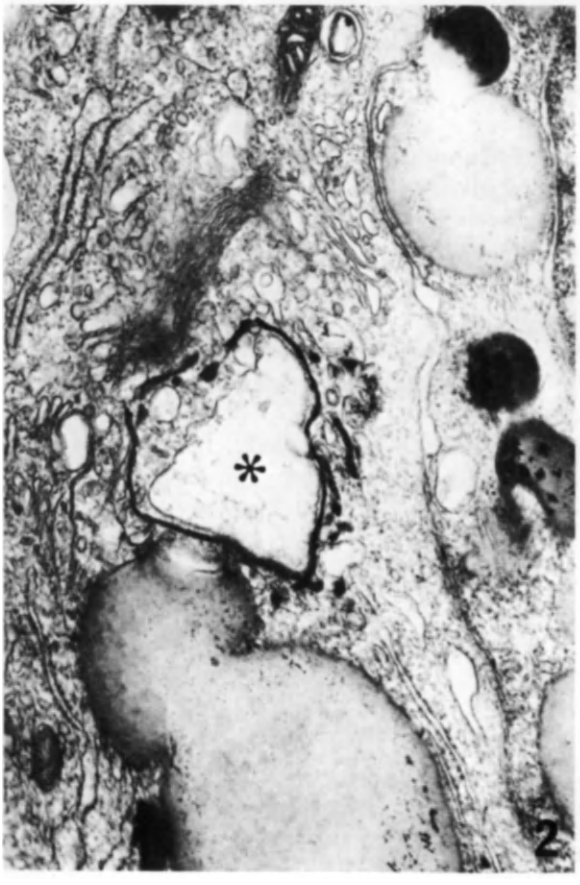
Epidermis of the external gills of the larval frog *Rana pipiens* during period of gill degeneration. One of the ways in which autolysosomes develop is depicted here. Tissue was treated by the Gomori technique for detection of acid phosphatase activity. *(From Michaels, Albright and Patt, 1971)*

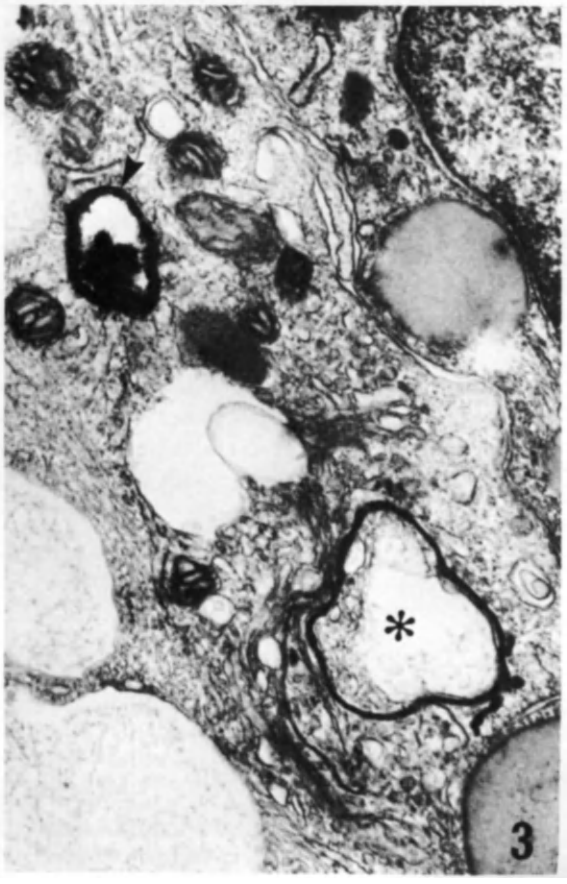
Fig. 1. A single cisterna (arrow) exhibiting acid phosphatase activity has separated from the forming face of the Golgi complex. Such a cisterna containing acid hydrolases may be looked upon as a lamellar variety of primary lysosome. × 40 000

Fig. 2. Lamellar primary lysosomes and vesicular primary lysosomes appear to be curling and joining together to form a cup-shaped primary lysosome which is in the process of sequestrating a portion of cytoplasm containing vesicles and vacuoles (★). × 28 000

Fig. 3. In another plane of sectioning (arrow) the lamellar lysosomes appear to have fused creating a ring-shaped profile sequestrating a vacuole (★) and cytoplasmic material. These two micrographs *(Figs. 2 and 3)* demonstrate a way in which some autolysosomes probably develop. At the top of *Fig. 3* is another lysosome (arrowhead), which would be interpreted as a further stage of development where the enzyme in the 'shell' of the lysosome has been discharged into its interior. × 30 000







Multivesicular bodies and R-bodies

The term 'multivesicular body' is employed to describe a vacuole containing vesicles set in a lucent or dense matrix *(Plate 258)*. Depending upon the density of the matrix, the body is referred to as either a light or a dark multivesicular body. Since acid phosphatase has been demonstrated in multivesicular bodies from various sites they are considered to be a variety of lysosome.

These bodies have been studied in: (1) oocytes (Rebhun, 1960); (2) duodenal epithelium of fetal rats (Moe *et al.*, 1965); (3) HeLa cells (Robbins *et al.*, 1964); (4) neurons (Rosenbluth and Wissig, 1964); (5) adrenal medulla of rats (Holtzman and Dominitz, 1968); (6) anterior pituitary of rat (Smith and Farquhar, 1966); (7) fibroblasts in culture (Gordon *et al.*, 1965); (8) glomerular epithelial cells (Farquhar and Palade, 1962); (9) *Leishmania donovani* (Djaczenko *et al.*, 1969); (10) various tissues of an insect during moult-intermoult cycle (Locke and Collins, 1967); (11) epithelium of vas deferens and epididymis of rat (Friend and Murray, 1965; Sedar, 1966; Friend and Farquhar, 1967; Friend, 1969); (12) choroid plexus of chicken embryo (Meller and Breipohl, 1971); (13) proximal convoluted tubules of rat kidney (Spors, 1971); (14) human monocytic cells *(Plate 258, Figs. 1 and 2)*; (15) human jejunum *(Plate 258, Fig. 3* and *Plate 259, Fig. 1)*; (16) vascular endothelial cells (*Plate 351*); (17) rat myelocytes incubated with chloroquine (Fedorko, 1968); (18) reticulosarcoma (Vasquez *et al.*, 1963); and (19) colonic mucosa of patients with melanosis coli (Ghadially and Parry, 1966b) *(Plate 259, Fig. 2)*.

Some of the above-mentioned studies have demonstrated that exogenous substances commonly employed as tracers, such as horseradish peroxidase and ferritin, are picked up in coated vesicles and are transported to multivesicular bodies, which then evolve into dense structures acceptable as lysosomes and residual bodies. In such instances the multivesicular body may be regarded as a variety of heterolysosome. In other instances, however, multivesicular bodies have been found to behave as autophagic vacuoles or autolysosomes and digest endogenous material such as secretory granules and thus regulate secretory processes within certain cells.

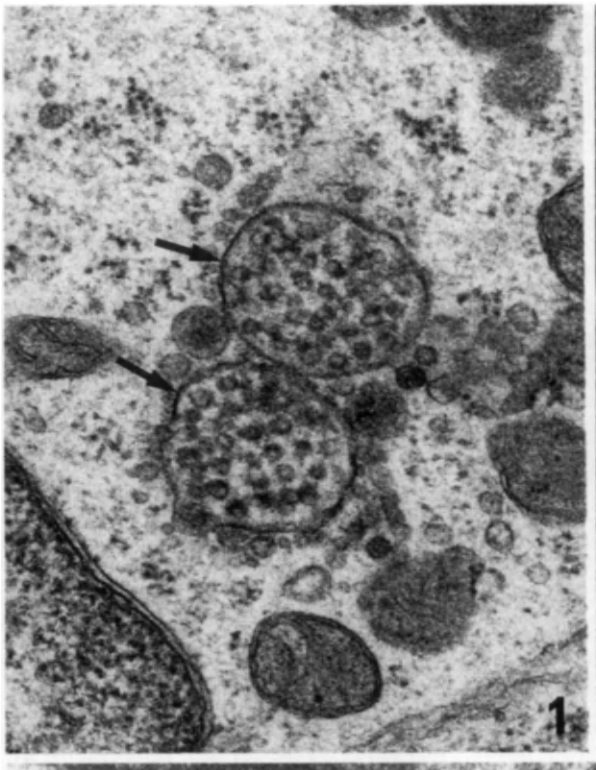
Several investigators believe that the vesicles within multivesicular bodies are primary lysosomes derived from the Golgi region (e.g. Novikoff *et al.*, 1964). In the rat epididymis, however, Friend (1969) has reported that acid phosphatase activity is seen in the matrix of the multivesicular body but not in the vesicles. According to him, the vesicles in the multivesicular body of the rat epididymis are similar in morphology and cytochemical properties to the outer Golgi vesicles (that is to say, the transitional vesicles lying between the endoplasmic reticulum and the outer convex face of the Golgi complex and the vesicles at the ends of the Golgi stacks). Such vesicles and some of the vesicles in the multivesicular body were stained after prolonged fixation in osmium but other vesicular structures in the cell were not. The function of these vesicles in the multivesicular body (mvb) is obscure but it is speculated (Friend, 1969) that 'the mvb vesicles contain enzymes or other substances responsible for the acidification of the mvb'.

Plate 258

Fig. 1. Multivesicular bodies (arrows) found in a circulating monocyte from a case of erythroleukaemia. Rounded vesicles are seen within and adjacent to these single-membrane-bound bodies. × 55 000

Fig. 2. Dark (arrow) and light (arrowhead) multivesicular bodies found in a monocyte from the peripheral blood of a patient with hepatic cirrhosis. Rounded vesicles are seen within and around these bodies. The appearance seen here could be interpreted as vesicles entering or leaving these bodies. × 50 000

Fig. 3. Jejunal biopsy from a seven-month-old infant with protein lymphangiectasis. Dark multivesicular bodies (arrows) are seen in an epithelial cell. The sectioned vesicles in these bodies present circular profiles. × 47 000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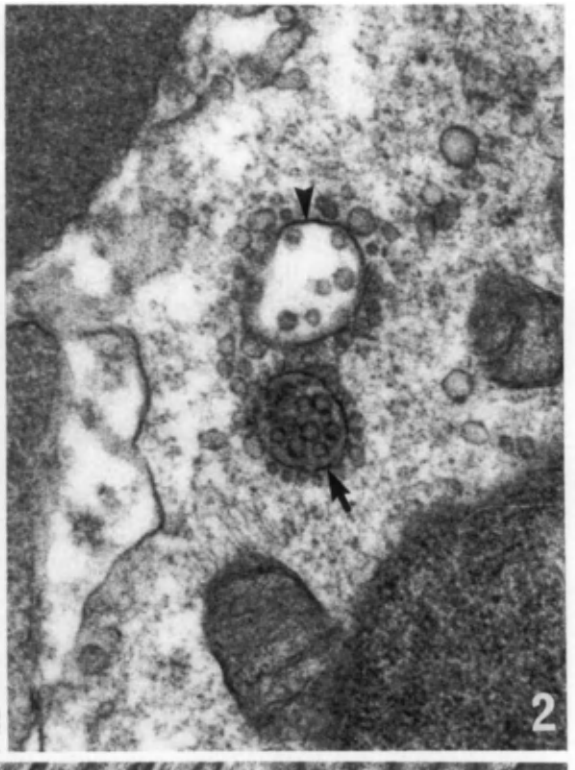
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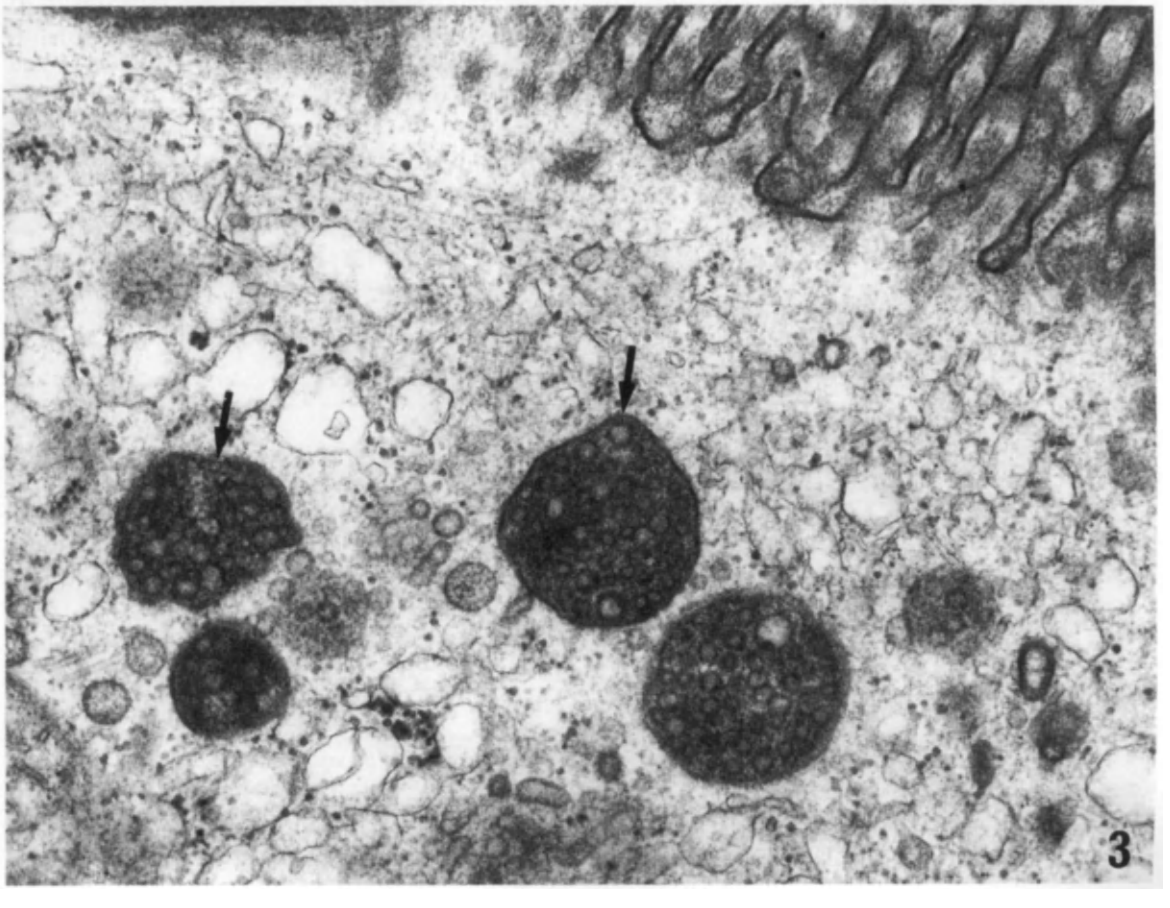
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Another theory regarding the origin of the vesicles within the multivesicular body is that they arise by a process of invagination and budding from the wall of the limiting vacuole. It has also been speculated that endocytic vesicles carrying exogenous material may traverse the wall of the vacuole and find their way into multivesicular bodies (Novikoff *et al.*, 1964; Gordon *et al.*, 1965). Yet another concept is that a multivesicular body may form by autophagy of a cluster of vesicles (Ericsson, 1964; Kessel, 1966). The various ideas mentioned above are not mutually exclusive, and there is no reason whatsoever to believe that all multivesicular bodies arise in precisely the same manner or perform identical functions in all cell types as will be evident from the text which follows.

It would appear that in the colonic mucosa there occurs a special type of multivesicular body which contains varying proportions of round and oval vesicles and also elongated rod-like particles and vesicles (50 nm wide and 100-250 nm long). At one end of the scale we have a body which contains little besides 'solid-looking' rods and at the other end we have a body which contains round and oval vesicles*. Transitional forms between such solid-looking rods and elongated vesicles are also seen and at high magnifications it becomes evident that all vesicles, be they round, oval or rod-shaped are bounded by a trilaminar membrane.

These structures were first seen by us (Ghadially and Parry, 1966b) in melanosis coli and we considered them to be a variety of multivesicular body. Later they were seen in the normal colonic mucosa and the term R-bodies (rod-containing bodies) has been used to describe them (Biempica *et al.*, 1976; Stone *et al.*, 1977; Marcus, 1981). R-bodies found in normal and pathological colon and rectum have diameters from 0.2–1.5 μm. R-bodies are said to be acid phosphatase negative, while multivesicular bodies are acid phosphatase positive (Biempica *et al.*, 1976). Therefore one may contend that the latter are lysosomes but the former are not, and that these are two distinct and different organelles. However, this would be difficult to reconcile with the occurrence of transitional forms between these bodies. A compromise could be reached by speculating that perhaps the R-body is an early or immature form lacking acid phosphatase, and that as it matures it acquires acid phosphatase and rods turn into vesicles to produce a variety of multivesicular body. The matrix of some R-bodies is remarkably similar to the contents of mucous granules *(Plate 259, Fig. 3)*, and at times images are seen which suggest that mucous granules fuse and discharge their contents into R-bodies (Biempica *et al.*, 1976). The significance of this phenomenon is obscure. The function of R-bodies is not known, but two suggestions have been offered in the literature: (1) that their contents are secreted and transformed into glycocalyceal bodies; or (2) that R-bodies are formed by endocytosis of glycocalyceal bodies (*see* pages 1072–1079 for details about glycocalyceal bodies).

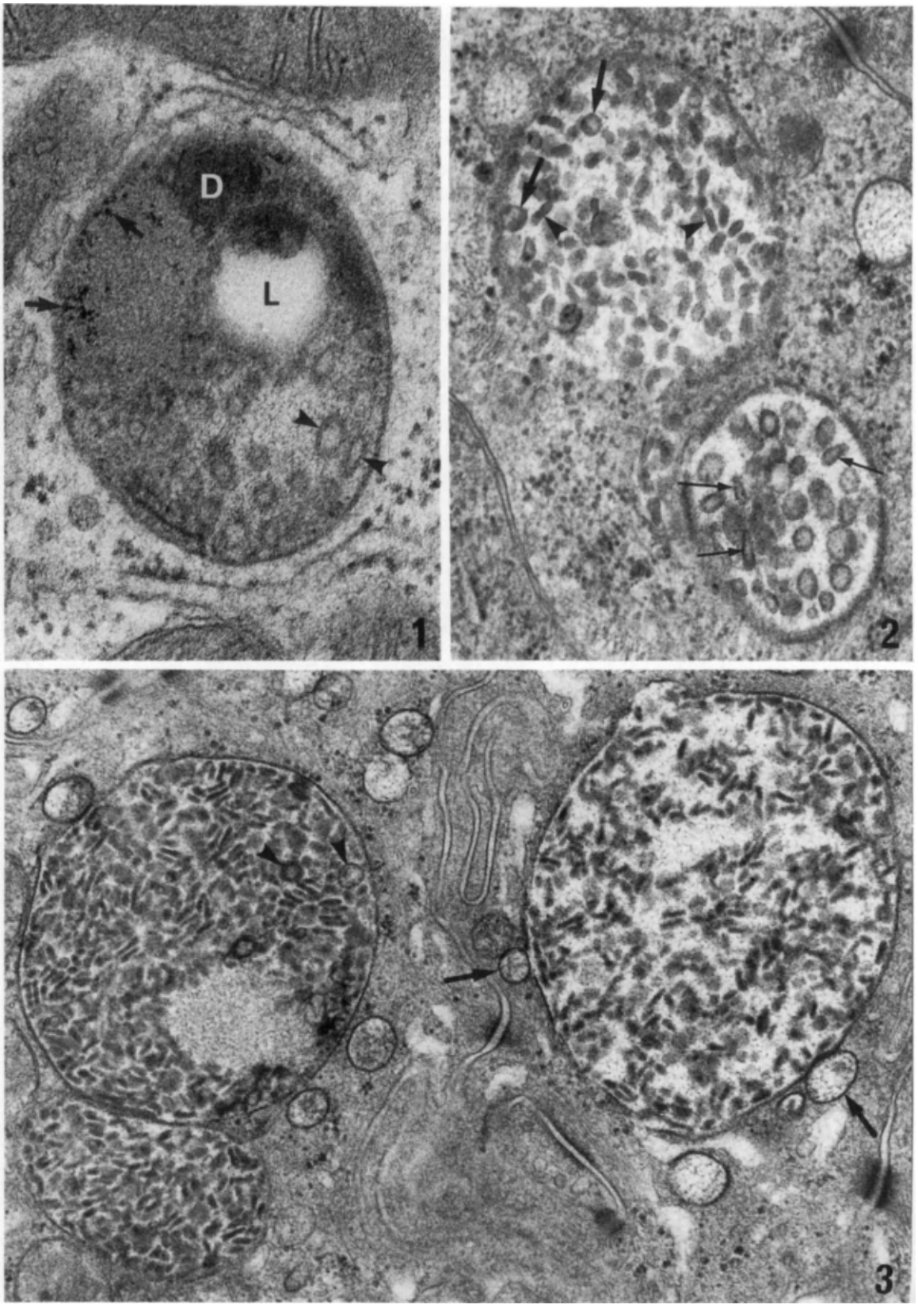
* Because of the occurrence of transitional forms it is at times difficult to know whether to call a body a 'multivesicular body' or a 'R-body'. I have adopted the attitude that if vesicles predominate it is a multivesicular body and if the rods predominate it is an R-body.

Plate 259

Fig. 1. A multivesicular body found in an epithelial cell in a jejunal biopsy from a patient who had received chrysotherapy. Some of the vesicles are elongated or oval (arrowheads). Note also the lipid droplet (L), electron-dense material (D) and electron-dense particles (arrows). × 94 000

Fig. 2. Epithelial cells from the colonic mucosa of a case of melanosis coli. In the top part of the picture is seen a R-body containing rod-shaped profiles (arrowheads) and a few circular profiles (thick arrows) which one may confidently diagnose as sections through vesicles because the diameter is too large for them to be transverse sections through the rods. In the bottom part of the picture we see a structure deserving to be called a multivesicular body because it mainly contains rounded vesicles. However, a few elongated vesicles (thin arrows) reminiscent of those at times seen in R-bodies are present. Bodies such as these make one wonder whether some multivesicular bodies derive from R-bodies. × 57 000

Fig. 3. Same case as *Fig. 2.* Seen here are three R-bodies which contain numerous 'solid-looking' rods and a few rounded vesicles (arrowheads). Two of these bodies are rather large. The one on the right has an average diameter of 1.5 μm. The matrix of the R-body on the right has the same flocculent appearance as the contents of mucous granules, two of which (arrows) are abutting its wall. × 45 000


D
L
1
2
3

I have not seen indubitable R-bodies filled with 'solid' rods and elongated vesicles in the jejunal biopsies that I have examined. However, multivesicular bodies containing rounded vesicles were encountered (*Plate 258, Fig. 3*) and at times, bodies containing oval and elongated vesicles (reminiscent of those seen in R-bodies) were also seen. In a jejunal biopsy from a patient with rheumatoid arthritis who had received chrysotherapy (Myochrysine), gold deposits of characteristic morphology (like those seen in *Plates 322–324*) were detected in the subepithelial macrophages but not in the multivesicular bodies.

Multivesicular bodies are of frequent occurrence in type II alveolar cells. Here the multivesicular body is involved in the production of the characteristic secretory granules of the type II alveolar cells. These bodies (i.e. secretory granules) which contain whorls (at times stacks) of myelinoid membranes (hence called 'myelinosomes' *see* page 358 for details) (Campiche *et al.*, 1963; Hatasa and Nakamura, 1965; Balis *et al.*, 1966; Sorokin, 1966; Goldenberg *et al.*, 1967, 1969) are rich in phospholipids and acid hydrolases (Balis and Conen, 1964; Hatasa and Nakamura, 1965; Goldfischer *et al.*, 1968; Kuhn, 1968; Vijeyaratnam and Corrin, 1972) and when exocytosed they contribute to the pulmonary surfactant.

The sequence of events leading to the formation of these secretory granules seems to be: (1) conversion of a light multivesicular body into a dark one; and (2) laying down of myelinoid membranes and a concomitant disappearance of vesicles in the dark multivesicular body (*Plate 260*). The precise manner in which the multivesicular bodies form in the type II alveolar cell is not clear, but it is thought that vesicles containing secretory material are pinched off from the rough endoplasmic reticulum and after their (probable) passage through the Golgi complex a cluster of vesicles is encompassed within a single limiting membrane, to form the multivesicular body.

Multivesicular bodies are among the many structures (listed on page 138) that have been mistaken for virus particles. Thus for example, the virus-like particles described in cases of AIDS (acquired immune deficiency syndrome) by Feremans *et al.* (1983) are in fact the vesicles in multivesicular bodies, as quite rightly pointed out by Onerheim *et al.* (1984). Ewing *et al.* (1983) described a non-membrane-bound structure composed of vesicles clustered around an ill-defined electron-dense core in cases of AIDS. They called this structure a 'vesicular rosette', and postulated that it might be a marker for AIDS and perhaps also a morphological expression of virus infection. Other workers (Onerheim *et al.*, 1984; Sidhu *et al.*, 1985) have quite rightly pointed out that the vesicular rosette is either an artefactual aggregation of vesicles or a disintegrated multivesicular body found in poorly preserved tissues and that it has nothing to do with AIDS or virus infections.

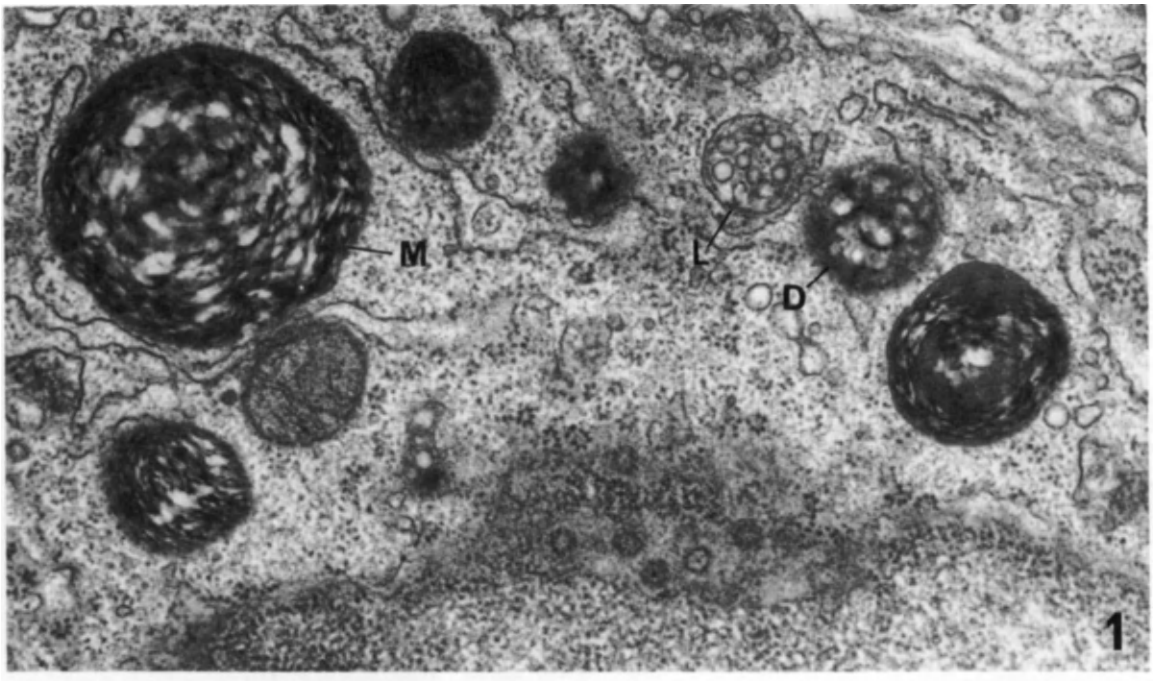
Plate 260

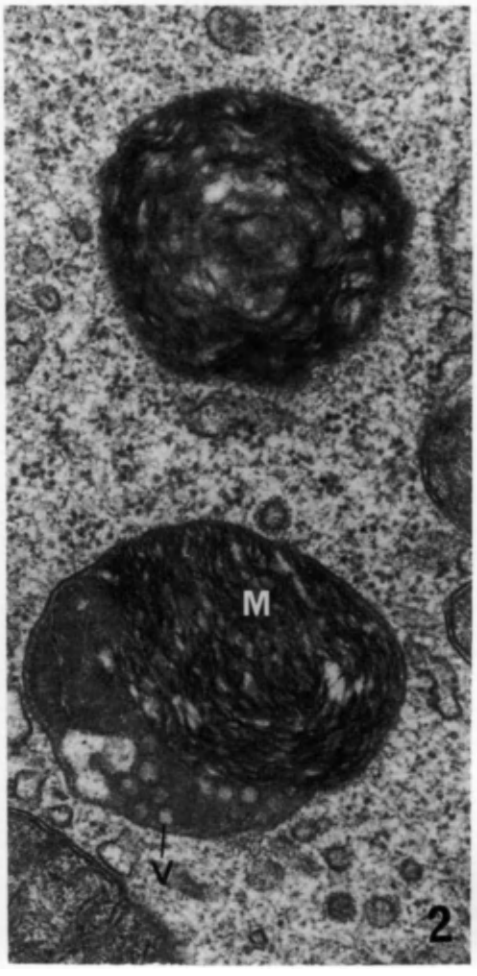
Type II alveolar cells from a newborn rat.

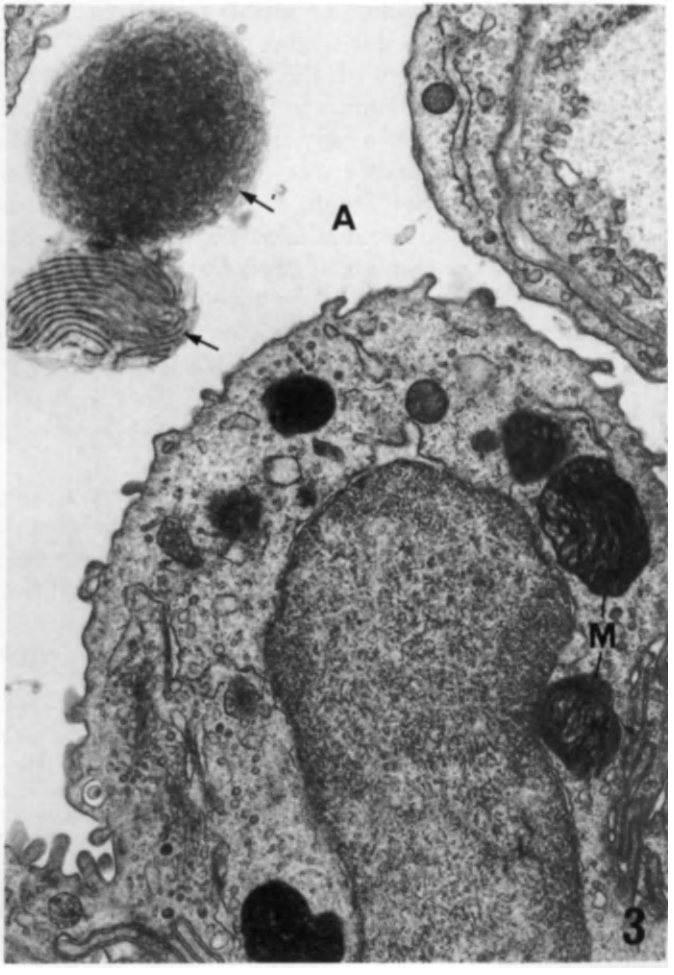
Fig. 1. Some of the stages of evolution of a myelinosome (M) from light (L) and dark (D) multivesicular bodies are seen in this electron micrograph. × 40 000

Fig. 2. A dark multivesicular body with vesicles (V) and dense membranes (M) is seen in company with another more mature form containing mainly laminated dense membranes. × 51 000

Fig. 3. Low-power view showing a type II alveolar cell with myelinosomes (M) and alveolar space (A) containing discharged secretory material (arrows). × 17 500


M
L
D
1


M
V
2


A
M
3

Lipofuscin (residual bodies)

Although the golden yellow or brown pigment we now call lipofuscin has been known to light microscopists for over a century, its nature and manner of formation could not be truly appreciated until the lysosome concept had evolved. There is now ample evidence that the granules of lipofuscin and the identical or closely related pigments, haemofuscin and ceroid, represent residual bodies left behind in the cell after lysosomal activity. Ultrastructural studies have also revealed that although the major deposits of lipofuscin occur in the nervous system, heart and liver, there is hardly an organ or tissue where some lipofuscin-containing residual bodies do not occur (*Plates 261-263*).

The presence of pigment granules we now call lipofuscin was first noted by Hannover (1842) in nerve cells. The term 'lipofuscin' (meaning 'dusky fat') was coined later by Borst (1922). The abundance of this pigment in older individuals soon led workers to refer to it as the 'age pigment','wear and tear pigment' and 'womb to tomb pigment'. The term 'haemofuscin' was used by von Recklinghausen (1883) to distinguish the iron-free pigment granules in cases of haemochromatosis from those containing iron which were called 'haemosiderin'. The term 'ceroid' was used by Pappenheimer and Victor (1946) to describe the pigment in the motor neurons of vitamin-E-deficient rats and later extended to pigment deposits seen in various pathological situations. Histochemical evidence supports the view that lipofuscin, ceroid and haemofuscin are essentially the same material in different stages of oxidation (Lillie, 1965; Pearse, 1972). The variations in staining reaction and ultraviolet fluorescence observed in such pigment deposits are explainable on this basis. Porta and Hartroft (1969) succinctly express current feeling in this matter when they state 'All the separate information gathered to date from investigations on the pigment related to ageing and to dietary factors favours their unification under lipofuscin pigment. To divide these closely related products into subentities seems unrealistic and only an accident of historic discoveries'.

Lipofuscin granules seem to be more abundant in humans than in other animals, but lipofuscin has been found in all vertebrates examined to date. It has also been found in gastropods (von Braunmuhl, 1957) and insects (Hodge, 1894). As clearly as 1886, Koneff had noted that the amount of lipofuscin in nerve cells increased with age. This was subsequently confirmed by many workers, for various species including man (Beams *et al.*, 1952; Sulkin and Kuntz, 1952; Sulkin, 1955; Wilcox, 1959; Brody, 1960; Samorajski *et al.*, 1965; Whiteford and Getty, 1966; Reichel *et al.*, 1968; Barden, 1969, 1970). Similar age-related increases of pigment also occur in the myocardium (Jayne, 1950; Strehler *et al.*, 1959; Munnell and Getty, 1968), liver (Bachmann, 1953), adrenal cortex and other organs (Planel and Guilhem, 1955; Samorajski and Ordy, 1967; Reichel, 1968; Ishii, 1977).

One might expect that in the premature ageing syndromes (e.g. progeria (Hutchinson-Guilford syndrome) and Werner's syndrome) there would be an increase in lipofuscin deposits in various organs. Students of progeria have expressed conflicting views on this point (Reichel and Garcia-Bunuel, 1970) but it would appear that in cases of progeria no excessive lipofuscin deposits occur when compared to age-matched controls (Spence and Herman, 1973; West,1979). However, curiously enough large numbers of lysosomes and residual bodies

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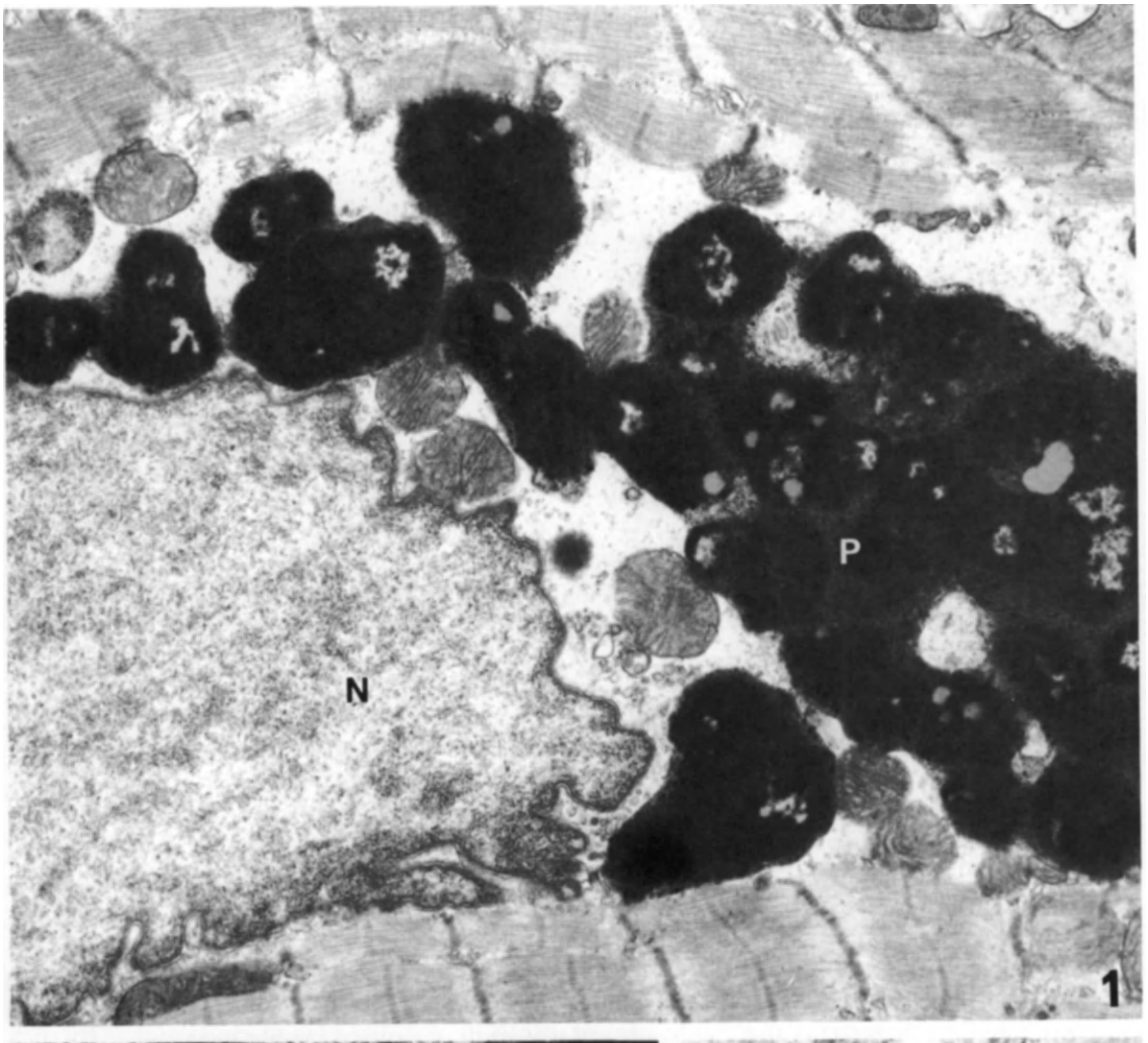
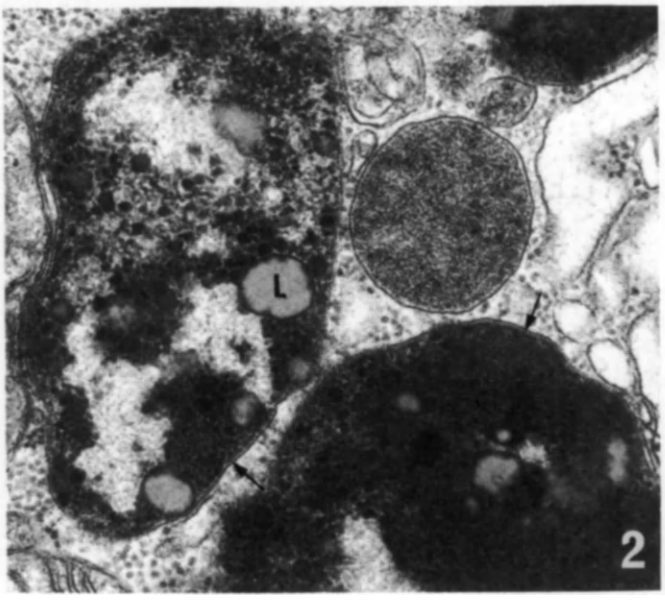
Plate 261

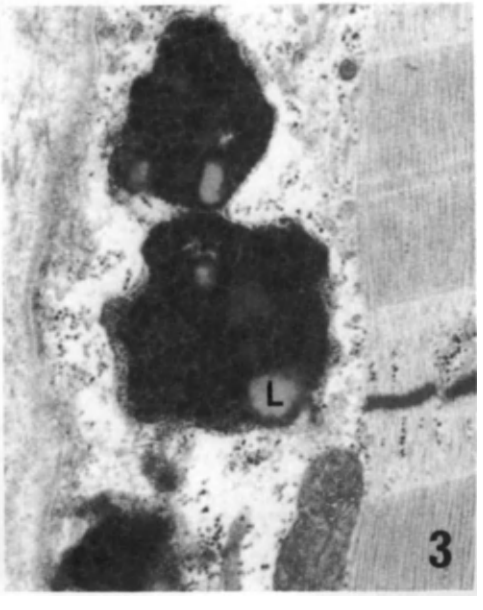
Fig. 1. Lipofuscin granules found in the myocardium of an elderly man. As pathology students well know, in the condition called brown atrophy of the heart such granules are abundant adjacent to the poles of the elongated nuclei of myocardial cells. This well known relationship of the pigment (P) to the nucleus (N) is demonstrated in this electron micrograph. × 18500

Fig. 2. High-power view of lipofuscin granules from the same specimen as *Fig. 1.* Note the characteristic dense granules, lipid droplets (L) and membrane surrounding the granules (arrows). × 45.000

Fig. 3. Lipofuscin granules found in an atrophic deltoid muscle of man. Note once again the presence of dense granular material and lipid droplets (L). × 23000


N
P
1


L
2


L
3

accumulate in strains of cultured fibroblasts from Werner's syndrome (Basler *et al.*, 1979). In this connection it is worth recalling that one of the degenerative changes that occurs in strains of human fibroblasts (from normal individuals) as they approach senescence in culture is an accumulation of lysosomes and residual bodies in their cytoplasm (Robbins *et al.*, 1970).

Besides ageing, lipofuscin accumulation can also be engendered by various noxious influences. Such examples include: (1) brown atrophy of the heart in patients with severe inanition or starvation, endocrine disturbances (e.g. Addison's disease and Simmond's disease) and in chronic wasting diseases such as tuberculosis and cancer (Scotti, 1971); (2) lipofuscin accumulation in the motor cortex of squirrel monkeys (*Saimiri scireus*) kept on a protein-deficient diet (Manocha and Sharma, 1977); (3) lipofuscin deposits in the anterior horn cells of the spinal cord of squirrel monkeys born to mothers kept on a protein-deficient diet (Manocha and Sharma, 1978); (4) melanosis coli where lipofuscin deposits are produced in the colon by anthracene purgatives (Ghadially and Parry, 1966b, 1967; and pages 672–675 in this chapter); (5) prolonged analgesic medication producing hepatic and renal lipofuscinosis (Wolman, 1975); (6) lipofuscin deposits in the kidneys of animals after administration of toxic doses of phenacetin (Schnitzer *et al.*, 1965) or oestrogens (Harris, 1966); (7) lipofuscin deposits in the adrenal cortex of genetically hypertensive rats (Tsuchiyama *et al.*, 1972); (8) acetanilide feeding producing generalized lipofuscinosis (Strehler, 1962); (9) local lipofuscinosis produced by topical applications of polyene antibiotics such as Nystatin (Weissmann, 1969); (10) lipofuscin deposits in animals on a vitamin-E-deficient diet (Mason and Telford, 1947; Tappel, 1972; Reddy *et al.*, 1973); (11) lipofuscin deposits in the smooth muscle of the bowel (brown-bowel syndrome) in cases of long-standing malabsorption of fat in man (Pappenheimer and Victor, 1946; Schnitzer and Loesel, 1968; Foster, 1979); and (12) several human diseases associated with vitamin E deficiency (Lazaro *et al.*, 1986).

In ultrathin sections lipofuscin granules present as rounded or irregularly shaped bodies limited by a single membrane. The contents are variable but in the main they consist of highly electron-dense granular material and one or more medium-density or lucent lipid droplets. On rare occasions recognizable remnants of organelles and/or electron-dense membranous material (myelinoid membranes) may be discerned in these residual bodies.

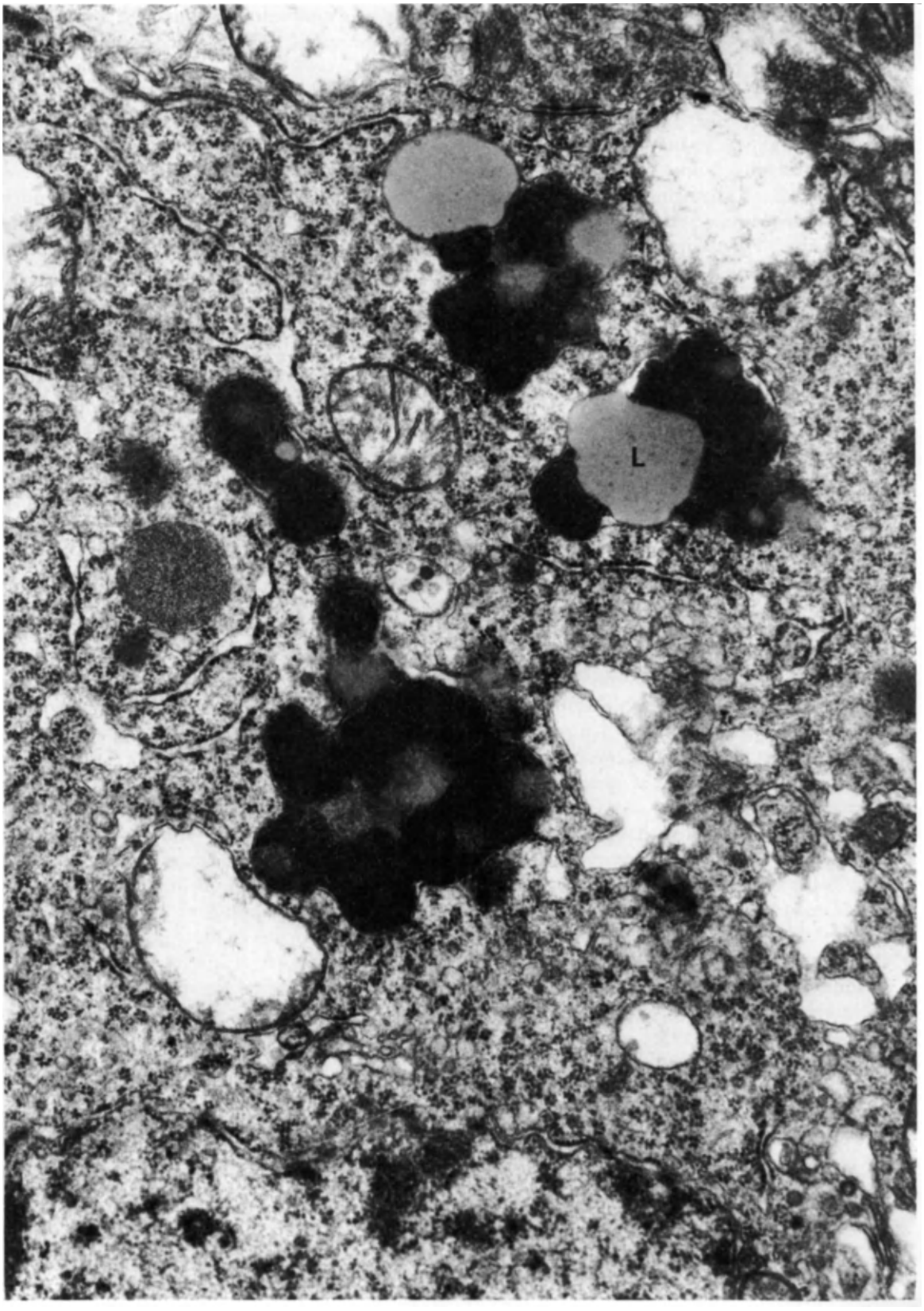
The view that lipofuscin granules are lysosomal bodies is supported by many studies which have demonstrated the presence of acid phosphatase and other acid hydrolases such as esterases and cathepsins in them. This has been achieved by histochemical and cytochemical methods employing tissue sections and also by chemical analysis of isolated lipofuscin granules. By such techniques the lysosomal nature of lipofuscin granules has been confirmed in the liver (Essner and Novikoff, 1960; Goldfischer *et al.*, 1966), mammary gland (Miyawaki, 1965), interstitial cells of the testis (Frank and Christensen, 1968) and neurons (Anderson and Song, 1962; Samorajski *et al.*, 1965; Barden, 1969, 1970).

Both the ultrastructural appearance and the histochemical analysis of lipofuscin granules show that they are not composed of a homogeneous substance or a single chemical compound. According to the lysosome concept, one may look upon lipofuscin as a conglomerate of undigested residues derived by lysosomal hydrolysis and modification of sequestrated material prior to and after digestion. Such modifications include lipid peroxidation, polymerization and cross-linking of lipoproteins. (For references, *see* Barden, 1970.) The power of lysosomal hydrolases to degrade lipids (Mahadevan and Tappel, 1968a, b; Foster, 1979) is said to be limited*, hence it is thought that the lipids are left behind and suffer stepwise oxidation

*Deficient lysosomal lipolytic activity has also been implicated in the overloading of lysosomes and formation of lipid-laden foam cells in atherosclerotic lesions of man and experimental animals (Coltoff-Schiller *et al.*, 1976).

Plate 262

Lipofuscin in a neuron from the cerebral cortex of a rat. Note the characteristic granular electron-dense contents and the lipid droplets (L). × 34 000


L

producing lipofuscin. It has also been argued that the reason why such residues are left behind is that the lipids suffer oxidation at a very early stage and are hence not broken down by lysosomal enzymes.

It is generally held that lipofuscin derives mainly or entirely from the degradation of endogenous and not exogenous material in the lysosome. In the rat myocardium lipofuscin granules appear to be formed by the breakdown of mitochondria, glycogen and lipid droplets within autolysosomes (Travis and Travis, 1972). In the dorsal root ganglia of mice (Duncan *et al.*, 1960) and human myocardium (Koobs *et al.*, 1978) lysosomal degradation of mitochondria is said to be the source of lipofuscin. Chio *et al.* (1969) have shown that lipid peroxidation of isolated mitochondria *in vitro* produces lipofuscin and this can be prevented by the addition of an antioxidant to the medium. However, there is little doubt that organelles and inclusions besides mitochondria also provide the substrate for lipofuscin formation *(see* page 672).

Be that as it may, the fact that residual bodies containing lipofuscin accumulate with age shows that the power of many mammalian cells to excrete these bodies or their contents is limited. It has been argued that such mounting accumulation of lipofuscin (which can at times constitute as much as one-third of the dry weight of the myocardium) may hamper the functional capacity of the organ (heart or brain) and may hence be a factor responsible for or involved in the ageing process. It remains for future work to determine whether suppression of the formation of such bodies or their elimination would be beneficial to the individual.

Several drugs appear to halt or reverse the accumulation of lipofuscin, perhaps the most effective is centrophenoxine (ANP-235) which is said to drastically reduce the amount of lipofuscin in the neurons of senile guinea pigs and squirrel monkeys (Nandy and Bourne, 1966; Nandy, 1968; Chemnitius *et al.*, 1970; Meier and Glees, 1971; Hasan and Glees, 1972; Hasan *et al.*, 1974a, b; Nandy and Lal,1978). According to Nandy *et al.* (1978) gradual accumulation of lipofuscin pigment occurs in cultured neuroblastoma cells and this can be reduced by the addition of centrophenoxine to the medium. However, the electron micrographs in this paper depict cells containing myelinosomes (i.e. lysosomal bodies containing myelin figures) and not lipofuscin granules of characteristic morphology. This is not too serious a criticism if one recalls that whether residual lipidic material in the lysosome presents as osmiophilic granules or as osmiophilic membranes depends mainly on the degree of hydration.

We noted above that animals fed a diet deficient in vitamin E accumulate excessive amounts of lipofuscin. Conversely, it has been shown (Constantides *et al.*, 1986) that prolonged treatment of mice with the natural anti-oxidant vitamin E, or the synthetic anti-oxidant butylated hydroxytoluene reduces significantly the age-associated increase in lipofuscin granules in the spinal cord. That a similar situation prevails in humans is suggested by Lazaro *et al.* (1986) who review various disease states where a deficiency of vitamin E is linked with an increase in lipofuscin in various tissues. They also report their studies on muscle biopsies from a case of Bassen–Kornzweig syndrome (a disorder of lipid metabolism) where administration of vitamin E for one year reduced the number of lipofuscin granules in muscle fibres.

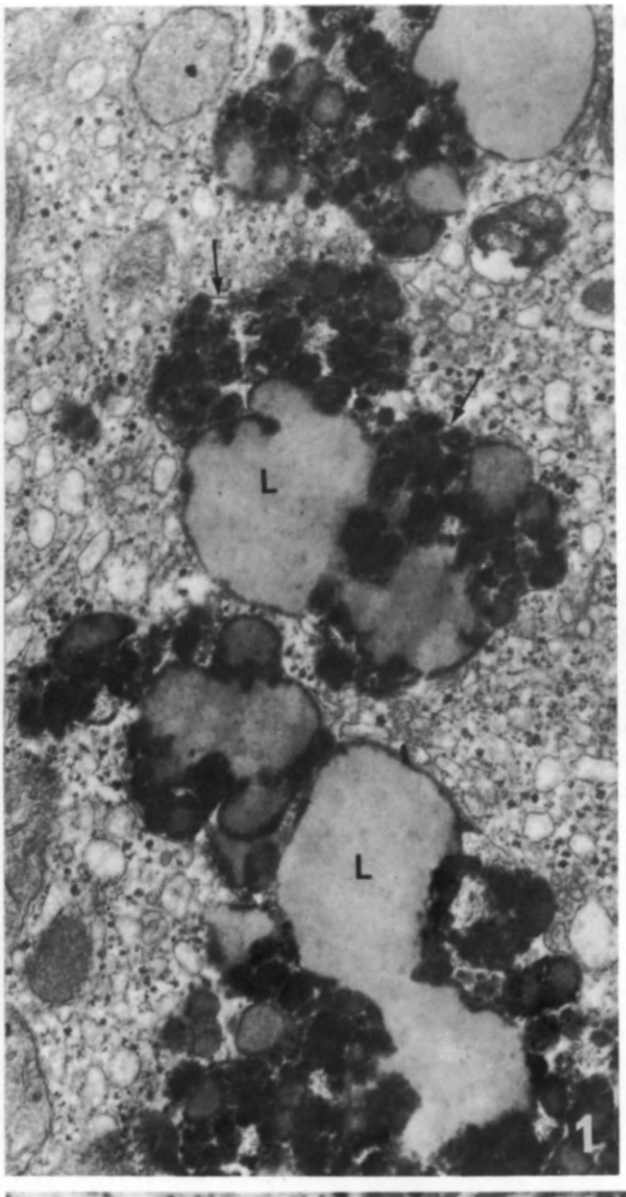
Little is known about the occurence of lipofuscin in various types of tumours. What little there is to note is recorded on page 644.

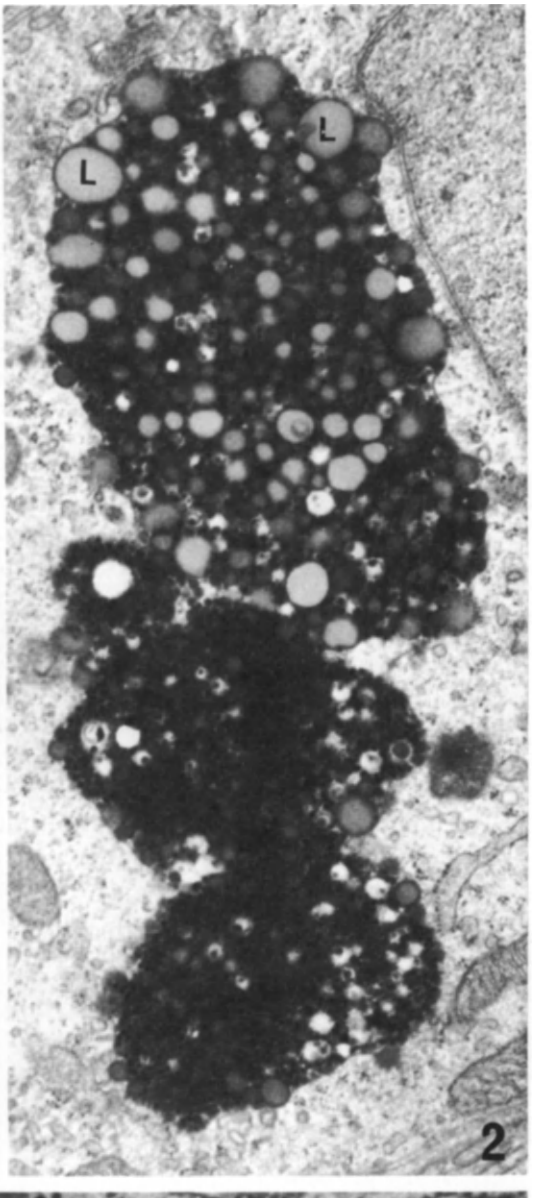
Plate 263

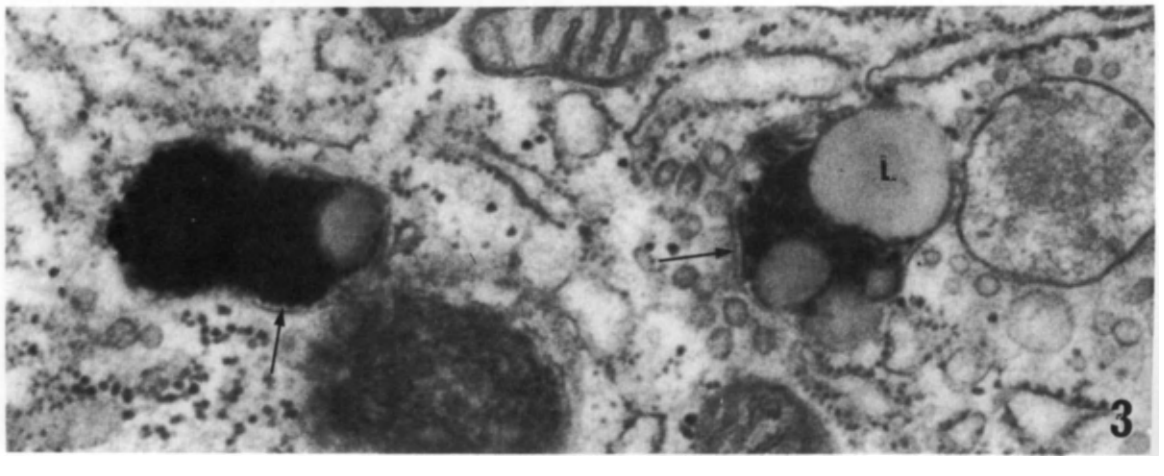
Fig. 1. These lipofuscin granules, composed of numerous dense granules and unusually large lipid droplets (L), were found in the liver tissue adjacent to a human hepatoma. The limiting membrane of these lysosomal bodies is discernible in some places (arrows). × 27 000 *(From Ghadially and Parry, 1966a)*

Fig. 2. Lipofuscin granules found in human gall-bladder epithelium. Here the lipid droplets (L) are small and numerous. × 26 000

Fig. 3. In these lipofusin granules, found in a human synovial intimal cell, the electron-dense granular material is compacted and difficult to discern. Note once again the characteristic lipid droplets (L) and the enveloping membrane (arrows). × 60 000 *(From Ghadially and Roy, 1969)*


L
L
1


L
L
2


L
3

Myelinoid membranes, myelin figures and myelinosomes

The term 'myelin' was coined by Virchow (1854) to describe certain fatty substances obtainable from vegetable and animal materials (e.g. white matter of brain and egg yolk) which, when mixed with water, produced a laminated membranous structure. The term 'myelin' has subsequently been used mainly in connection with the medullary sheath of nerve fibres which, with the electron microscope, is seen to be an osmiophilic laminated membranous structure. Today the term 'myelinoid membranes' is used to describe markedly osmiophilic membranes, and the term 'myelin figures' is employed when such membranes show a stacked, reticulated or whorled arrangement (*Plates 264* and *265*). However, it should be remembered that the electron density realized in electron micrographs of myelin figures depends much on the tissue processing and photographic techniques employed so that at times the electron density may fall short of the expected ideal. Furthermore, the membranes comprising the myelin figure may not at times be too clearly visualized owing to obliquity of sectioning or other reasons* (*Plate 264*).

Single-membrane-bound bodies containing myelin figures have frequently been encountered (*see below*) and acid phosphatase activity has been demonstrated in them. The term 'myeloid bodies' has been proposed to describe such lysosomal bodies (Hruban *et al.*, 1965, 1972), but it seems more appropriate to use the term 'myelinoid bodies' or 'myelinosomes' since the word 'myeloid' means resembling or pertaining to the marrow,

Virchow's observations on *in vitro* formation of myelin figures have been extended by some more recent studies. Such figures have been produced by Stoeckenius (1962) from brain phospholipid, by Revel *et al.* (1958) from egg lecithin and by Samuels *et al.* (1964) from a mixture of gangliosides, cholesterol, phospholipids, cerebrosides, amino acids and protein.

This mixture, based on the composition of myelinoid bodies found in Tay-Sachs disease (*see below*), produced myelin figures comparable to those found *in vivo* within lysosomes in this condition. Such experiments suggest that at least one of the ways in which myelin figures may be produced in lysosomes is by hydration of lipidic component within these organelles. Thus one may speculate that lipid residues may present either as osmiophilic granules and droplets or, if hydrated, as myelin figures within the lysosome.

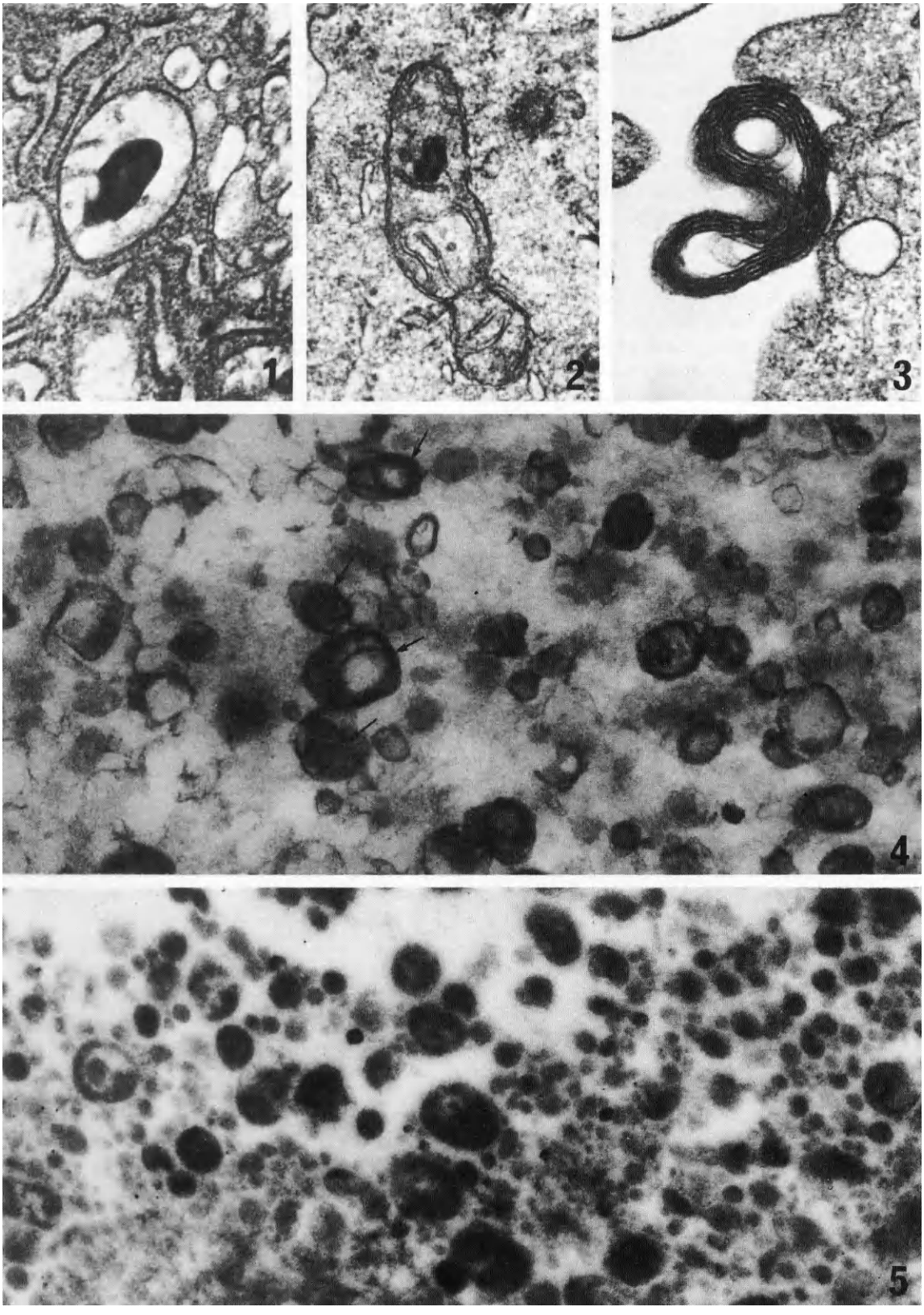
* Myelin figures develop by hydration of lipidic material. Thus, there is a stage of development when only a few myelinoid membranes are seen embedded in, or at the periphery of, an osmiophilic lipid droplet. In such partially formed or not fully developed myelin figures, it is also difficult to visualize myelinoid membranes.

Plate 264

Figs. 1–3. Cells from a ganglion of the wrist. Myelin figures produced by glutaraldehyde fixation. *Figs. 1 and 2* show poorly developed myelin figures in mitochondria. The membranous substructure is difficult to see here but it could be better discerned in the original electron micrographs. *Fig. 3* shows a well-developed myelin figure where the membranous substructure is clearly visualized. × 48 000; × 44 000; × 85 000 *Ghadially and Mehta, unpublished electron micrograph)*

Fig. 4. Matrix of rabbit articular cartilage. The osmiophilic lipidic debris contains myelinoid membranes (arrows). *See also Plates 541* and *542* where membranous and granular lipidic debris is shown in the matrix of articular cartilage. × 48 000 *(From Ghadially, Meachim and Collins, 1965)*

Fig. 5. Matrix of human articular cartilage, showing lipidic debris which presents as osmiophilic granular material. × 48 000 *(From Ghadially, Meachim and Collins, 1965)*


1
2
3
4
5

Myelinoid membranes and myelin figures are readily produced in tissues fixed in glutaraldehyde (Ericsson and Biberfeld, 1967), particularly if the fixation is prolonged for many hours or days. Since lipids are not fixed by glutaraldehyde, some lipids leach out of the cytomembranes. Mixture of such liberated lipid with the aqueous environment produces membranous formations which are fixed by the osmium used as a post-fixative agent. These then present as myelinoid membranes and myelin figures at electron microscopy. Such myelinoid membranes and myelin figures may be found in extracellular sites and also within the cell, particularly common being myelin figures in the cytoplasmic matrix and mitochondria (*Plates 264* and *265*). The latter are likely to be mistaken for pathological or degenerative changes in the cell or mitochondrion by the novice.

Another interesting example of extracellular myelin figures or myelinoid membrane formation is found in the matrix of articular cartilage. The *in situ* necrosis of chondrocytes and the shedding of chondrocyte cell processes which normally occur in this tissue liberate much osmiophilic lipidic debris into the matrix (for references and details, *see* Ghadially, 1983). Such material usually presents as electron-dense amorphous or granular material or as small highly osmiophilic membranous formations (more often myelinoid membranes rather than the more complex myelin figures) (*Plates 264, 541* and *542*).

Since the normal nucleus contains no membranous structures, one does not expect to find myelinoid membranes or myelin figures in the nucleus. Nevertheless, at times these structures are seen in the nucleus. The commonest form of intranuclear myelin figure is that seen in a double-membrane-bound intranuclear pseudoinclusion (*Plate 265, Fig. 3*) where presumably degradation of sequestrated cytomembranes liberates lipids which are then transformed into myelin figures (either *in vivo* or *in vitro* during processing). Myelinoid membranes and/or myelin figures may also develop from lipids liberated from intranuclear tubulovesicular inclusions and intranuclear microtubuloreticular structures (*Plate 226*). Enigmatical, however, is the very rare finding of one or two myelin figures in an otherwise unremarkable nucleus (*Plate 265, Fig. 4*), for here one is not sure where the lipid which gave birth to the myelin figure came from. One may, however, speculate that it could have come from the total disintegration of a small membrane-rich pseudoinclusion or that it stemmed from hydration of an intranuclear lipid or phospholipid inclusion.

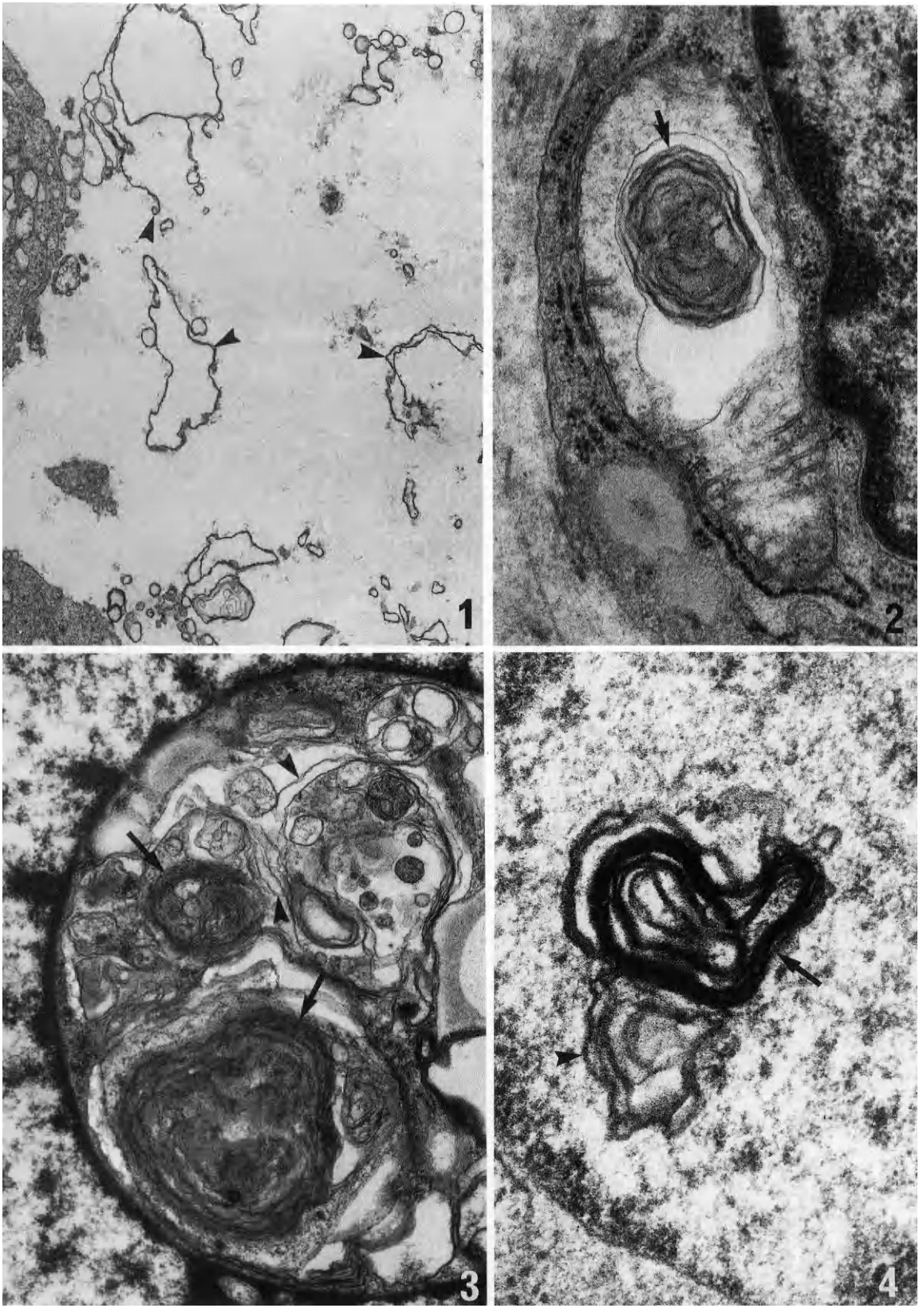
Plate 265

Fig. 1. An undiagnosed tumour thought to be either a malignant haemangiopericytoma or a neuroendocrine carcinoma (same case as *Plate 211*). Primary fixation was in formalin. Note the extracellular myelinoid membranes (arrowheads). × 7200

Fig. 2. Angiosarcoma. A mitochondrion containing a myelin figure (arrow). × 42 000

Fig. 3. Phaeochromocytoma. An intranuclear pseudoinclusion where the included organelles have suffered degenerative changes and the released lipid has formed myelinoid membranes (arrowheads) and myelin figures (arrows). × 22 000

Fig. 4. Lymphoma. A highly electron-dense myelin figure (arrow) and a not so electron-dense myelin figure (arrowhead) are seen lying free (i.e. true inclusions) in the nuclear matrix. × 78 000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Myelinosomes are pleomorphic structures which show infinite diversity of form. This is illustrated in *Plate 266* where myelinosomes from a herpesvirus-infected cell, a rat sarcoma, a human ovarian carcinoma and a synovial intimal cell after an intra-articular injection of egg yolk are depicted. In the last-mentioned instance it would appear that the myelinosomes represent heterolysosomes containing egg yolk.

A rare myelinosome is occasionally found in a variety of normal cells but in type II alveolar cells of the lung they are of constant and frequent occurrence. These myelinosomes (which are often referred to by undesirable terms such as 'cytosomes' or 'multilamellar bodies') represent the secretory granules normally produced by these cells (*Plate 260*).

The manner in which these myelinosomes develop has already been dealt with, as also their role in the production of pulmonary surfactant (pages 358–363). It is, however, worth recalling that the secretory granule of the type II alveolar cell contains phospholipids (in the form of myelin figures) and acid hydrolases. Therefore, it qualifies as a variety of myelinosome (i.e. a lysosomal body containing myelin figures). As is to be expected, myelinosomes are also seen in neoplastic type II alveolar cells and this is a point of great value in diagnosing alveolar cell carcinoma (*see* pages 358–363).

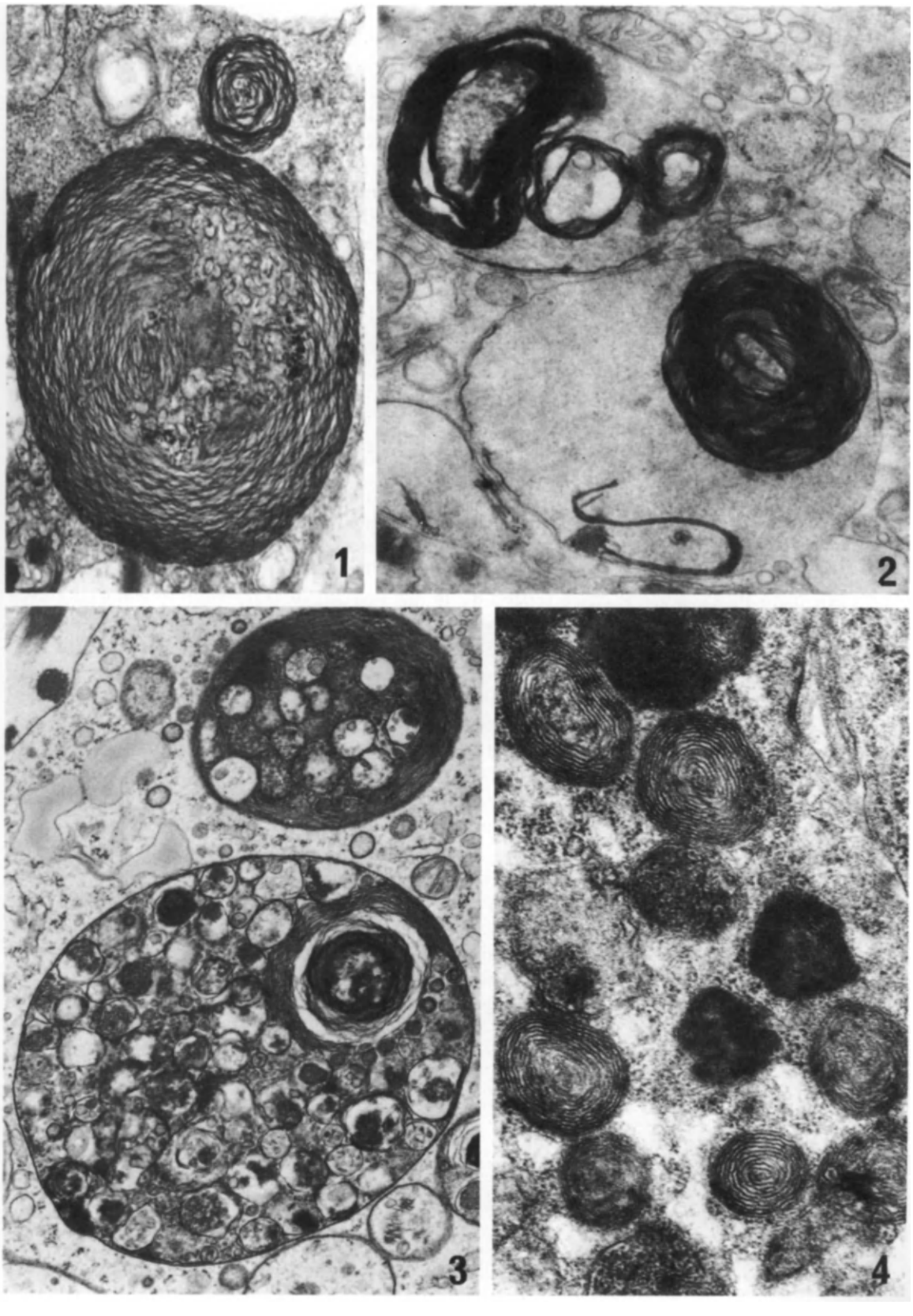
Plate 266

Fig. 1. Myelinosomes found in a herpesvirus-infected monkey kidney cell in culture. × 28 000

Fig. 2. Single-membrane-bound myelinosomes containing unicentric and multicentric myelin figures found in a sarcoma cell. From a carcinogen-induced subcutaneous sarcoma produced in a rat. × 38 000 *(Parry and Ghadially, unpublished electron micrograph)*

Fig. 3. It would appear that in these autolysosomes, cytomembranes are being transformed into myelinoid membranes and figures. From an ovarian carcinoma. × 23 000

Fig. 4. Myelinosomes found in a synovial intimal cell. From a rabbit knee joint after intra-articular injection of egg yolk. × 32 000 *(Ghadially and Mehta, unpublished electron micrograph)*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Myelinosomes in great numbers are found in certain inborn errors of metabolism or storage diseases, which are now also referred to as 'inborn lysosomal diseases' or 'lysosomal storage diseases'. These bodies have been given various names such as 'lipid cytosomes', 'multimembranous bodies', 'lamellar bodies', 'membranous cytoplasmic bodies' and 'zebra bodies'. All of these contain stacked, reticulated or whorled membranes. The last appellation (zebra bodies) is used to describe lysosomal bodies containing stacks of osmiophilic membranes, where adjacent membranes are closely apposed or fused, so that fairly wide alternating dense and light bands are produced. Myelinosomes of the zebra body type and myelinosomes containing whorled membranes have been seen in the neurons of cases of Tay-Sachs disease (Terry and Weiss, 1963) and also in some other (but not all) varieties of amaurotic idiocy (Menkes *et al.*, 1971). Acid phosphatase activity has been demonstrated in such bodies (Wallace *et al.*, 1964) and they have also at times been observed in various sites besides the brain in cases of Tay-Sachs disease (Hers and Van Hoof, 1969). Myelinosomes similar to those seen in man have also been noted in dogs with amaurotic idiocy (Diezel *et al.*, 1967; Karbe, 1968). In certain types of mucopolysaccharidoses (page 708) mucopolysaccharide deposits occur in the liver, but myelinosomes are found in neurons (*see* review by Hers and Van Hoof, 1969).

In Fabry's disease, intralysosomal glycolipid deposits occur in various tissues. Myelinosomes containing stacked membranes (i.e. zebra body type) and myelinosomes containing membranous whorls have been seen in kidney epithelial cells, vascular endothelial cells, pericytes, skeletal muscle cells, perineurial cells and neurons, and acid phosphatase has been demonstrated in them (Rae *et al.*, 1967; Tarnowski and Hashimoto, 1968; Grunnet and Spilsbury, 1973; Vital *et al.*, 1984; Rodriquez *et al.*, 1985; Farge *et al.*, 1985).

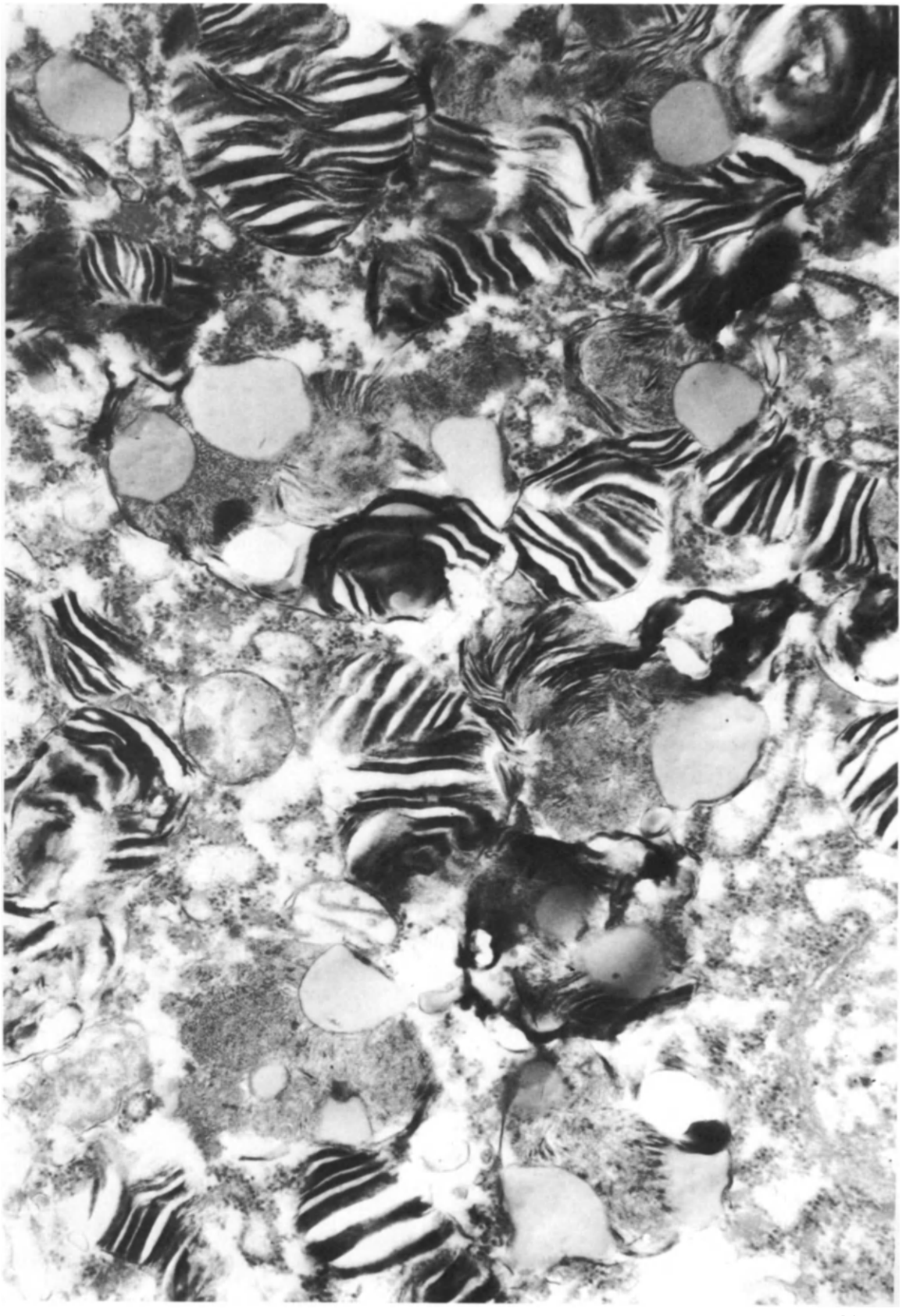
Niemann-Pick disease is characterized by sphingomyelin storage in the reticuloendothelial system and in various other sites. Myelinosomes containing mainly concentric whorled membranes have been described in this condition and acid phosphatase activity has been demonstrated in these bodies (Tanaka *et al.*, 1963; Lynn and Terry, 1964; Wallace *et al.*, 1965; Volk and Wallace, 1966; Lazarus *et al.*, 1967; Luse, 1967).

In Gaucher's disease (page 698), however, the abnormal glucocerebroside accumulation in the reticuloendothelial system (particularly the spleen) takes the form of tubular material in single-membrane-bound bodies (De Marsh and Kautz, 1957; Salomon and Caroli, 1962; Jordan, 1964; Toujas *et al.*, 1966). This is quite characteristic of the Gaucher cell. However, the neurons in some cases of this condition contain myelinosomes of the zebra body type (Adachi *et al.*, 1967).

It is now thought (Hers and Van Hoof, 1969; de Duve, 1970) that in most of the above-mentioned disorders there is a deficiency or absence of a specific lysosomal hydrolase. As a result of this, substances which need the deficient enzyme for their breakdown accumulate in the lysosome, which becomes overloaded with undigested material and increases considerably in size. However, enzymic defects outside the lysosome may be operative in some of these conditions, and these defects may lead to an accumulation of metabolite and its subsequent sequestration in the lysosome.

Plate 267

Electron micrograph showing myelinosomes of the zebra body type in a neuron from a case of Fabry's disease. × 34 000 *(From Grunnet and Spilsbury, 1973)*



Myelinosomes are at times seen in cells that have phagocytosed erythrocytes or their breakdown products (*Plate 268*) (for a review and references *see* Ghadially, 1979a). Examples of this include: (1) splenic macrophages that impound effete erythrocytes from the circulation; (2) macrophages which remove extravasated blood after a haemorrhage; and (3) synovial intimal cells and subsynovial macrophages in haemarthrosis.

The endocytosis and lysosomal degradation (in heterolysosomes) of entire erythrocytes, erythrocyte fragments, erythrocyte ghosts (i.e. lysed erythrocytes), crumpled erythrocyte membranes and haemoglobin leads to the formation of three types of lysosomal or residual bodies (*Plate 268*): (1) myelinosomes which contain myelin figures, representing hydrated lipidic residues derived from the erythrocyte membrane; (2) siderosomes which contain haemosiderin (resulting from the digestion of haemoglobin) which presents as electron-dense iron-containing particles (pages 636–643); and (3) myelinosiderosomes which contain myelinoid membranes or myelin figures and haemosiderin.

However, myelinosomes are not seen in all sites and situations where erythrophagocytosis occurs. For example, myelinosomes were rarely encountered by Edwards and Simon (1970) in rat splenic macrophages, but they were abundant in the same situation in the rabbit (Simon and Burke, 1970). In the many human bone marrow specimens I have examined, rarely have I seen a good example of a myelinosome in macrophages breaking down erythrocytes but they are quite common in the synovial intimal cells of man in cases of haemarthrosis and in the rabbit after intra-articular injection of autologous blood. It is also my experience that myelinosomes do not persist indefinitely. For example, such bodies are seen in the synovial cells for a few days after a single intra-articular injection of blood into the rabbit knee joint but a few months later only siderosomes are seen and myelinosomes are absent.

It would appear that myelinosomes stemming from erythrophagocytosis represent hydrated lipidic residues derived from the presumably difficult-to-digest, cholesterol-rich erythrocyte membrane. Since the power of lysosomal hydrolases to degrade lipids is limited and variable, one may speculate that only in those circumstances where the lipid residue from the cholesterol-rich erythrocyte membrane persists in a suitably hydrated or altered form do myelin figures develop, while in other instances the lipid presents as osmiophilic droplets or electron-dense granules.

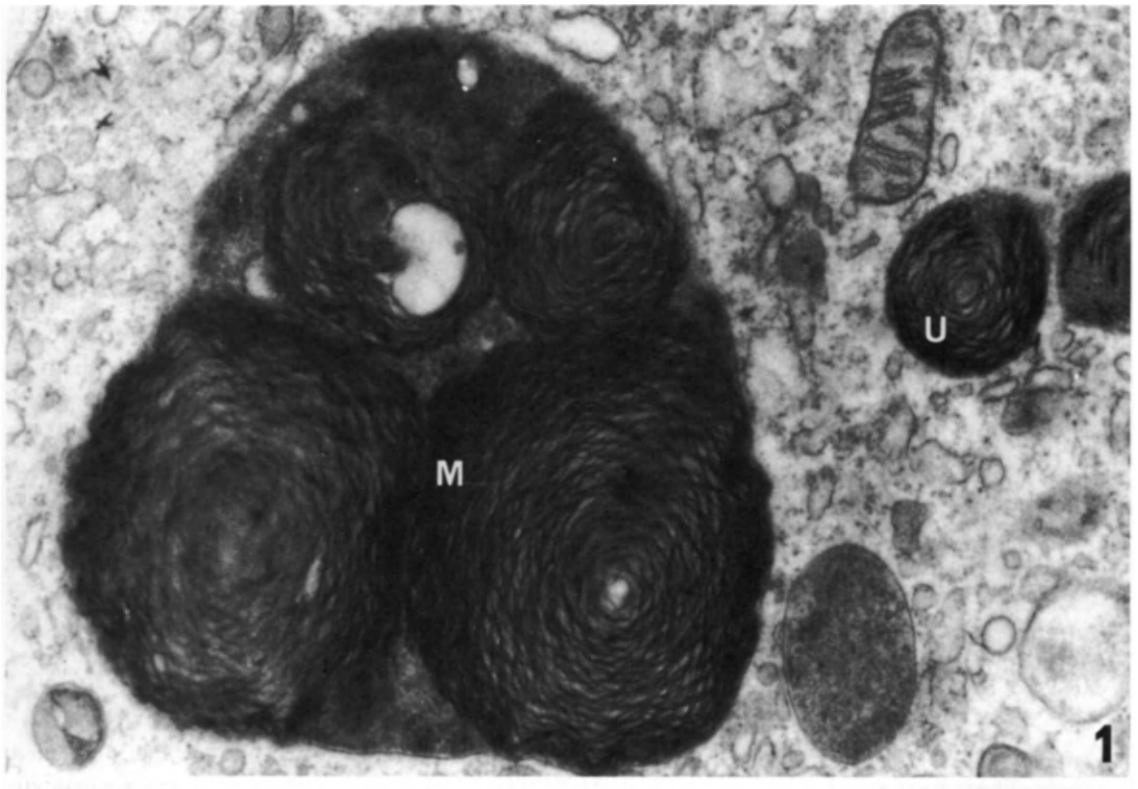
It is now clear that ingestion of other formed elements of the blood besides red blood cells can also lead to lipid storage in the form of myelinosomes and/or lipofuscin granules in macrophages or histiocytes (mainly in the spleen). Such cells called 'sea-blue histiocytes' have been seen in: (1) leukaemias (Sundberg *et al.*, 1964; Kattlove *et al.*, 1970; Clark and Davidson, 1972; Dosik *et al.*, 1972); (2) idiopathic or autoimmune thrombocytopenic purpura (Chandra *et al.*, 1973); (3) thalassemia (Beltrami *et al.*, 1973; Quattrin *et al.*, 1975); (4) sickle cell anaemia (Kattlove *et al.*, 1970); (5) iron deficiency anaemia and megaloblastic anaemia (Clark and Davidson, 1972); (6) drug-induced phospholipidosis (Takahashi *et al.*, 1977); (7) chronic granulomatous disease of childhood (Takahashi *et al.*, 1977); (8) histiocytic granuloma of gall bladder (Takahashi *et al.*, 1976); and (9) in a patient with coeliac sprue, thrombocytopenia and splenomegaly (Shnitka, personal communication).

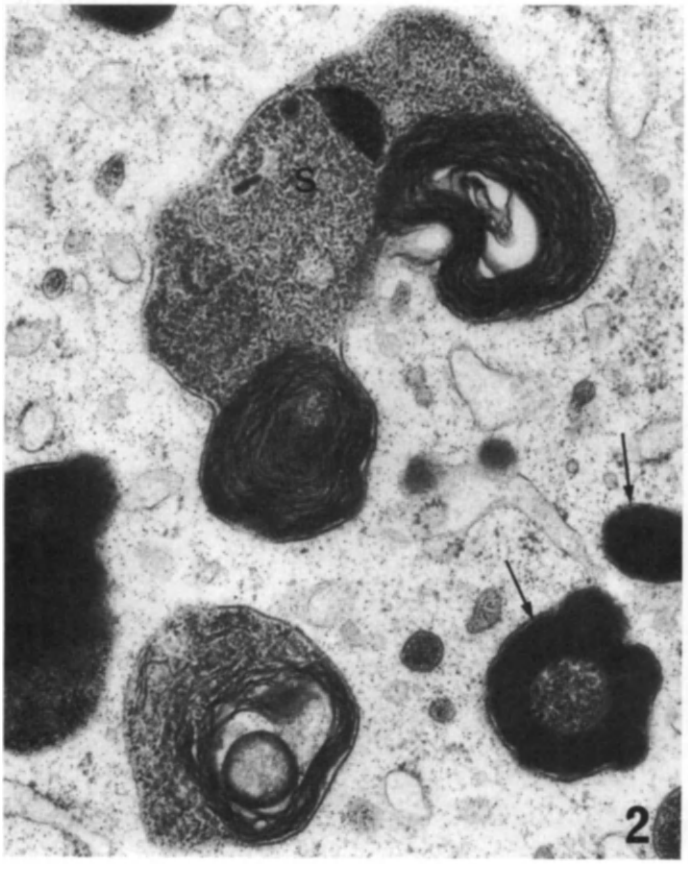
Plate 268

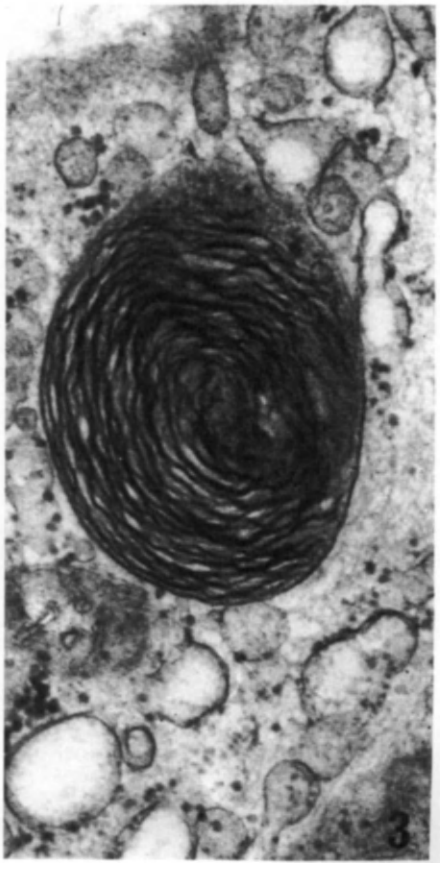
Fig. 1. Unicentric (U) and multicentric (M) myelinosomes found in a synovial intimal cell of a haemarthrotic rabbit knee joint. × 29 000 *(From Roy and Ghadially, 1966)*

Fig. 2. Subcutaneous haematoma produced by injection of autologous blood in the rabbit. Note myelinosiderosome (S) containing electron-dense membranes and electron-dense particles. Similar particles are just discernible in the cytoplasmic matrix. In some of these lysosomal bodies the lipid presents as homogeneous electron-dense material (arrows) rather than as myelinoid membranes. × 38 000 *(From Ghadially, 1979a)*

Fig. 3. A myelinosome found in the synovial intimal cell from a case of acute traumatic haemarthrosis in man. × 68 000 *(From Roy and Ghadially, 1967)*


M
U
1


S
2


3

The ultrastructural morphology of the granules in the sea-blue histiocyte resembles that of lipofuscin granules and myelinosomes (*Plate 269*)* and histochemical studies are in keeping with the idea that the pigment resembles lipofuscin. These lysosomes and residual bodies appear to be derived from the breakdown of ingested cells (mainly leucocytes but also some erythrocytes and thrombocytes) for lysosomes containing cell debris and cells in various stages of disintegration are also seen in sea-blue histiocytes. There is no evidence to suggest that the enzyme content of these lysosomes is deficient as in some lysosomal storage diseases. It seems more likely that accelerated breakdown of leukaemic cells and enhanced phagocytosis leads to an overloading and overwhelming of the lysosomal apparatus with resultant accumulation of lipidic material in the sea-blue histiocyte.

In certain cases of leukaemia, Gaucher-like cells and sea-blue histiocytes are seen and also cells with an intermediate morphology (Takahashi *et al.*, 1977) — that is to say macrophages containing angulate lysosomes of the type seen in Gaucher-like cells (*Plate 308*) and lysosomes of the type seen in sea-blue histiocytes. Furthermore, transitional forms suggesting that both these types of lysosomal bodies derive from ingested blood cells (mainly leucocytes) have been noted (Dosik *et al.*, 1972; Hopfner *et al.*, 1974; Kattlove *et al.*, 1968, 1969, 1970; Keyserlingk *et al.*, 1972).

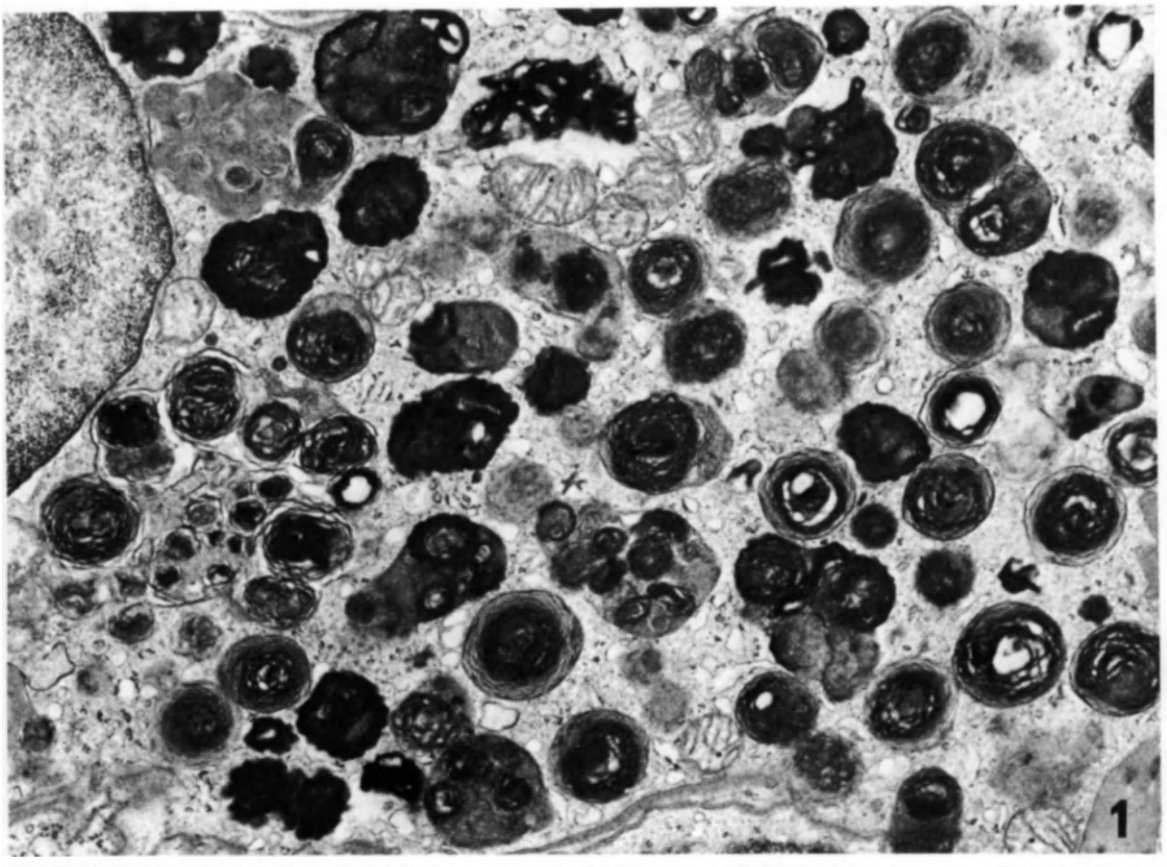
Myelinosomes are produced in numerous cell types of man and experimental animals (and also in cultured cells) by a variety of drugs, particularly amphiphilic drugs. Currently some 50 amphiphilic drugs are known. The unique pharmacological properties of some of these drugs necessitate clinical use despite undesirable side effects. Two excellent reviews of drug-induced myelinosomes have been published (Hruban *et al.*, 1972; Hruban, 1984), so a detailed list of references and drugs which produce myelinosomes is not presented here. Some of the better known drugs which produce myelinosomes are: amiodarone, chlorcyclizine, chloroquine, chlorphentermine, clindamycin, diazocholesterol, 4,4-diethylamino-ethoxyhexoestrol, erythromycin, imipramine, norchlorcyclizine, paraquat and tripanol.

*It will be noted that here, as in several other situations, lipid can present either as electron-dense granules or as myelin figures.

Plate 269

Fig. 1. Numerous lysosomes (mainly myelinosomes) are seen in this macrophage (sea-blue histiocyte) from the spleen of a patient with coeliac sprue, thrombocytopenia and splenomegaly. × 14 000 *(From a block of tissue supplied by Dr T. K. Shnitka)*

Fig. 2. A sea-blue histiocyte from a leukaemic patient. The lysosomes in this macrophage bear some resemblance to lipofuscin granules. × 22 000 *(From Takahashi, Terashima, Kojima, Yoshida and Kimura, 1977)*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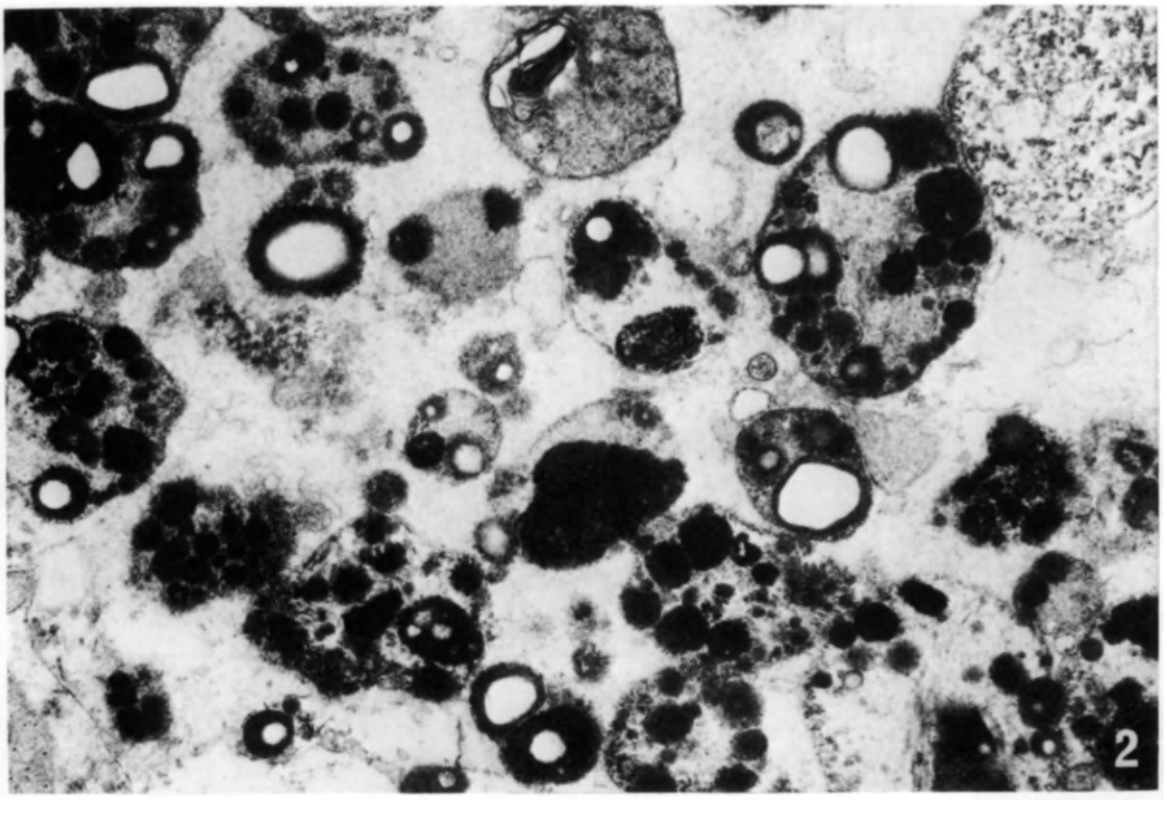

2

It is thought that drug-induced myelinosomes are formed because the drugs bind to lipids in cytomembranes and to other lipids, particularly phospholipids (e.g. in pulmonary surfactant). This alters the physicochemical properties of phospholipids and renders them difficult to digest when they are sequestrated in lysosomes. Another not mutually exclusive hypothesis is that the drugs selectively inhibit lysosomal enzymes. Be that as it may, the net result is that undigested lipidic material accumulates in lysosomes and when hydrated forms myelin figures in these lysosomal bodies which we then call 'myelinosomes'. This widespread accumulation of lipid or drug-lipid complexes in various organs is now often called 'drug-induced lipidosis' or 'drug-induced phospholipidosis'.

No amphiphilic drug has been the subject of greater interest and study than amiodarone, which is used to treat angina pectoris and cardiac arrhythmias. This drug produces myelinosomes* in cells of many organs (Delage *et al.*, 1975; Meier *et al.*, 1979; D'Amico *et al.*, 1981; Chew *et al.*, 1982; Colgan *et al.*, 1984; Costa-Jussa *et al.*, 1984; Poucell *et al.*, 1984; Costa-Jussa and Jacobs, 1985) and abnormalities to match.

These include: (1) keratopathy (Toussaint and Pohl, 1969; D'Amico *et al.*, 1981); (2) blue-grey skin pigmentation of areas exposed to light (Geerts, 1971; Wanet *et al.*, 1971; Matheis, 1972; Morand *et al.*, 1972; Delage *et al.*, 1975; Alinovi *et al.*, 1985); (3) hepatic dysfunction and fatty liver (Morand *et al.*, 1972; Groh *et al.*, 1980; Poucell *et al.*, 1984); (4) muscular weakness and neuropathy (Meier *et al.*, 1979; Butany *et al.*, 1983); (5) hyperthyroidism (Heger *et al.*, 1981); and (6) pulmonary disease, comprising interstitial pneumonitis, pulmonary fibrosis and intra-alveolar accumulation of foam cells (i.e. macrophages loaded with myelinosomes derived from the endocytosis of drug-lipid complexes produced by reaction of drug with pulmonary surfactant) (Marchlinski *et al.*, 1982; Riley *et al.*, 1982; Sobol and Rakita, 1982; Dake and Golden, 1983; Jirik *et al.*, 1983; Suarez *et al.*, 1983; van Zandwijk *et al.*, 1983). The neurological and pulmonary side effects of amiodarone can be quite serious, and may prove fatal. The toxicity is dose-related but remission usually occurs upon withdrawal of the drug.

As mentioned at the beginning of this section, single-membrane-bound lysosomal bodies containing whorls or stacks of myelinoid membranes are best referred to as myelinoid bodies or myelinosomes. Hruban (1984) also urges that these terms be adopted. He also quite rightly points out that 'Names which do not differentiate between membrane-bound myelinoid bodies and myelin figures without limiting membranes should be avoided'. Terms such as 'body', 'inclusion', 'structure' preceded by adjectives such as 'dense', 'cytoplasmic', 'lamellar', 'lamellate', 'multilamellate', 'membranous', 'honeycomb-like', 'finger print' are undesirable for just about all of them have been used for several different structures (e.g. whorls of endoplasmic reticulum). Perhaps the most idiotic term is 'cytoplasmic body', which could refer to just about anything including mitochondria and lipid droplets; next in line being 'dense body', which makes one think of lysosomes, osmiophilic lipid droplets and several other structures.

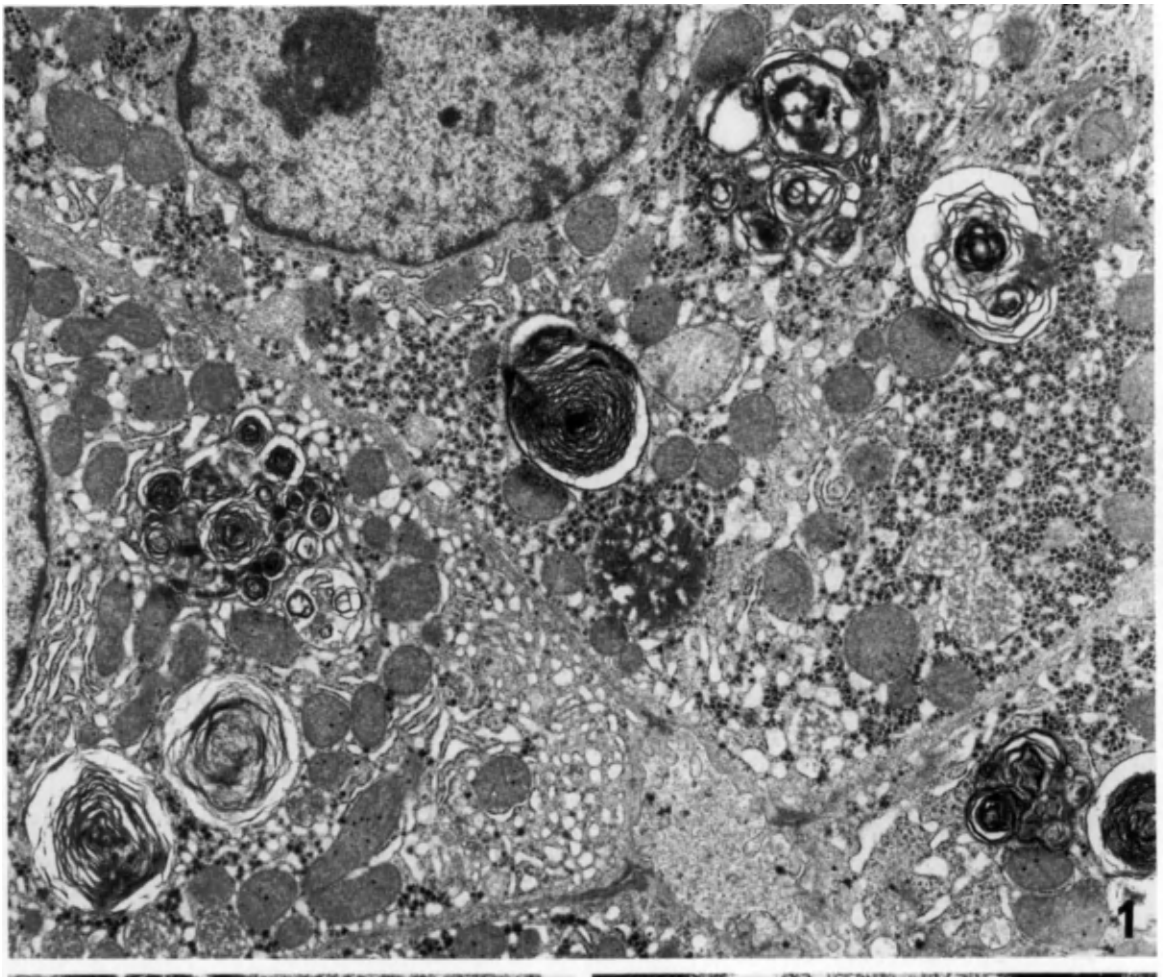
*Amiodarone produces lysosomes of various morphologies. If the lipid is not hydrated, it will present as electron-dense granules or as lipofuscin granules. When hydrated, the lysosomes contain whorled, stacked or reticulated myelinoid membranes. When such membranes are well ordered a crystalloid or crystalline structure is apparent.

Plate 270

Liver biopsy from a patient who had received amiodarone for 3.5 years.

Fig. 1. A low-power view showing several myelinosomes in hepatocytes × 10 500

Fig. 2 and 3. Higher-power view of myelinosomes containing electron-dense masses and myelinoid membranes deployed in concentric and reticulated patterns. × 27 000; × 20 000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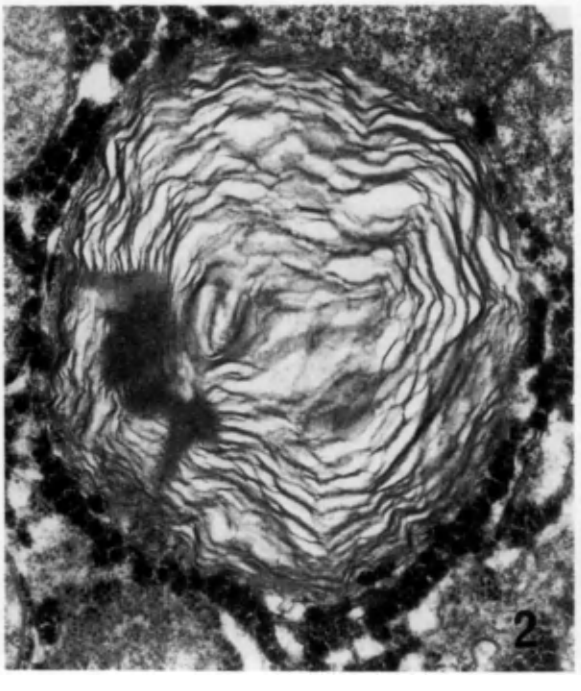
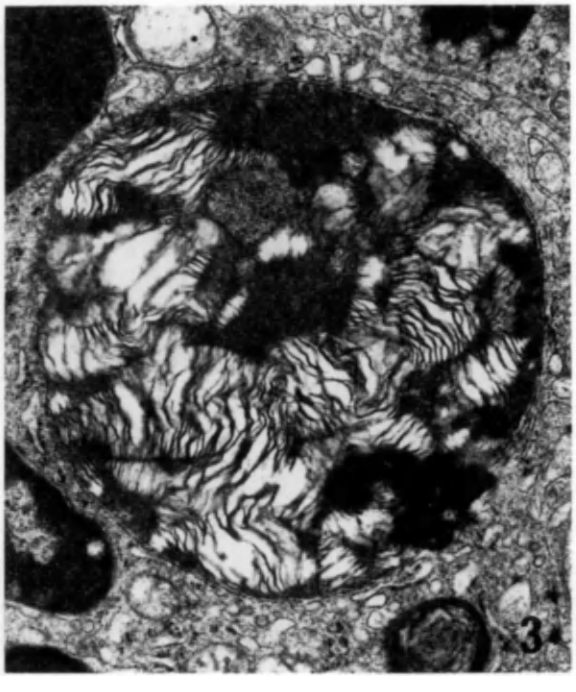

2


3

Erythrophagosomes and erythrophagolysosomes

Splenic erythrophagocytosis was first described by Kolliker is 1849. It is now known that this is the mechanism by which aged and atypical erythrocytes are removed from the circulation by cells of the reticuloendothelial system, particularly the macrophages in the spleen and bone marrow. Similarly, erythrophagocytosis is also one of the processes by which erythrocytes are removed after haemorrhage (*Plates 271–274*). In both instances the ingested erythrocytes are broken down by lysosomal enzymes, but while in the former case most of the iron released forms ferritin and is re-utilized, in the latter case much of the iron is converted to haemosiderin and stored for indefinite periods of time in residual bodies called siderosomes (pages 636–643).

Many studies dealing with erythrophagocytosis in the spleen, bone marrow, lymph nodes and lungs of man and other animals in normal and pathological states have been published (Rous and Robertson, 1917; Rous, 1923; Smith, 1958; Crosby, 1959; Koyama *et al.*, 1964; Simon and Pictet, 1964; Bessis, 1965; Rifkind, 1965; Wennberg and Weiss, 1968; Pictet *et al.*, 1969; Edwards and Simon, 1970; Simon and Burke, 1970; Aikawa and Sprinz, 1971; Adachi, 1977; Atwal and Saldanha, 1985).

The manner in which shed blood (e.g. haemorrhage after mechanical trauma or diseases in man) or injected blood (experimental studies in animals) is removed has also been extensively investigated. This includes studies on: (1) erythrophagocytosis by peritoneal macrophages in rats bearing the Novikoff ascites hepatoma (Essner, 1960); (2) erythrophagocytosis of extravasated maternal blood by chorionic epithelial cells of the placenta in the ewe (Burton *et al.*, 1976; Myagkaya and Vreeling-Sinderlarova, 1976; Lawn *et al.*, 1969; Davis and Wimstett, 1966; ferret (Gulamhusein and Beck, 1975); antarctic seal (Sinha and Erickson, 1974); and the paraplacenta in the cat (Malassiné, 1977); (3) erythrophagocytosis and siderosome formation by macrophages and endothelial cells of the trabecular meshwork and keratocytes after intraocular haemorrhage in man (Grierson and Lee, 1973, 1978; Ghadially, 1981a) and after injection of autologous blood in monkeys (Shabo and Maxwell, 1972); (4) erythrophagocytosis by alveolar macrophages after injection of blood in the trachea (Collet and Petrik, 1971; Petrik and Collet, 1971); (5) erythrophagocytosis by rat thyroid epithelial cells after microhaemorrhages produced by the administration of thiouracil (Zeligs and Wollman, 1977); (6) erythrophagocytosis by epithelial cells of the urinary bladder after necrosis and haemorrhage produced by various cytotoxic drugs (Wakefield and Hicks, 1974); (7) erythrophagocytosis and siderosome formation in synovial intimal cells and subsynovial macrophages after single or multiple injections of autologous blood in the rabbit knee joint (Roy and Ghadially, 1966, 1969; Ghadially and Roy, 1969; Ghadially *et al.*, 1976a); (8) erythrophagocytosis and siderosome formation in acute traumatic haemarthrosis in man (Roy and Ghadially, 1967); (9) erythrophagocytosis and siderosome formation in diseases where a state of chronic haemarthrosis prevails such as haemophilic arthropathy and villonodular synovitis (Ghadially *et al.*, 1976b; Ghadially *et al.*, 1979); (10) erythrophagocytosis and siderosome formation after the production of a subcutaneous haematoma by injection of autologous blood in the rabbit (Lalonde and Ghadially, 1977); and (11) erythrophagocytosis and siderosome formation after the production of an intramuscular haematoma by injection of autologous blood in the rabbit (Lalonde *et al.*, 1978).

A review of the above mentioned literature shows that whenever, wherever and however extravasation of blood occurs certain basic mechanisms are deployed by the organism for its

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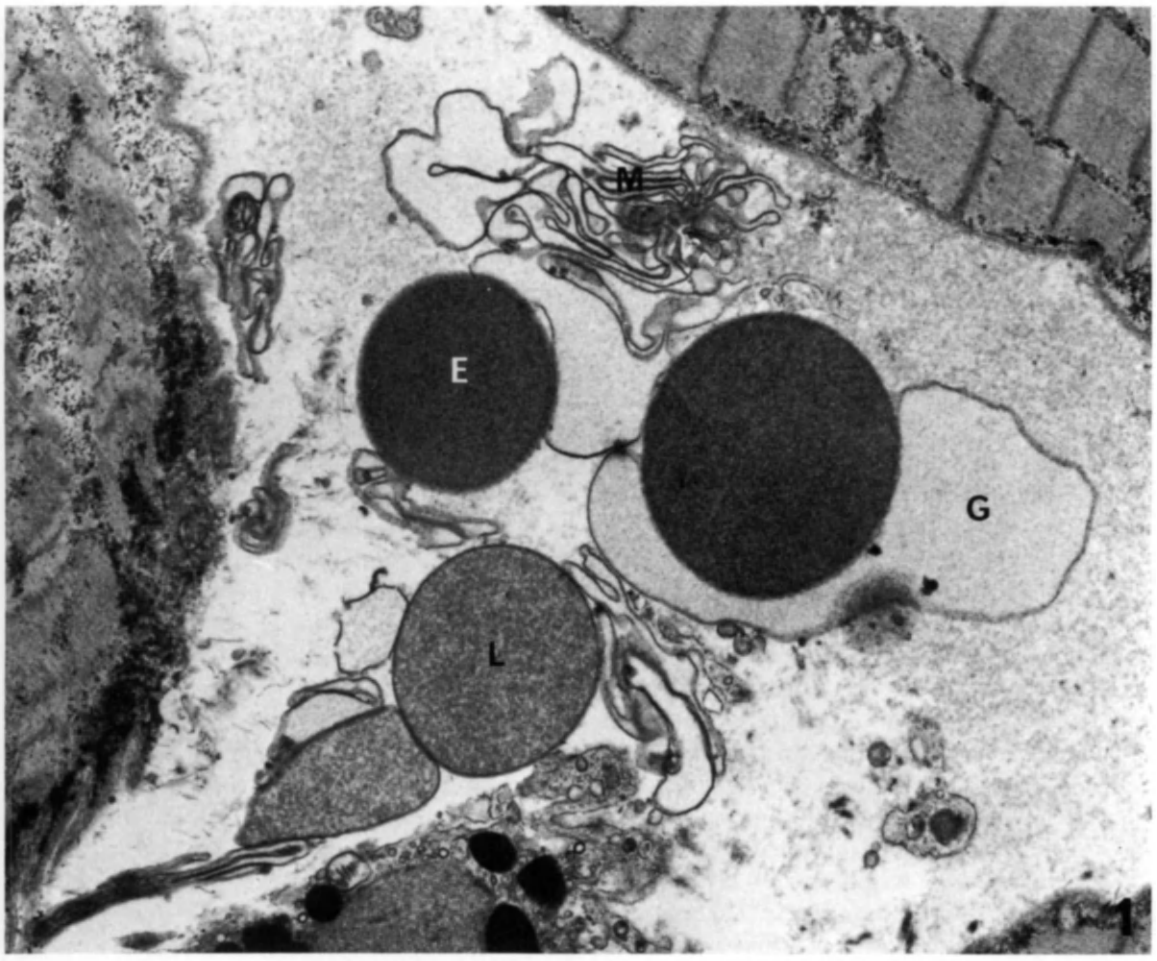
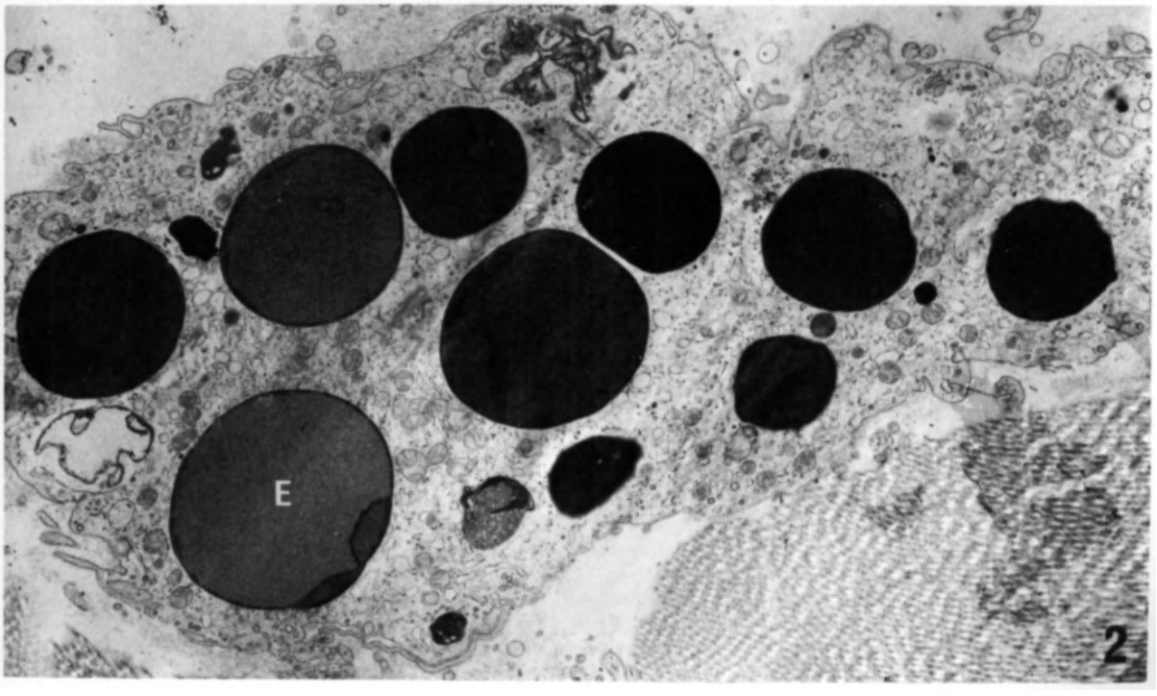
Plate 271

Fig. 1. Erythrocytes (E), partially lysed erythrocytes (L), a distorted erythrocyte ghost (G) and crumpled erythrocyte membranes (M) are seen one day after the production of an intramuscular haematoma in a rabbit by an injection of autologous blood. × 11 000

Fig. 2. Some nine or more erythrocytes (E) are seen in this macrophage four days after a subcutaneous haematoma had been produced in a rabbit by injection of autologous blood. × 6500 (*From Lalonde and Ghadially, 1977*)


M
E
G
L
1


E
2

disposal and that the key mechanism is phagocytosis of erythrocytes and their breakdown products by macrophages and other cells. There are, however, some site dependent differences worthy to note.

Not all the erythrocytes in shed blood* are removed by phagocytic cells or end up as haemosiderin deposits. There is now ample evidence that after a haemorrhage many erythrocytes can escape from various 'cavities' such as the peritoneal cavity, joint cavity or the eye (for references and more details *see* Ghadially, 1979a) but this probably does not happen to any great extent in 'solid' tissues (e.g. muscle or subcutaneous tissues), where usually a haematoma develops (Lalonde and Ghadially, 1977; Lalonde *et al.*, 1978). Clotting of blood and lysis of erythrocytes† is also more likely to occur when haemorrhage occurs in a 'solid' tissue than in a 'cavity' (*Plate 271, Fig. 1*).

This may be so, but from the point of view of removal of shed erythrocytes by phagocytic cells a deciding factor is whether lysis occurs or not. Thus, when lysis does not occur, the phagocytic cells will have to cope with entire erythrocytes or erythrocyte fragments, but when lysis does occur the phagocytic cell will have to tackle these and also the products of lysis, namely, erythrocyte ghosts, crumpled erythrocyte membranes and the liberated haemoglobin. It should be clearly understood that erythrocyte fragments are small rounded or teardrop shaped membrane-bound structures containing haemoglobin and that they are derived from the erythrocyte prior to or during ingestion by the phagocytic cell. This is quite different from lysis of erythrocytes, where haemoglobin escapes leaving behind a red cell ghost or the crumpled remnants of erythrocyte membrane after escape of its contents.

Fragmentation of the erythrocyte may be looked upon as a mechanism whereby the erythrocyte is reduced to more manageable smaller units which would be more easily ingested and digested by the phagocytic cell. How erythrocyte fragmentation occurs prior to ingestion is not clear but Essner (1960) presents excellent illustrations of numerous small spherical fragments derived from erythrocytes in a haemorrhagic ascites in rats bearing the ascites form of the Novikoff hepatoma. Sometimes a phagocytic cell pinches off a portion of an erythrocyte (referred to as 'fragmentation during ingestion') and later also the unengulfed schizocyte that remains. This piecemeal erythrophagocytosis by macrophages is seen in normal spleen engaged in the removal of old and effete erythrocytes (Rous, 1923; Crosby, 1959) and this phenomenon has also been studied *in vitro* using phase contrast microcinematography (Bessis, 1973). Fragmentation of erythrocytes (during ingestion) is also witnessed when blood shed in the thyroid follicle is phagocytosed by thyroid epithelial cells (Zeligs and Wollman, 1977). Fragmentation prior to or during ingestion are, however, rather rare phenomena, they were not witnessed in most of the studies listed at the beginning of this section.

In the pre-electron microscopic era there was much doubt as to whether breakdown of red blood cells occurred extracellularly or intracellularly. It is now abundantly clear that in some sites and situations there is virtually no change in the shed erythrocytes (e.g. after injection of autologous blood in a normal rabbit joint) and that entire erythrocytes are phagocytosed and broken down within the phagocytic cell but in others, fragmentation and/or lysis occurs, so

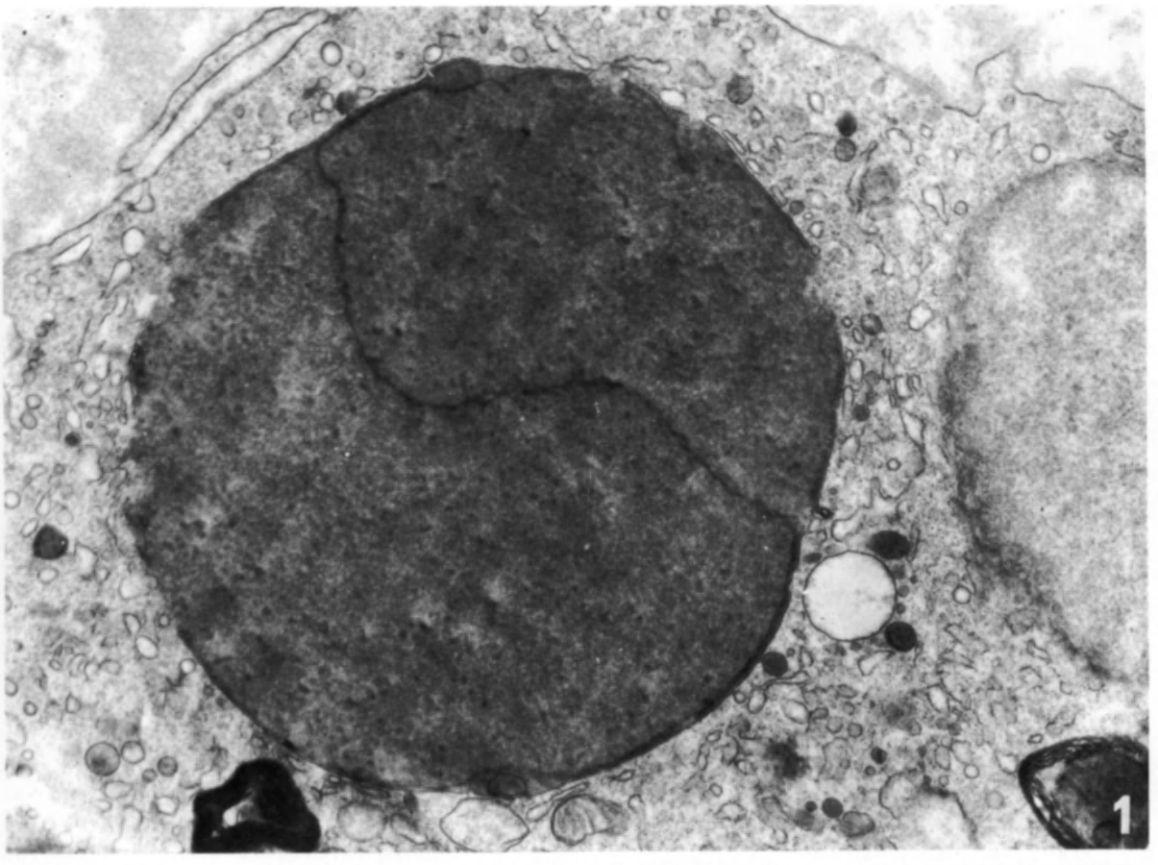
*In the text that follows the terms 'shed blood' or 'extravasated blood' is used to cover not only haemorrhage engendered by trauma, disease processes or administration of drugs, but also autologous blood injected into experimental animals.

†Discocyte to spherocyte transformation is quite common and no doubt precedes lysis.

Plate 272

Fig. 1. Haemarthrosis produced by injection of autologous blood into the rabbit knee joint. A subsynovial macrophage has phagocytosed an erythrocyte which is showing segmentation by a partition which was found to be double-membraned at higher magnification. × 23 000 (*From Ghadially and Roy, 1969*)

Fig. 2. An erythrophagosome or erythrophagolysosome found in a Kupffer cell. The probable stages (A, B, C, D) of evolution of satellite erythrophagosomes or erythrophagolysosomes are easily discerned. × 26 000 *From Ghadially and Lalonde, 1977)*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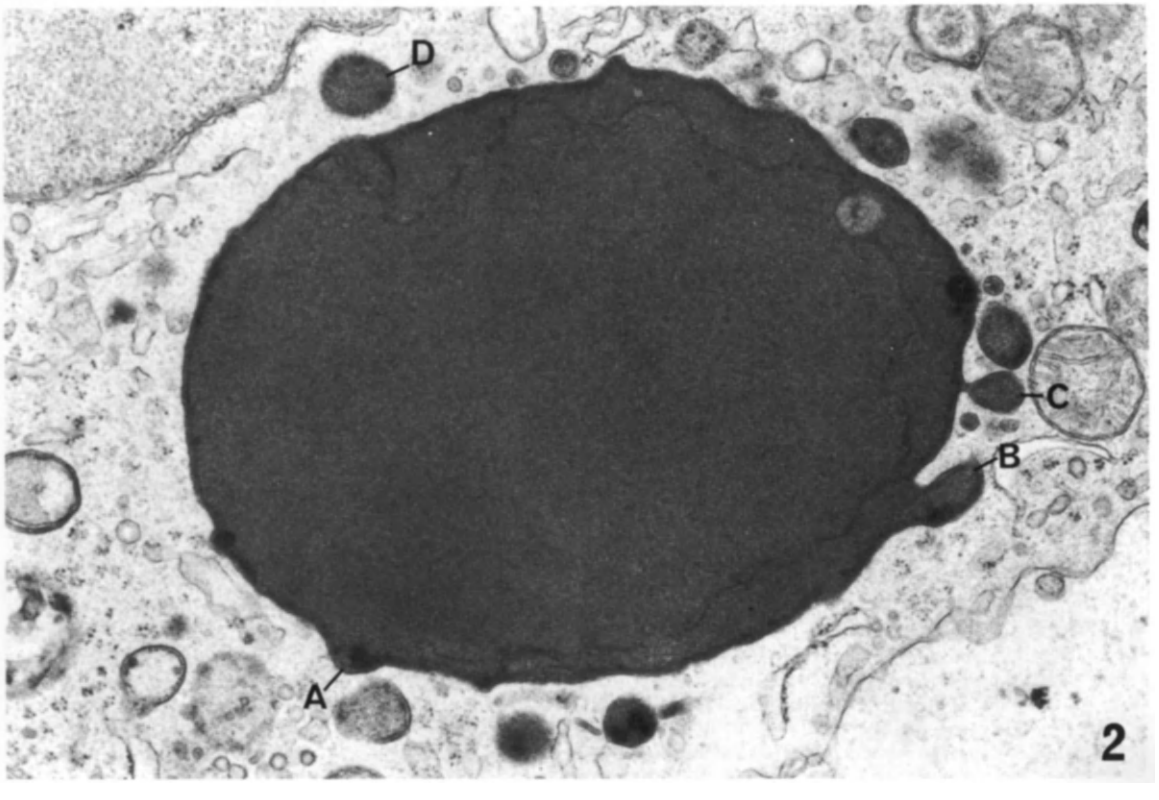
D
C
B
A
2

that one also finds phagosomes containing erythrocyte ghosts and crumpled membranes. However, it must be stressed that there is no evidence whatsoever that haemoglobin is degraded to haemosiderin in an extracellular site and then picked up by phagocytic cells. The formation of haemosiderin from haemoglobin occurs within the heterolysosomes (erythrophagolysosomes) that develop in the phagocytic cell. Quite a few types of cells can at times phagocytose erythrocytes (e.g. synovial intimal cells, bladder epithelial cells and thyroid epithelial cells), but in most instances it is the mácrophage which performs this function.

There is now ample evidence that erythrophagosomes (also phagosomes containing material derived from lysed erythrocytes) fuse with primary lysosomes to produce heterolysosomes where digestion of the erythrocyte ensues, for acid phosphatase has been demonstrated in such structures in many situations. (For references *see* Myagkaya and Vreeling-Sinderlarova, 1976.)

Various morphological changes in phagocytosed erythrocytes make one speculate that the entire erythrocyte may be a bit too large to digest in a single large heterolysosome and that special mechanisms have been evolved to tackle this problem. For example, it is at times noted that the erythrocyte lying within the phagosome or phagolysosome is segmented by one or more double-membraned partitions (*Plate 272*) which on closer examination appear to be continuous with the erythrocyte membrane. The enveloping phagosomal membrane does not trail into these septae nor does this appear to be a prelude to cleavage and fission leading to formation of smaller phagosomes or heterolysosomes. However, one may speculate that such septae may provide channels which permit the hydrolytic enzymes to reach the deeper parts of the endocytosed erythrocyte. The idea that this appearance (in man and common laboratory rodents studied so far) of 'septae' in erythrocytes is due to the presence of more than one erythrocyte in a phagosome is not attractive because of the circular shape and the consistent failure to find an erythrophagosome so large that its size would necessitate one to postulate that it contained more than one erythrocyte. However, an exception to this is seen in the sheep placenta where multiple erythrocytes (several circular profiles) may be found lying in a single very large heterolysosome (Myagkaya and Vreeling-Sinderlarova, 1976).

Yet another peculiar morphological alteration that occurs in erythrophagosomes or erythrophagolysosomes has been termed 'tunnelization' by Edwards and Simon (1970) who observed this phenomenon in splenic macrophages. This is a rather rare phenomenon but we (Ghadially and Roy, 1969) have seen this in the synovial intimal cells also (*Plate 273, Fig. 3*). As its name implies, the phagocytosed erythrocyte mass is riddled by invagination of the ensheathing membrane of the erythrophagolysosome. This then may be looked upon as yet

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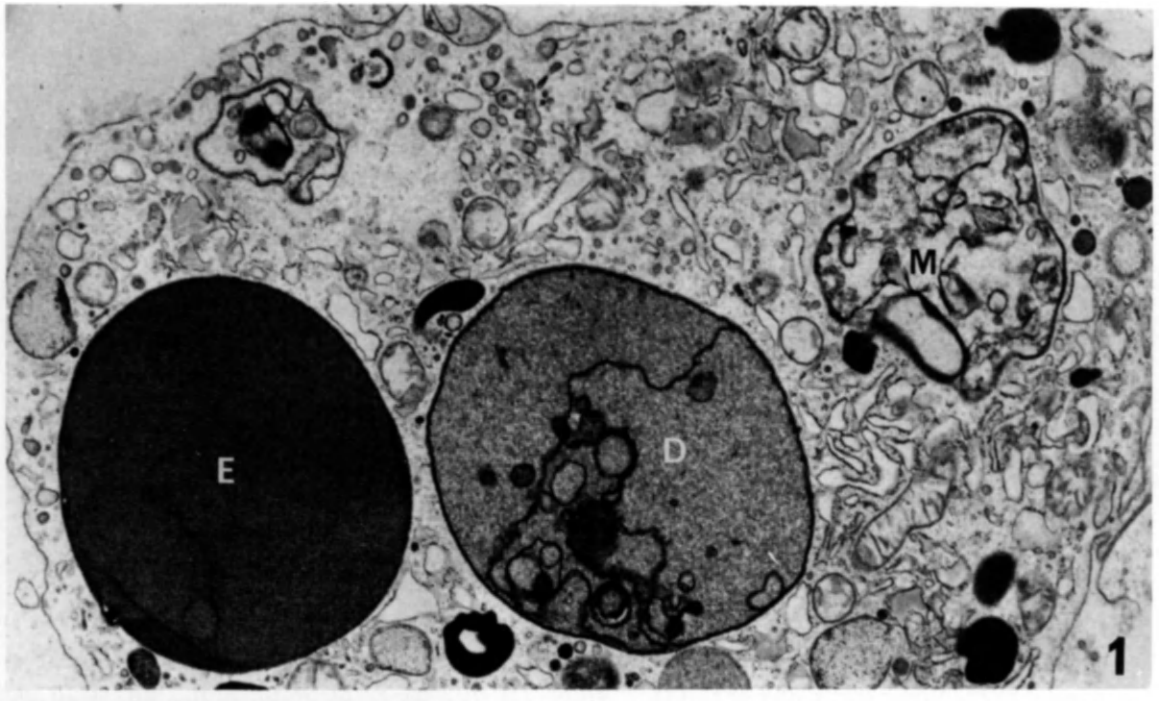
Plate 273

Fig. 1. A macrophage found four days after an intramuscular haematoma had been produced by injection of autologous blood in the rabbit. It contains a phagocytosed erythrocyte (E), a partially dehaemoglobinized erythrocyte (D) and crumpled erythrocyte membranes (M). × 16 000 (*From Lalonde, Ghadially and Massey, 1978*)

Fig. 2. A macrophage found seven days after the production of a subcutaneous haematoma by injection of autologous blood. It has phagocytosed, crumpled erythrocyte membranes (M) and a ghost. The membrane (arrows) of the ghost is disintegrating and has receded from the wall (arrowheads) of this heterolysosome. × 20 000 (*From Lalonde and Ghadially, 1977*)

Fig. 3. Haemarthrosis produced by injection of autologous blood into the rabbit knee joint. A tunnelled erythrophagolysosome is seen in a synovial intimal cell. × 17 000 (*From Ghadially and Roy, 1969a*)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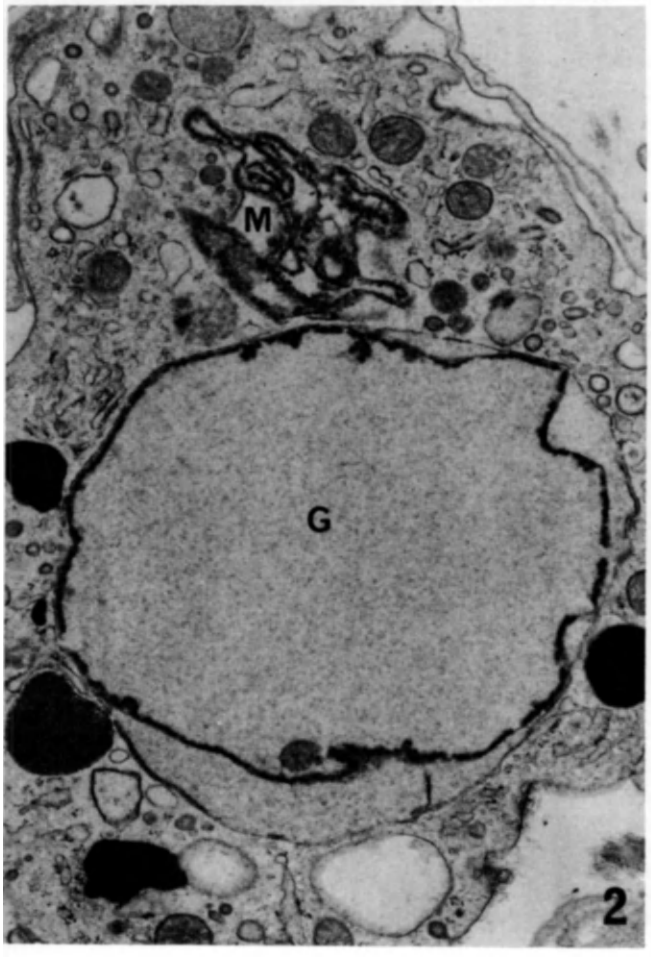
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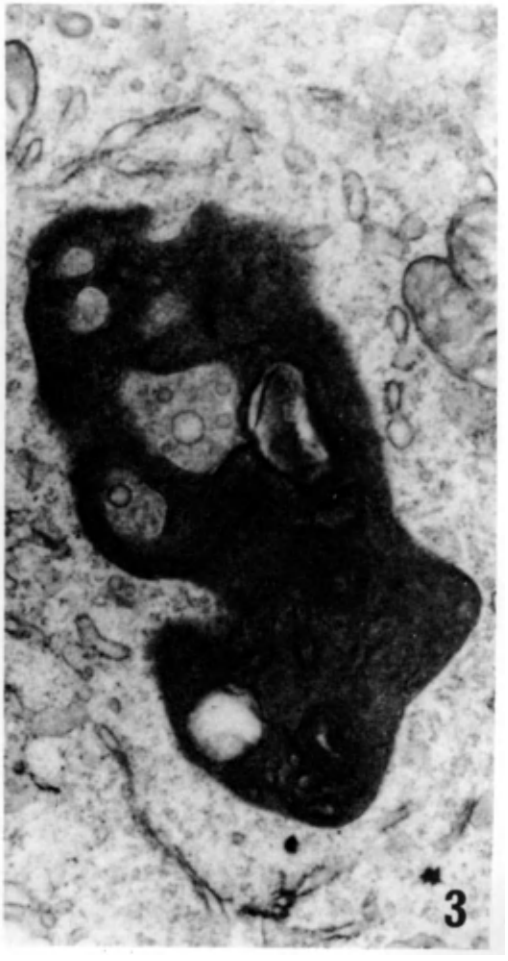

3

another device whereby a large increase in surface area occurs and once more this too would facilitate digestion by producing contact between hydrolytic enzymes (which are delivered to the periphery of the erythrophagosome by the fusion with primary lysosomes) and the substrate.

Yet another phenomenon which seems to have no parallel in other situations (i.e. after phagocytosis of materials other than erythrocytes) is the formation of what might be called satellite or daughter heterophagosomes or heterophagolysosomes from the main phagocytosed erythrocyte mass (*Plate 272, Fig. 2*). This phenomenon described by us in synovial intimal cells (Roy and Ghadially, 1966; Ghadially and Roy, 1969; Ghadially, 1975a) has also been seen in epithelial cells of the urinary bladder that have ingested erythrocytes (Wakefield and Hicks, 1974) and in a Kupffer cell that had ingested an erythrocyte (Ghadially and Lalonde, 1977). The probable sequence of events leading to the formation of the satellite structures seems to be the production of a mound-like elevation which elongates into a tear-drop-shaped structure which then detaches to form the satellite heterophagosomes or heterophagolysosomes. Serial sections show that these satellite structures are indeed truly separate from the main mass and not connected with it in another plane of sectioning.

This phenomenon may be looked upon as an example of 'intracellular fragmentation' not just of the erythrocyte but the entire phagosome or phagolysosome containing the erythrocyte and one may speculate that such 'fragmentation' creates smaller units where digestion is facilitated by bringing about better contact between the enzymes and substrates.

However, a common phenomenon seen in erythrophagolysosomes in many sites (*Plate 274*) suggests that after the fusion of primary lysosomes with the erythrophagosome, digestion and partial disintegration of the erythrocyte membrane occurs so that the erythrocyte membrane recedes from the wall of the erythrophagolysosome (i.e. the membrane bounding the lysosome) and the escaped haemoglobin comes to lie between the receded erythrocyte membrane and the wall of the lysosome. In our study on villonodular synovitis (Ghadially *et al.*, 1979) we saw appearances which suggest that digestion and disintegration of haemoglobin in this region leads to the formation of electron-dense iron-containing particles which then form aggregates. Such aggregates (which might be considered nascent siderosomes) probably escape later on by a process of budding, acquiring a limiting membrane from the wall of the erythrophagolysosome. Thus a single-membrane-bound body containing electron-dense-iron-containing particles (viz. a siderosome) would be born.

In the spleen the situation is somewhat similar in that very fine electron-dense particles (thought to be ferritin by Edwards and Simon, 1970) form along the wall of the erythrophagolysosome but iron does not accumulate to form haemosiderin deposits but escapes into the cell and then into the circulation to replenish the iron stores in the body.

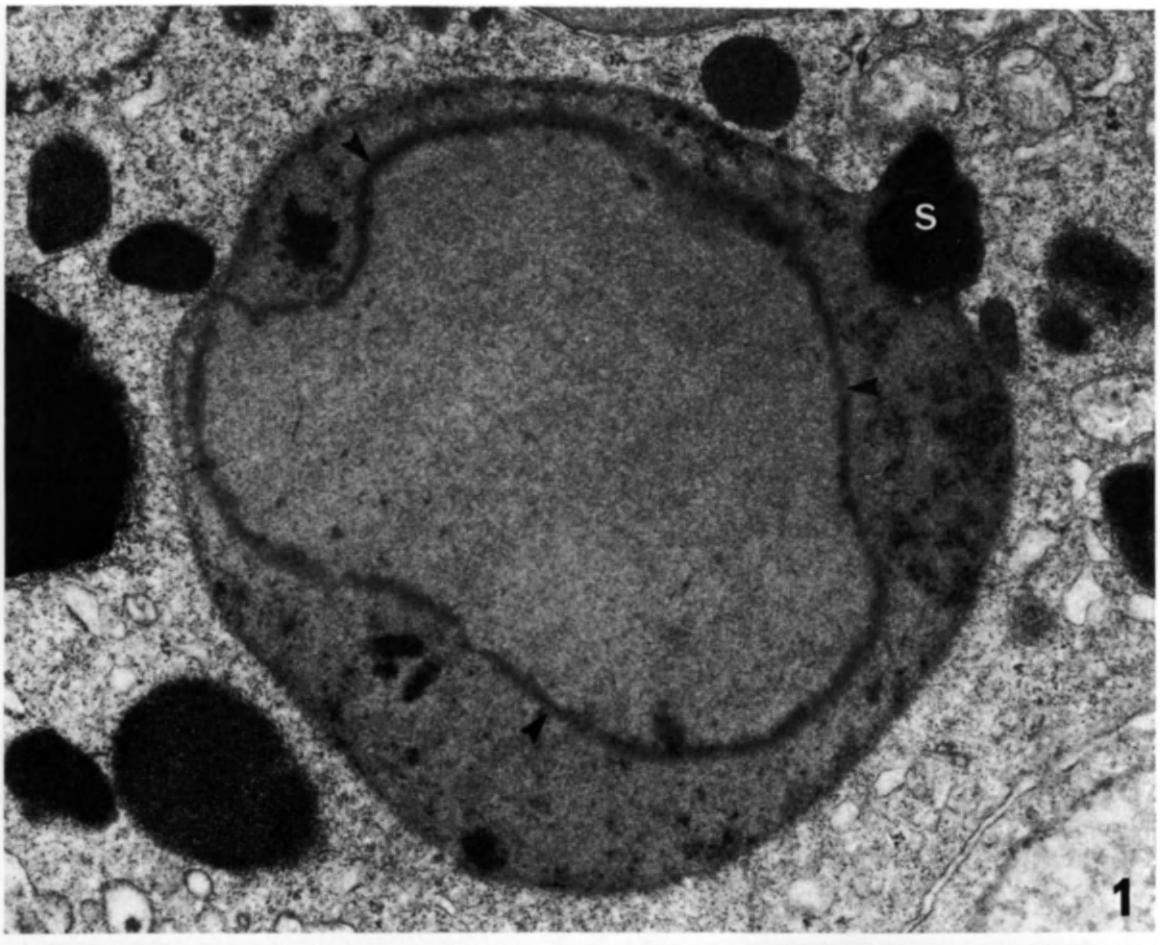
The endocytosis and digestion of entire erythrocytes, erythrocyte fragments, erythrocyte ghosts, crumpled erythrocyte membranes and haemoglobin lead ultimately to the formation of three types of lysosomal or residual bodies, myelinosomes and myelinosiderosomes which are dealt with on page 622 and *Plate 268* and siderosomes which are considered in the next section.

Plate 274

From Ghadially, Lalonde and Dick, 1979.

Fig. 1. Villonodular synovitis. Erythrophagolysosome showing electron-dense particles lying between the receded erythrocyte membrane (arrowheads) and the wall of the lysosome. Also seen is a structure thought to be a nascent siderosome (S). × 33 000

Fig. 2. Same specimen as above. High-power view of structures which may be interpreted as nascent siderosomes (S). The limiting membrane of the erythrophagolysosome (arrow) and the disintegrating, receded erythrocyte membrane (arrowheads) are easily identified. The cytoplasmic matrix is peppered with electron-dense particles. × 69 000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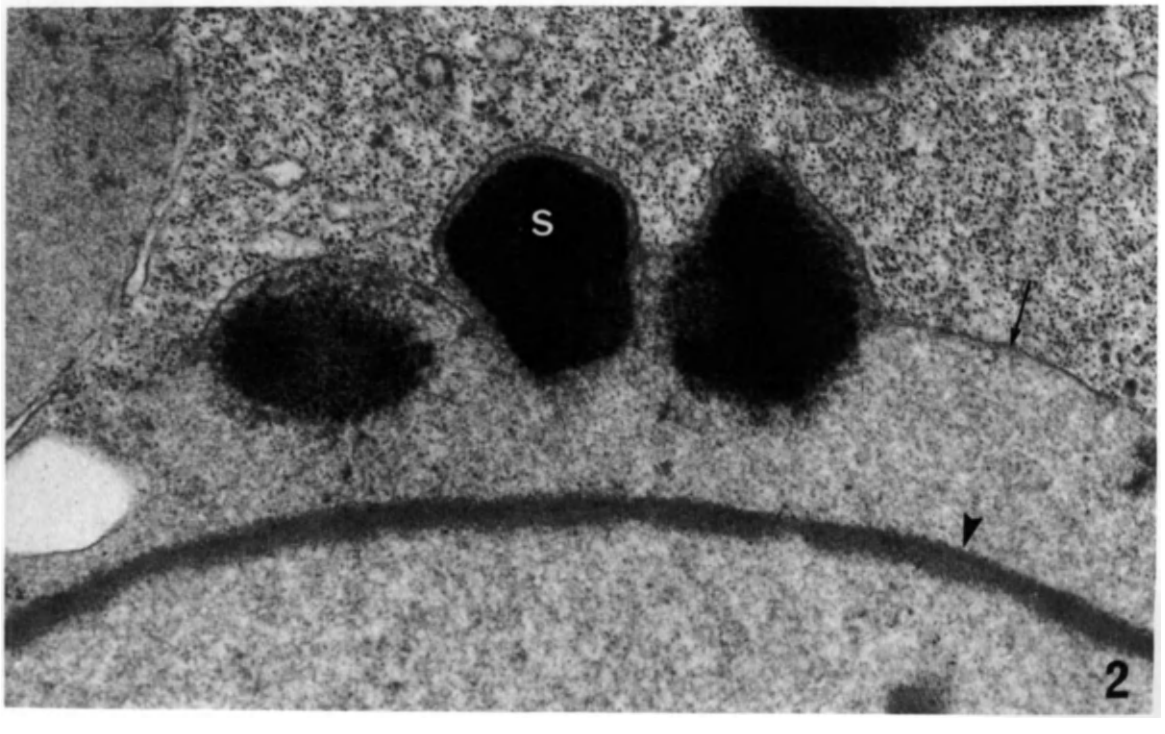
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Siderosomes, haemosiderin and ferritin

The term 'siderosome' was coined by Richter (1957) to describe single-membrane-bound bodies containing aggregates of electron-dense haemosiderin particles. It is now clear that the Prussian-blue-positive haemosiderin granule of the light microscopist represents either a large siderosome, a clump of smaller siderosomes or compounds siderosomes. Occasional siderosomes are normally found in the cells of the reticuloendothelial system but many more are encountered in diseases or experimental situations where an iron-overload is operative (e.g. pathological iron assimilation, blood transfusion, parenterally administered iron, etc.). In such states, the parenchymatous cells of various organs, particularly the liver, also come to contain siderosomes (*Plate 320*). Siderosomes are also encountered in tissues after haemorrhage or injection of blood in experimental animals (for details and references *see* page 628) (*Plates 275–278*). In all the above-mentioned situations (systemic iron overload or local haemorrhage), the iron deposits occur mainly in single-membrane-bound lysosomal bodies (residual bodies) called siderosomes, but in some cells the cytoplasm may also be peppered with innumerable electron-dense iron-containing particles.

At one time it was thought that haemosiderin was deposited in mitochondria (Arnold, 1900; Gillman and Gillman, 1945) but it is now clear that, except in certain special instances (page 323) iron is not deposited in mitochondria and siderosomes do not form in this fashion. Current opinion regards the siderosome as a lysosomal body, since acid phosphatase can be demonstrated in it (Novikoff *et al.*, 1956; Ericsson, 1965; Hultcrantz *et al.*, 1979). The mature siderosome is, in fact, a residual body in which indigestible iron residues have been left behind after most of the lysosomal enzyme activity has subsided.

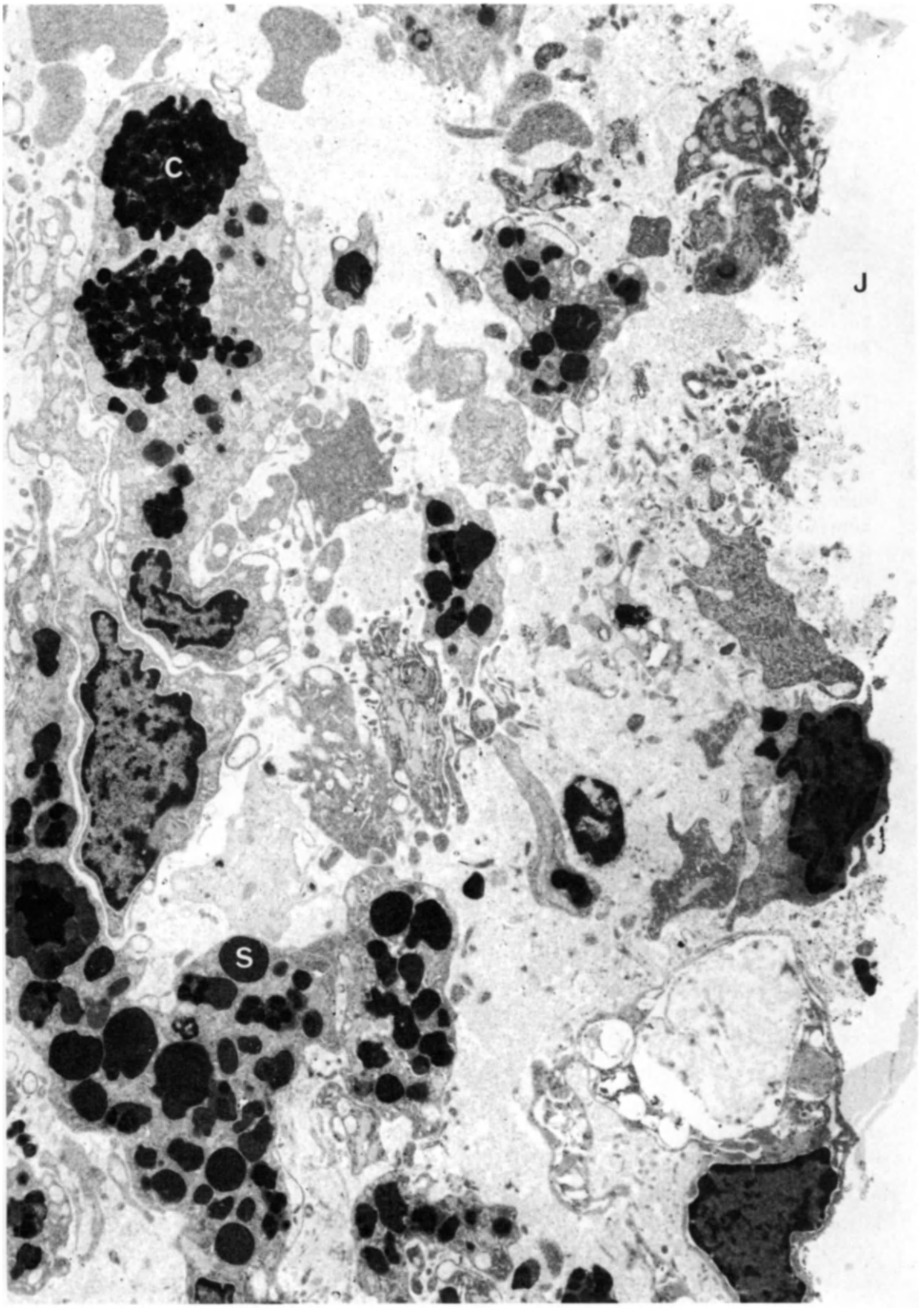
Despite massive ultrastructural evidence to the contrary, studies claiming that iron deposits occur in mitochondria continued to appear for some time. For example, even in 1975, on the basis of cell fractionation studies on the liver of rats treated with iron-dextran, Hanstein *et al.* claimed that virtually all the iron was located in the mitochondria. The experiments of Hanstein *et al.* (1975) were repeated by Bonkowsky *et al.* (1979) and they demonstrate quite clearly that the so-called 'mitochondrial pellet' contains not only mitochondria but siderosomes and that further fractionation into 'light' and 'heavy' fractions does not eliminate this problem (for a more detailed critique, *see* Ghadially, 1979b).

The solitary siderosomes (*Plates 275–277*) seen in the synovial membrane in acute or chronic haemarthrosis or after injection of blood into the joint, skin or muscle of experimental animals vary in size from small to quite large, but they show a fairly uniform distribution of electron-dense iron-containing particles within them, so there is no morphological ground for suspecting that the larger siderosome has arisen by the fusion of smaller ones. However, such a process of fusion is clearly evident in many of the very large siderosomes seen in such states. We (Roy and Ghadially, 1967) have coined the term 'compound siderosome' to describe these structures where a conglomeration of fused siderosomes is seen (*Plates 275, 276* and *278*).

The exact manner in which a siderosome develops is not too clear but certain steps in the genesis of siderosomes can be seen with the electron microscope. Thus, after parenteral administration of iron compounds, electron-dense iron-containing particles have been seen to accumulate in the secondary lysosomes (Richter, 1958, 1959; Bradford *et al.*, 1969; Arborgh *et al.*, 1974) in the hepatocyte (i.e. peribiliary dense bodies) while after intra-articular injection of ferritin into the joint space, endocytosis of this material by synovial intimal cells has been witnessed (Muirden, 1963).

Plate 275

Haemophilic human synovial membrane. Numerous solitary (S) and compound (C) siderosomes are present in the synovial intimal cells. Joint space (J). × 8500 (*From Ghadially, Ailsby and Yong, 1976a*)


C
J
S

After haemorrhage, the endocytosis and digestion of entire erythrocytes, and breakdown products of erythrocytes in heterolysosomes leads to the formation of a variety of lysosomal or residual bodies (page 622) including siderosomes which no doubt stem from the degraded haemoglobin.

The intermediate steps between the phagocytosed erythrocyte on the one hand and the final siderosome on the other are not easy to trace in all instances, but in a case of villonodular synovitis we (Ghadially *et al.*, 1979) saw appearances (*Plate 274*) which suggest that in the erythrophagolysosome digestion and partial disintegration of the erythrocyte membrane occurs first, so that the membrane recedes from the wall of the erythrophagolysosome and the escaped haemoglobin comes to lie between the receded membrane and the wall of the lysosome. Digestion and disintegration of the haemoglobin in this region presumably leads to the formation of electron-dense iron-containing particles which then form aggregates. Such aggregates (which might be considered nascent siderosomes) probably escape later on by a process of budding, acquiring a limiting membrane from the wall of the erythrophagolysosome. Thus a single membrane-bound body containing electron-dense iron-containing particles (viz. a siderosome) would be born.

Electron microscopic studies have helped clarify certain aspects of the staining reaction of haemosiderin seen with light microscopy. When tissues containing haemosiderin are subjected to the Prussian-blue test, discrete blue granules, and at times also a diffuse blue hue, is seen in the cytoplasm. The latter type of staining has been ascribed in the past to a diffusion of Prussian blue from the granules or to differences in the diffusion of various constituents of haemosiderin (e.g. Lillie, 1954). Such hypotheses are now unnecessary, for electron microscopic studies provide a much more plausible explanation of this phenomenon. It is clear that at times numerous electron-dense iron-conatining particles similar to those found in siderosomes are also found scattered in the cytoplasm (*Plate 277*). These are too small to be resolved by light microscopy but when present in sufficient numbers are likely to produce enough Prussian blue to give the cell a faint blue tint. Groups of small siderosomes, large solitary siderosomes and compound siderosomes are sizeable enough to produce the characteristic discrete blue granules or the larger blue masses seen in the Prussian blue test for haemosiderin.

The term 'haemosiderin' was coined by Neumann (1888) to describe the iron-rich pigment that developed after extravasation of blood. He states: 'so möchte ich mir erlauben, die Bezeichnung "Haemosiderin" vorzuschlagen' (I will allow myself to suggest the name of 'haemosiderin').'

Since haemosiderin is in essence a product of red cell destruction the idea was born that haemosiderin is an iron-rich organic compound derived from haemoglobin. This notion was supported by the histochemical studies of Gedigk and Strauss (1953) who concluded that besides iron, haemosiderin contains polysaccharide, protein and lipid, and Gössner (1953) who believed that haemosiderin contained glycoprotein.

At the ultrastructural level haemosiderin presents as electron-dense particles in the siderosome. There has been much controversy regarding the nature and chemical composition of these particles. Some authors have called them 'ferritin' or 'ferritin aggregates', others have

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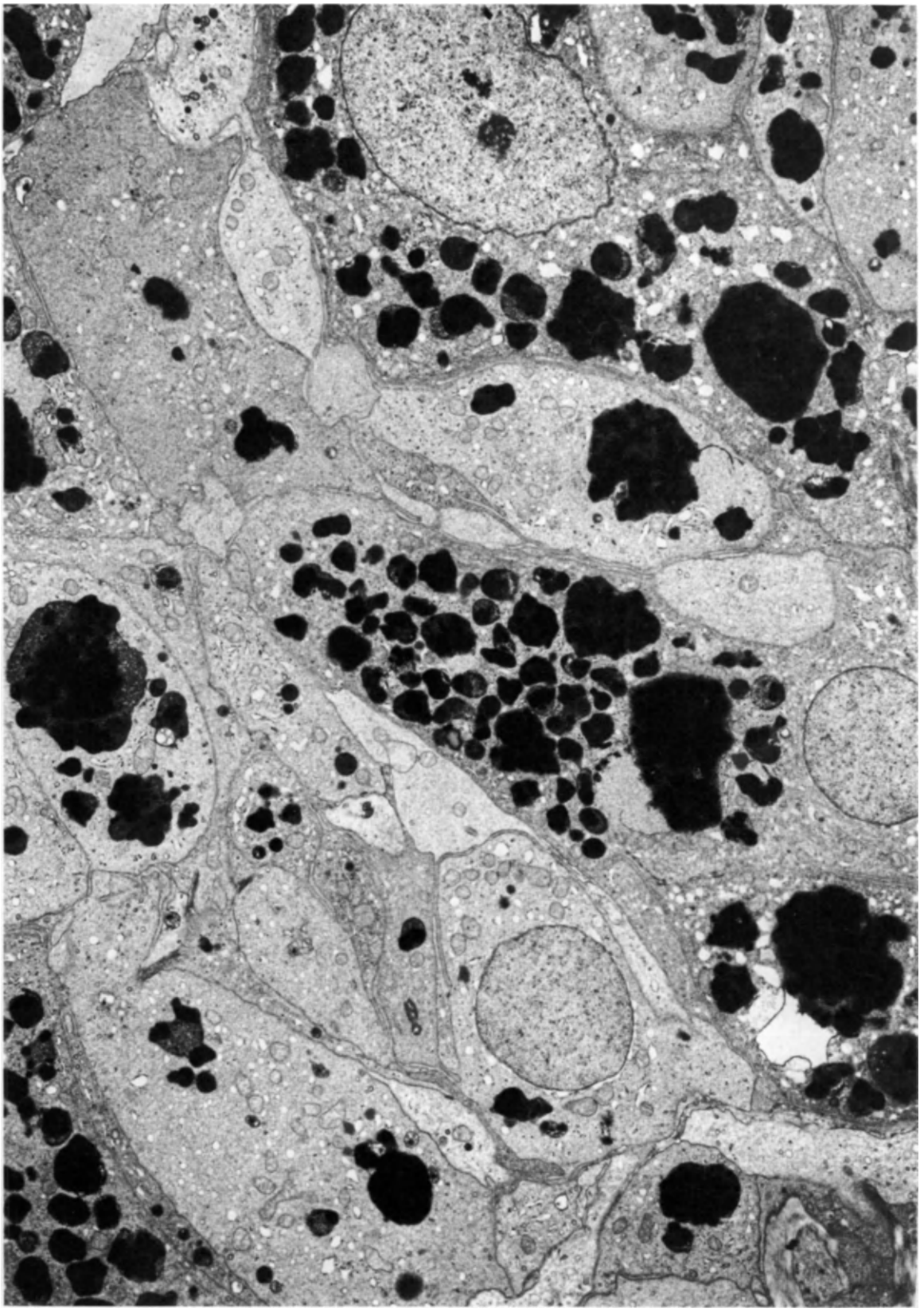
Plate 276

Villonodular synovitis. The subsynovial macrophages are loaded with solitary and compound siderosomes. × 8000 (*From Ghadially, 1979a*)



called them 'haemosiderin', while we (Ghadially and Roy, 1969) refer to them by the non-committal term 'electron-dense iron-containing particles'. The problem stems from differences of opinion regarding the morphology of the ferritin molecule as seen with the electron microscope and the differences between haemosiderin and ferritin. About the latter, Richter (1958) stated that: (1) 'the micelles of haemosiderin and ferritin are composed of the same sub-units'; and (2) 'ferritin is a component of haemosiderin and at times a prominent one'. On the other hand, Shoden and Sturgeon (1961) have suggested that the haemosiderin granule is a relatively amorphous condensate of ferric hydroxide virtually free of protein, and that it probably arises from a process of degradation of the protein (apoferritin) matrix of ferritin.

Ferritin is a biochemically well-defined iron-containing protein which was isolated from the spleen on the basis of its solubility in water by Laufberger (1934, 1937). By contrast, haemosiderin is insoluble in water and it is generally thought, on the basis of biochemical and histochemical studies that it is probably not a single substance but varies in its composition depending upon the anatomical source, the stage of maturation of the pigment and the iron preparation used to induce its formation. For example, as pointed out before, besides iron, haemosiderin is thought to contain polysaccharides, protein and lipid (e.g. Gedigk and Strauss, 1953). As Sturgeon and Shoden (1969) quite rightly point out 'it is highly improbable that any of these substances other than iron constitute an integral part of haemosiderin' and the different results obtained on chemical analyis could be attributable to the impossibility of obtaining 'pure' haemosiderin uncontaminated by other cell components. Yet another explanation of the alleged heterogeneous nature of haemosiderin would be that haemosiderin granules are lysosomal bodies which, at least in the earlier stages of their evolution, may contain erythrocyte membranes and cytoplasm. Little wonder then, that proteins and lipids are found when such granules are analysed, or that with ageing and maturation of the pigment the iron concentration is found to increase★.

Ultrastructural studies have shown that the ferritin molecule is roughly spherical in shape. It comprises a protein shell (apoferritin) of about 11 nm diameter with a centrally located 5.5 nm diameter core containing iron.

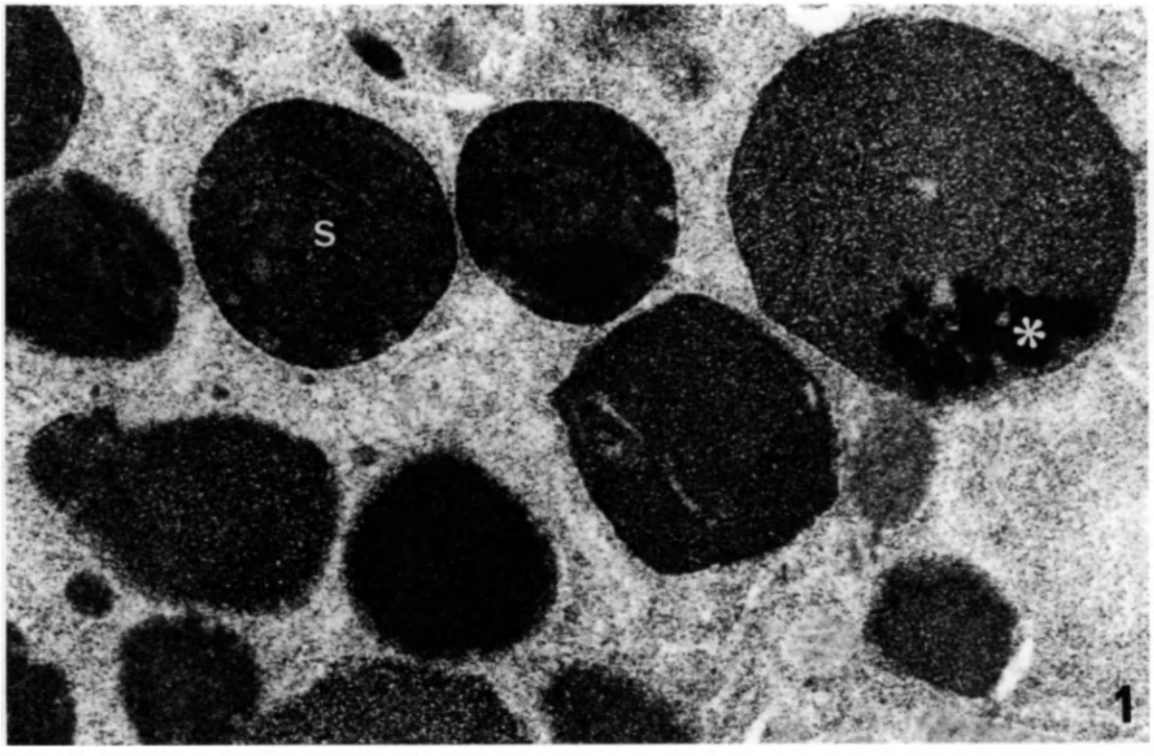
The core as seen with the electron microscope is thought to consist of four to six subunits (called iron micelles) of about 2.7 nm in diameter (Farrant, 1954; Richter, 1959; Kerr and Muir, 1960; Bessis and Breton-Gorius, 1959, 1960; van Bruggen *et al.*, 1960). Although much has been made of such images and the so-called tetrads and hexads have been considered to be the characteristic 'signature' of ferritin, it is now evident that this appearance is an artefact. Haydon (1969) has demonstrated that: '(1) At a potential resolution of 5 Å near-focus electron micrographs of ferritin molecules show no substructure in the core and (2) by defocusing the microscope a computed amount, electron micrographs of the same ferritin molecule can be produced which show apparent core substructure similar to that shown in several patterns reported in the literature'. Such contentions are supported by x-ray diffraction studies (Harrison *et al.*, 1967) which show that hundreds of iron 'micelles' do exist in the ferritin core but they are randomly distributed and too small to be resolvable by currently available electron microscopes.

★This is supported by morphological evidence also. In the early siderosomes produced after injection of blood, the electron-dense iron-containing particles lie in a medium density (presumably proteinaceous) matrix. At a later stage little beside compacted electron-dense iron-containing particles are seen in the siderosome.

Plate 277

Fig. 1. Haemophilic human synovial membrane. Synovial intimal cell containing numerous solitary siderosomes (S) with fairly uniformly dispersed electron-dense iron-containing particles within them. Similar particles are seen also in the cytoplasmic matrix. One of the siderosomes also contains highly electron-dense material (★) acceptable as osmiophilic lipid. × 41 000 (*From Ghadially, Ailsby and Yong, 1976a*)

Fig. 2. Same case as above. Unstained section from unosmicated material (i.e. fixed in glutaraldehyde but not post-fixed in osmium). As is to be expected cellular details are not visualized but the electron-dense particles (arrows) in the cytoplasm and in the siderosomes (S) are easily visualized because of their iron-content. × 90 000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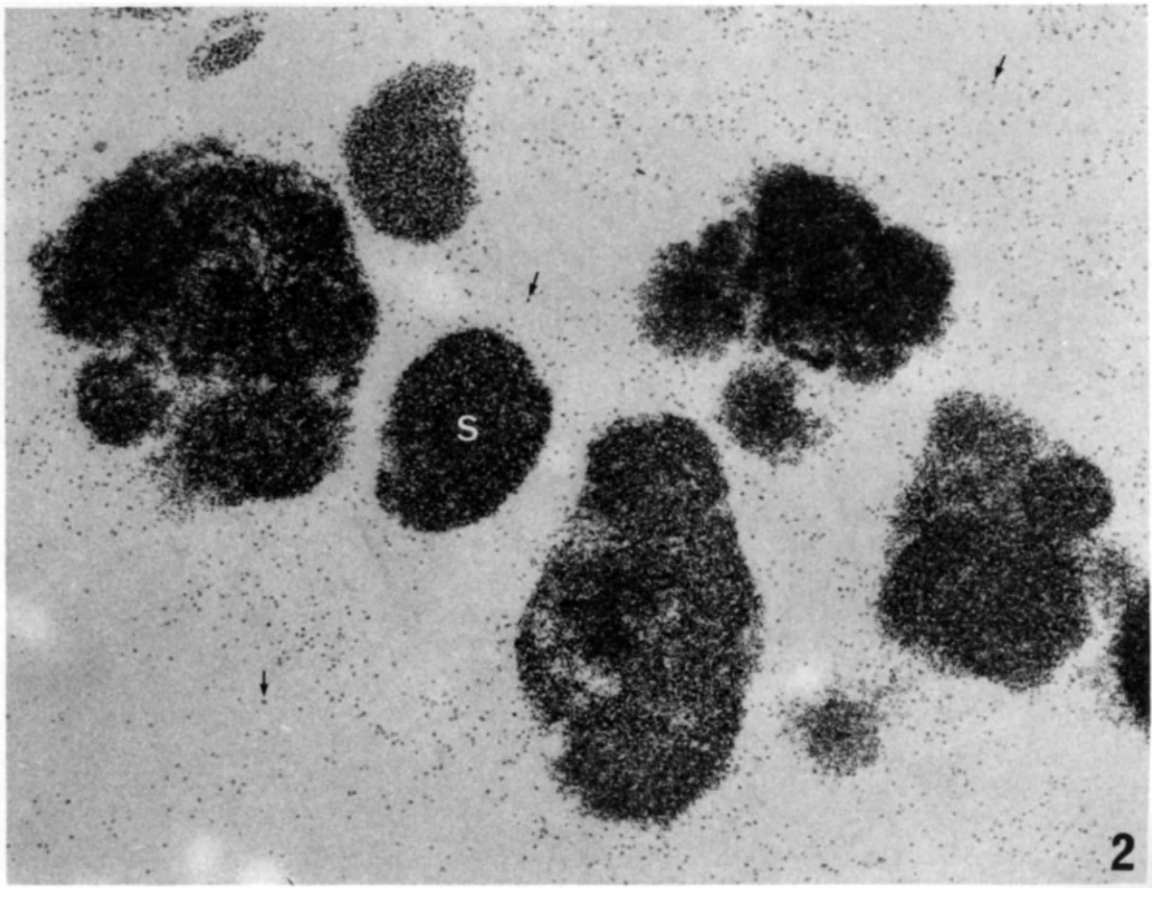
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Such considerations and the fact that the electron-dense particles in siderosomes are often tightly packed and overlie each other so that it is difficult to resolve them (let alone discern any substructure in them) have led us to use the noncommittal term 'electron-dense iron-containing particles' to describe them. Despite much evidence to the contrary (as discussed above) the idea that ferritin* occurs in lysosomes (e.g. Muirden and Rogers, 1978) and that it is the same as haemosiderin (e.g. Robbins, 1974) persists to this day. However, it is difficult to see how ferritin could be derived from the breakdown of haemoglobin or how a protein such as ferritin could 'survive' for any length of time in the potent proteolytic enzyme-rich milieu of the lysosome.

With the advent of electron probe x-ray analysis (*see* review by Ghadially, 1979d), which permits detection of small amounts of elements (as little as 10^{-18} g) within minute areas (as small as approximately 5 nm in diameter) in the cell, the problem of the chemical composition of haemosiderin has been re-examined by this new technique.

Electron-probe x-ray analysis has now been carried out on siderosomes found in: (1) haemophilic human synovial membrane (Ghadially *et al.*, 1976a); (2) rabbit synovial membrane after repeated intra-articular injections of autologous blood (Ghadially *et al.*, 1976b); (3) experimentally produced intramuscular haematomas in the rabbit (Lalonde *et al.*, 1978); and (4) siderotic cornea after intraocular haemorrhage (Ghadially *et al.*, 1981a). Such studies show that all siderosomes contain iron. Phosphorus is detectable in many but not all siderosomes. In siderosomes where phosphorus and iron occur the P:Fe ratio varies from about 1:10 to 1:500. In a few instances traces of sulphur are also detectable in the siderosome. On the basis of these results one may conclude that there just is not enough phosphorus present in the siderosome for all the iron to occur as ferritin or an inorganic compound of phosphorus. If all the iron was present as ferritin† one would have expected to find a P:Fe atomic ratio of approximately 1:9, while if it was ferric phosphate ($FePO_4$) or ferrous phosphate ($Fe_3(PO_4)_2$) the atomic ratio would have been 1:1 or 1:1.5 respectively. Therefore one may speculate that haemosiderin is either a condensate of ferric hydroxide ($Fe(OH)_2$) or, as seems more likely, it is a condensate of some more stable, less reactive compound such as ferric hydroxide oxide‡ ($FeO(OH).nH_2O$). The presence of phosphorus or sulphur in some siderosomes may be explained by assuming that they reflect the presence of as yet undigested organic material in the siderosome.

Perhaps the last word on this matter has not been said yet. Our electron-probe x-ray analytical studies were carried out on sections from unosmicated, unstained Epon-embedded material. One may therefore argue that either a loss or a gain of phosphorus or sulphur could have occurred during tissue processing. However, it seems likely that this problem may be resolved by electron-probe x-ray analysis performed on frozen or freeze-dried sections prepared with a cryo-ultramicrotome from fresh unfixed tissues.§

* By its very definition ferritin is water soluble, hence not much is likely to remain in processed tissues.

† The probable composition of the colloidal iron complex in ferritin (i.e. the core of the molecule) is said to be $(FeOOH)_8.(FeO.PO_3H_2)$ (Crichton, 1971).

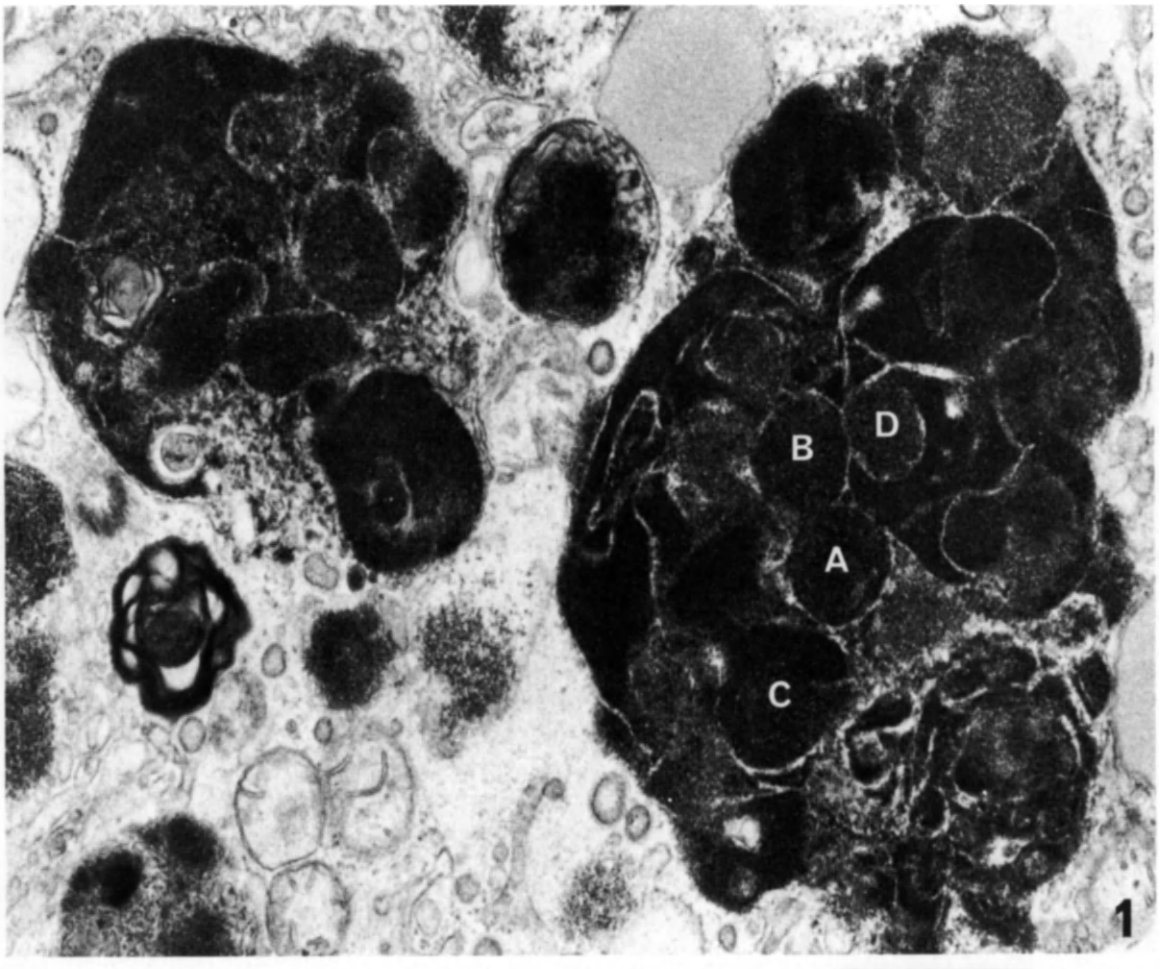
‡ The idea that ferritin cannot hope to survive in the acid milieu of the lysosome and that haemosiderin is ferric hydroxide oxide and not the same as ferritin has at last been accepted by Richter (1984).

§ We have just completed a preliminary electron-probe x-ray analytical study comparing siderosomes (produced in liver and muscle by intramuscular injections of iron dextran) in sections of Epon embedded tissues and cryosections of fresh unfixed frozen (in liquid nitrogen) tissues. No significant difference in the P:Fe ratio in these two types of preparations was detected. More detailed studies are in progress, but it would appear that our contention that haemosiderin is ferric hydroxide oxide is correct.

Plate 278

Fig. 1. Subcutaneous haematoma produced by injection of autologous blood in the rabbit. A macrophage containing compound siderosomes presumably formed by the conglomeration of solitary siderosomes, some of which are indicated as A, B, C and D. × 37 000 *(From Lalonde and Ghadially, 1977)*

Fig. 2. Subcutaneous haematoma produced by injection of autologous blood in the rabbit. A macrophage containing a myelinosiderosome (M). × 51 000 *From Lalonde and Ghadially, 1977)*


B
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A
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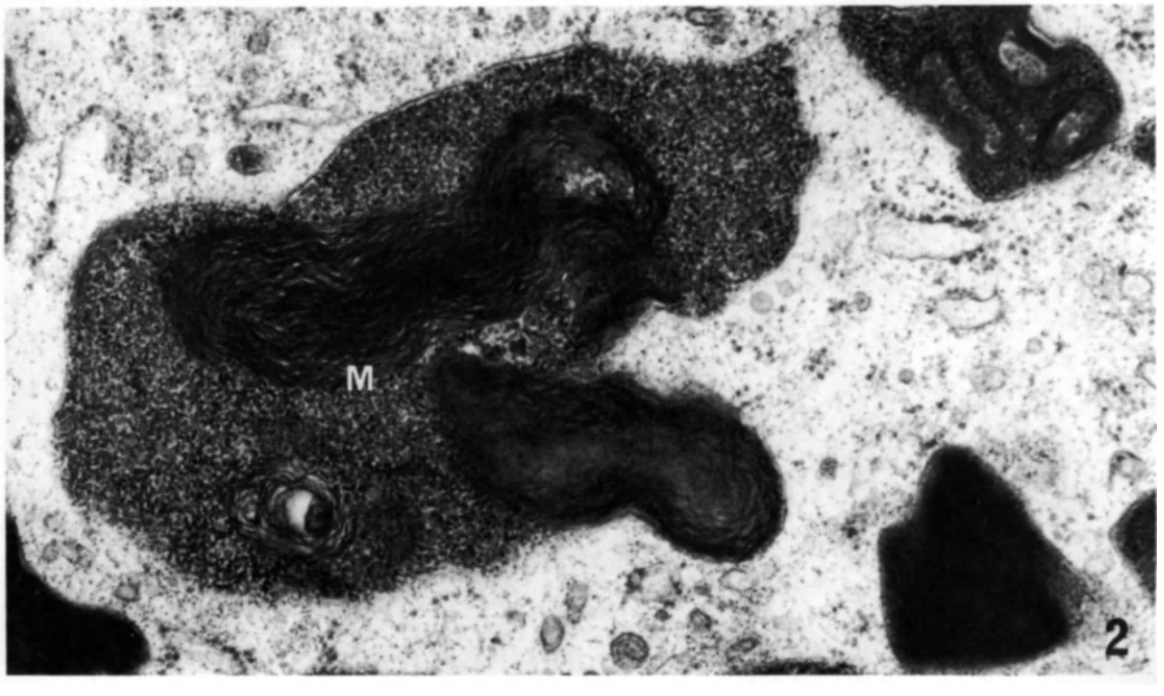
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Lysosomes and residual bodies in tumours

Little is known about the occurrence of primary lysosomes in tumours, since acid phosphatase reaction would be needed to confidently identify them. Only a few electron-dense bodies acceptable as secondary lysosomes are as a rule seen in most tumours, but in certain granular cell tumours the cytoplasm is loaded with lysosomes (*Plate 299–301*).

An occasional autolysosome may on rare occasions be found in any tumour, but I have not encountered substantial numbers in any human tumour. However, carcinogen-induced sarcomas in the rat often contain numerous autolysosomes, as also residual bodies (lipofuscin granules) derived from them (*Plate 255*). Contrary to some past beliefs, tumour cells are not avidly phagocytic and do not infiltrate surrounding tissues by devouring neighbouring cells. Therefore, heterolysosomes are rarely found in tumour cells. Phagocytic activity is generally diminished or lost, even in malignant histiocytes, *vis-à-vis* their normal counterpart. However, I have seen a secondary deposit of adenocarcinoma in a lymph node (primary site not known) where the tumour cells contained many heterolysosomes which appeared to be derived from endocytosis of fibrin and cell debris. Structures acceptable as residual bodies were also present. They contained electron-dense material and membranous formations (*Plate 279, Fig. 1*), but they lacked the characteristic lipid droplets seen in lipofuscin granules.

It is my experience that cells of most malignant tumours lack lipofuscin granules; only rarely is a small lipofuscin granule seen. In contrast to this are the cells of many adenomas (including oncocytomas) and some not too aggressive tumours like well differentiated carcinoids where lipofuscin granules are constantly found (*Plate 279, Fig. 2*). and can at times be quite large and/or numerous. Perhaps the most florid example of this is the black adenoma of the adrenal cortex, where the blackening is due to massive deposits of lipofuscin in the tumour.

Myelinosomes containing whorled or stacked membranes are very rarely seen in benign or malignant tumour cells except of course in alveolar cell carcinoma where they are often quite abundant (*Plate 157*), and useful in the diagnosis of this tumour. However, I have seen a malignant bronchial carcinoid (*Plate 279, Fig. 3*) where several tumour cells contained myelinosomes of the zebra body type. Zebra bodies are seen in some lipoidoses (e.g. Fabry's disease, *see Plate 267*) but the patient did not suffer from any such disease. Zebra bodies have been seen in non-EC cells (M cells?) in the duodenum of a patient with long-standing cholelithiasis and jaundice (Solcia *et al.*, 1980) so one may speculate that some non-neoplastic and neoplastic neuroendocrine cells may at times store lipids in the form of zebra bodies.

Most tumour cells do not endocytose erythrocytes so that erythrophagosomes and siderosomes are rarely seen. However, in some tumours, such as Kaposi's sarcoma and some cases of malignant fibrous histiocytoma, erythrophagosomes are commonly seen as also are some siderosomes derived from them (*see* illustrations in Ghadially, 1985).

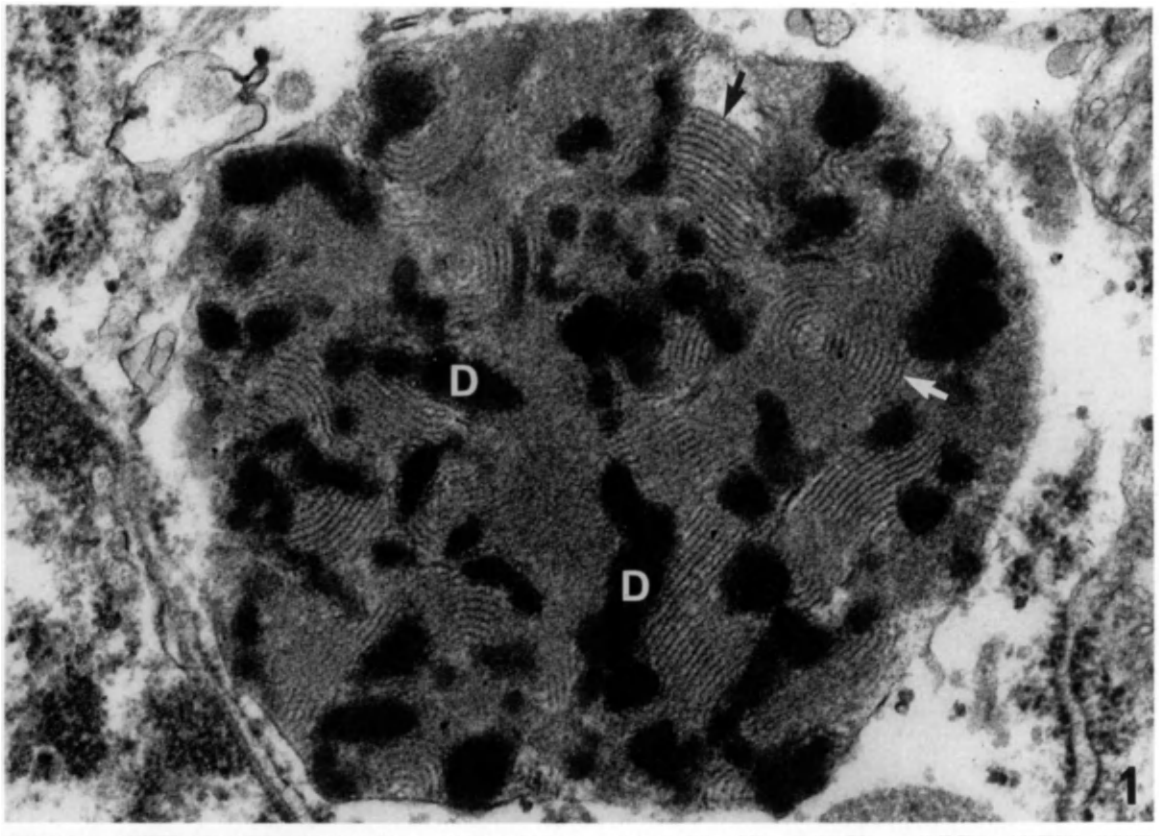
The remarks made in this section of the text relate to the tumour cells themselves and not to macrophages found in tumours. In the latter, lysosomes and/or residual bodies of various types are of constant occurrence.

Plate 279

Fig. 1. A lysosome found in a tumour cell from a secondary deposit of adenocarcinoma (unknown primary) in a lymph node. The lysosome contains electron-dense material (D) and membranous stacks (black arrow) and whorls (white arrow). × 52 000

Fig. 2. A cell from a parathyroid adenoma (chief cell type) containing a residual body which shows the characteristic ultrastructural morphology of lipofuscin, in that it contains electron-dense material (D) and lipid droplets (L) (compare with lipofuscin granules in *Plates 261–263*). Note also the neuroendocrine granules (arrowheads) one expects to see in this tumour. × 17 000

Fig. 3. A cell from a malignant bronchial carcinoid containing several neuroendocrine granules (arrowheads) and two zebra bodies (arrows) × 40 000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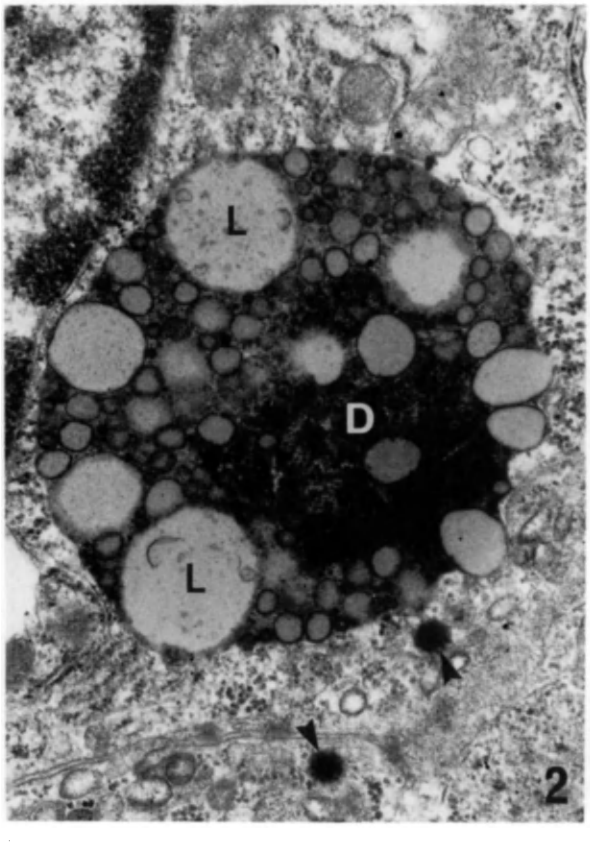
D
1


L
D
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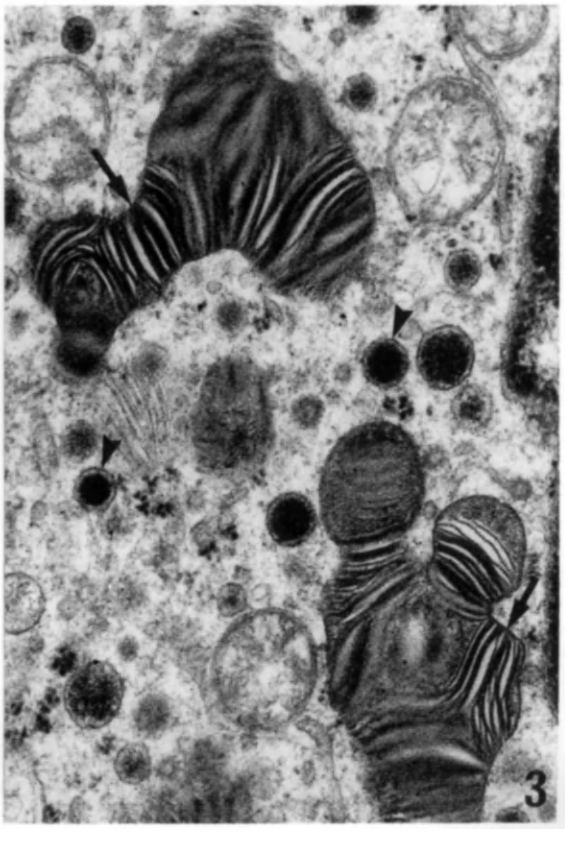
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Lysosomes in erythrocytes

The ability of many mammalian cells to rid themselves of residual bodies seems to be strictly limited. The mounting accumulation of residual bodies (lipofuscin) with age in various organs and the indefinite persistence of siderosomes (haemosiderin) after haemorrhage bear clear testimony to this fact. However, the autolysosomes (autophagic vacuoles) and residual bodies (*see below*) that occur in erythrocytes are an exception. Appearances are seen which suggest that these cells can expel such bodies, and there is also evidence that they surrender them to the spleen during their passage through this organ.

Many and varied are the appearances of the contents of autolysosomes seen in erythrocytes and reticulocytes (*Plates 280* and *281*). In some instances a portion of the haemoglobinized cytoplasm is seen sequestrated in a single-membrane-bound autophagic vacuole. This no doubt represents an early stage of formation of one variety of autolysosome (*Plate 281, Fig. 1*). Later stages of evolution are represented by bodies containing membranous structures, myelin figures and electron-dense material. Other autolysosomes, particularly those seen in reticulocytes, at times contain recognizable organelle remnants derived from ribosomes, mitochondria and cytomembranes. This could be one of the ways in which the reticulocyte disposes of its organelles as it transforms into an erythrocyte.

It would appear that fully mature residual bodies, in which digestion of contents has been truly completed, rarely evolve from autolysosomes in erythrocytes. Perhaps the enzyme content is not sufficiently potent or the autolysosomes are expelled by the cell or removed by the spleen long before digestion is complete. The situation in the reticulocyte appears to be different, because siderosomes, which give a positive Prussian blue reaction and contain little else besides electron-dense iron-containing particles, are formed in these cells. Siderosomes found in erythrocytes are more likely to be those carried from the normoblast or reticulocyte stage rather than new ones formed after the erythrocyte has matured.

Particularly intriguing are appearances which suggest that the contents of the autophagic vacuole are being or have been discharged. Such an appearance is depicted in *Plate 280, Fig. 2*, where an autolysosome is seen to communicate with the exterior of the cell via a small opening. In other instances one sees 'holes' in erythrocytes (*Plate 281, Figs. 1 and 3*) which may appear empty or contain some residual lysosomal material and/or fibrillary proteinaceous precipitate identical in appearance to that seen outside the cell. Such appearances suggest that the contents of the autolysosome have been discharged and the pit or vacuole created by this is now occupied by blood plasma.

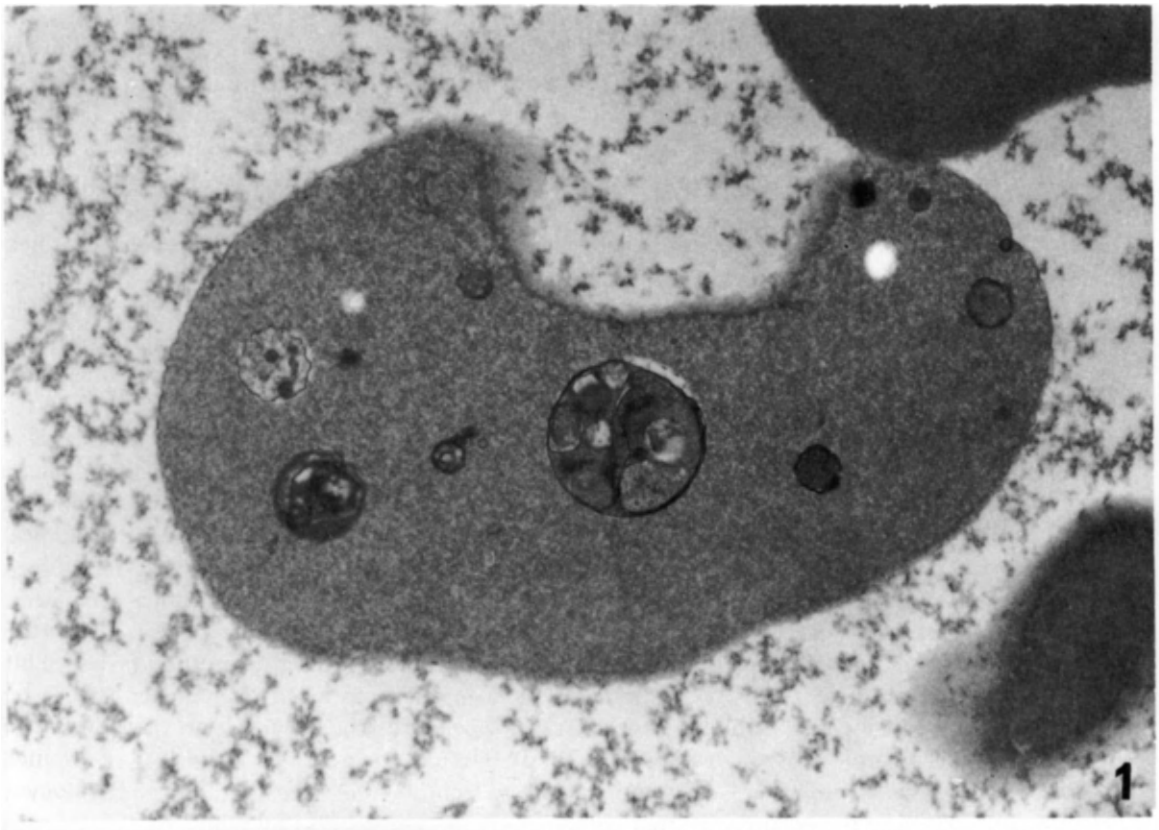
Occasional erythrocytes containing autolysosomes are found in normal individuals but many more are seen in patients with haematological disorders and in splenectomized individuals. We (Ghadially and Skinnider, unpublished observations) have seen them in the blood of various experimental animals, and they were found to be particularly frequent in the blood of some day-old rats we examined. Autolysosomes have also been observed in the nucleated erythrocytes of amphibians (Tooze and Davies, 1965). Acid phosphatase activity has been demonstrated in the autolysosome of human erythrocytes (Kent *et al.*, 1966; Schaeffer *et al.*, 1970).

Plate 280

From the peripheral blood of a splenectomized patient with hepatic cirrhosis

Fig. 1. Numerous pleomorphic autolysosomes are seen in the cytoplasm of this erythrocyte. × 43 000

Fig. 2. Appearances seen here (arrow) suggest that an autolysosome is about to discharge its contents. × 43 000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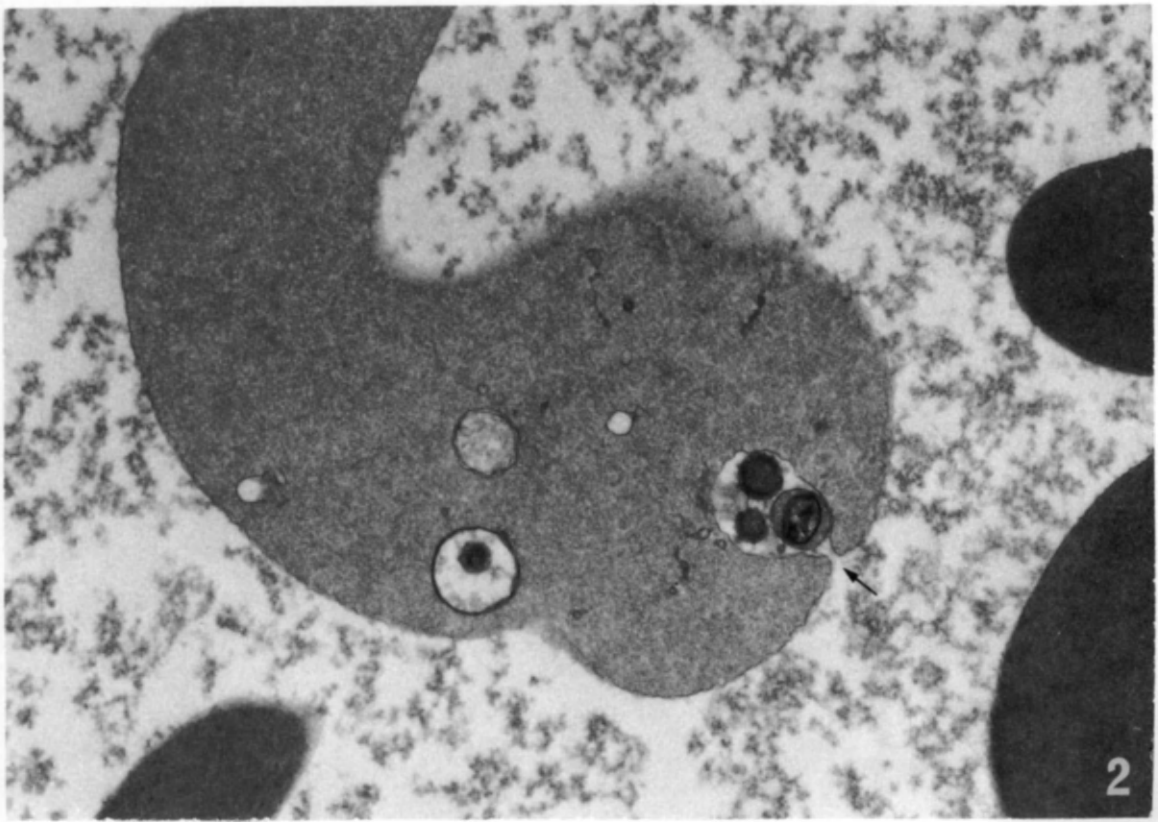
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A detailed study of autolysosomes in human erythrocytes and reticulocytes by Kent *et al.* (1966) has revealed many points of interest. According to them, 0.1–0.3 per cent of erythrocytes from normal individuals contain autolysosomes. A tenfold increase in the percentage of erythrocytes containing autolysosomes was noted in patients with haematological disorders and intact spleen, and an increase of similar magnitude was found in persons whose spleen had been removed after traumatic rupture and who had no haematological disorder.

The most profound increase, however, was noted in individuals whose spleen had been removed as a treatment of certain haematological disorders. Thus 16.3 per cent of the erythrocytes contained autolysosomes in a post-splenectomized case of haemolytic anaemia, while the largest increase (36.5 per cent) was seen in a post-splenectomized case of haemoglobin C disease. These values were obtained by counting red blood cell profiles in ultrathin sections, so it stands to reason that the true incidence of autolysosomes must be far greater than these figures.

The increase in autolysosomes seen after splenectomy, both in normal individuals and in patients with haematological disorders, clearly shows that the spleen plays an important role in their removal and that in its absence autolysosomes in erythrocytes are not eliminated efficiently. The situation here is reminiscent of the removal of siderotic granules (siderosomes) from erythrocytes. Siderocytes (erythrocytic cells containing siderosomes) are only rarely seen in normal blood but they are of common occurrence in post-splenectomized cases of haemolytic anaemia. The experiments of Crosby (1959) where ^{15}Cr-labelled siderocytes were transfused into normal and splenectomized subjects have shown that the number of detectable siderocytes diminishes rapidly in normal but not in splenectomized individuals. The loss, however, is in iron granules (siderosomes) and not cells, for the reduction in the number of Prussian-blue-positive siderocytes is not associated with a concurrent reduction of labelled donor cells. This demonstrates that the spleen can remove siderotic granules (siderosomes) without destroying the erythrocyte that contained them. The process by which this is achieved is referred to as 'pitting'. Crosby (1959) has defined the pitting function of the spleen as 'its ability to remove a solid particle from the cytoplasm of a red cell without destroying the cell itself much as a housewife plucks the stone from a cherry without crushing the fruit'.

The pitting function of the spleen, however, is not specific for siderosomes. In the absence of this organ various 'solid particles' such as Howell-Jolly bodies, Heinz bodies and malarial parasites are apt to be more numerous in the circulating erythrocytes. Thus, although the actual uptake of autolysosomes by the spleen has not been demonstrated, it is obvious that the spleen is involved in their removal.

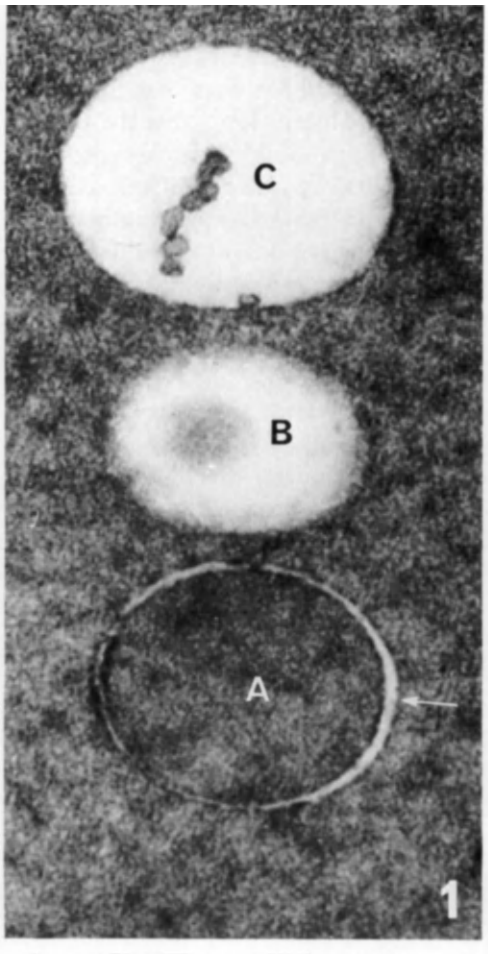
Plate 281

Fig. 1. A portion of erythrocyte cytoplasm, showing an early stage of autolysosome formation (A) where a portion of the haemoglobinized cytoplasm appears sequestrated in a vacuole. The clear space (arrow) is probably a shrinkage artefact. Two other lucent vacuoles (B and C) are interpreted as cavities left behind after most of the lysosomal contents have been discharged. From a splenectomized case of thrombocytopenic purpura. × 85 000

Fig. 2. Sequestrated haemoglobinized cytoplasm (H) and myelin figures (arrow) form the contents of this autolysosome in an erythrocyte. From the same case as *Plate 280.* × 85 000

Fig. 3. Transverse (T) and longitudinal (L) sections through pits left behind after discharge of lysosomal contents. From the same case as *Plate 280.* × 17 000

Fig. 4. An autolysosome (C) and a siderosome (S) are seen in this reticulocyte from a case of Hodgkin's disease with sideroblastic anaemia. × 50 000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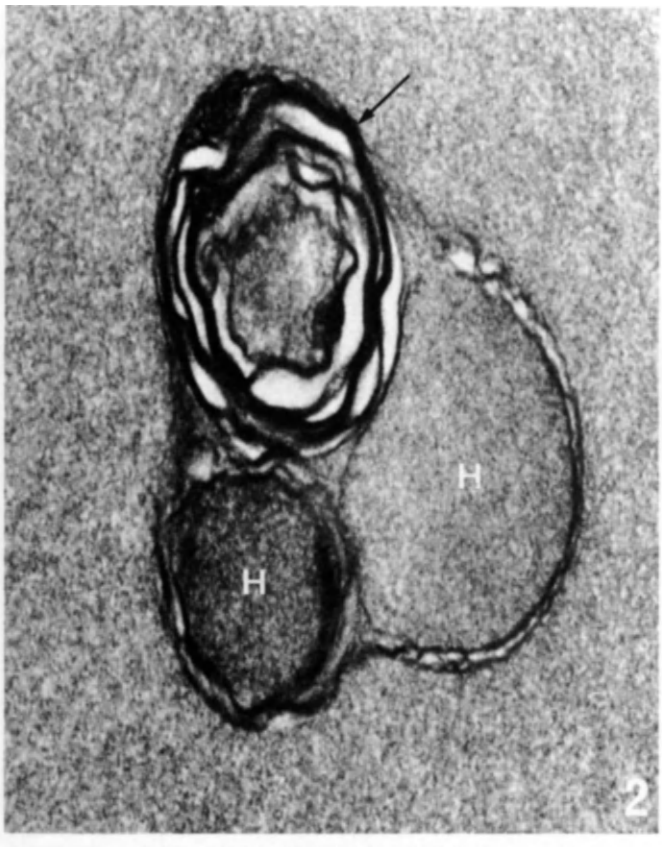
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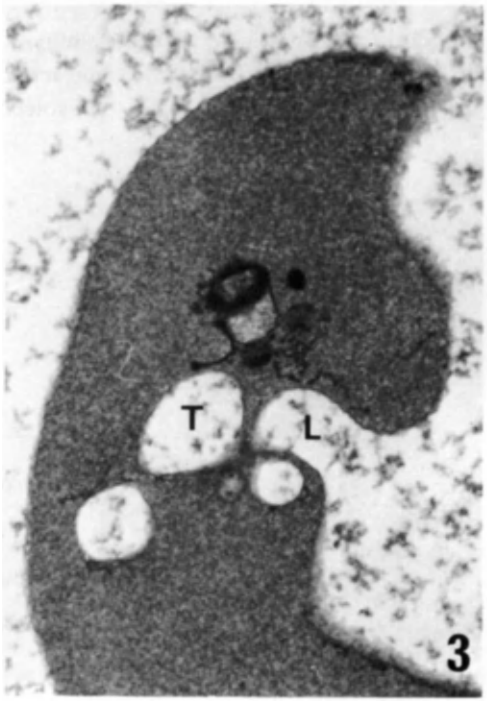
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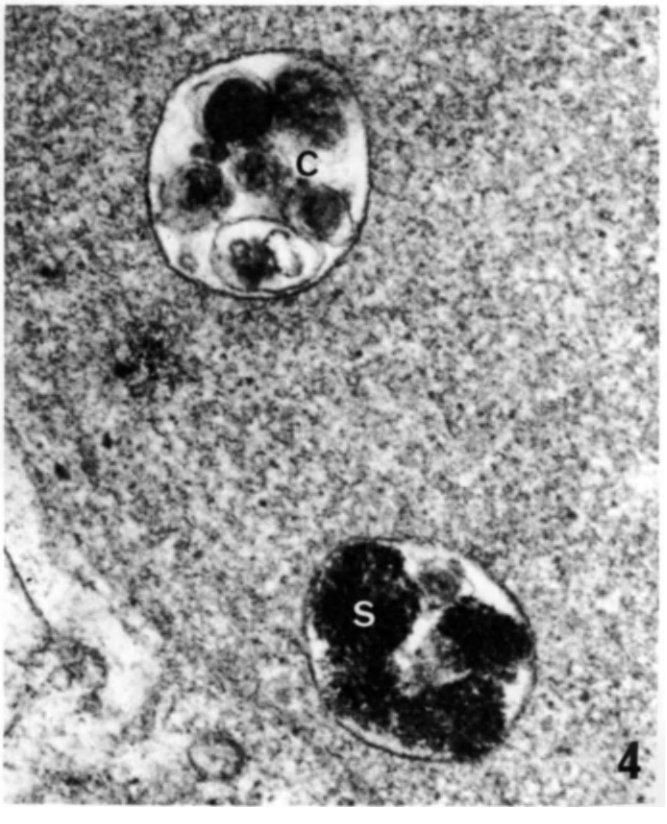
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Lysosomes in neutrophil leucocytes

The morphological heterogeneity of the granules of the neutrophil leucocyte has long been recognized (Florey, 1962). At first, it was thought (Bessis and Thiery, 1961) that the various forms of granules represented different developmental stages of but a single granule type. Later work has shown this view to be incorrect, but there is still uncertainty as to how many different varieties of granules are present. Various lysosomal enzymes and also a variety of antibacterial substances such as lysozyme and phagocytin are present in these granules, but the distribution of these among the various types of granules is not established unequivocally.

It is now universally accepted that neutrophils of rabbit and man contain at least two distinct types of granules: a primary or azurophilic granule, and a secondary or specific granule (*Plate 282*) (Bainton and Farquhar, 1966, 1968; Wetzel *et al.*, 1967a, b; Dunn *et al.*, 1968). It is also clear that they are formed quite independently at different periods of cell maturation. The primary granule is formed during the promyelocyte phase, while secondary granules are produced during later stages of neutrophil maturation (metamyelocyte, myelocyte and band form) after primary granule formation has virtually ceased. Subsequent ultrastructural, cytochemical and biochemical studies have indicated the existence of a tertiary granule which develops at a relatively late stage of neutrophil development (segmented forms) (Baggiolini *et al.*, 1969; Scott and Horn, 1970a).

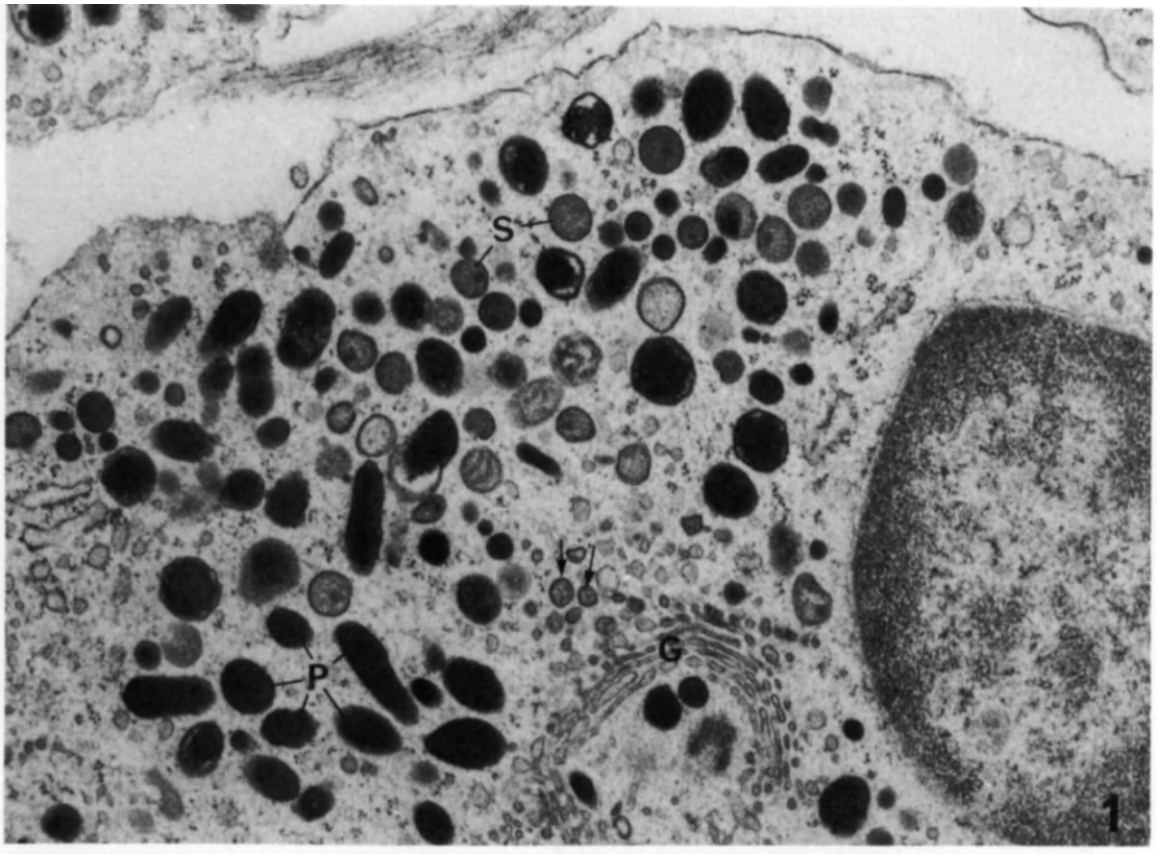
Rabbit neutrophil (heterophil) leucocytes have been studied more extensively than any other. Here the three types of granules have been isolated and analysed. It has been shown that both primary and tertiary granules contain sulphated glycosaminoglycans and numerous acid hydrolases, including acid phosphatase, but myeloperoxidase is found only in the primary granules. There is evidence that the primary granules of human neutrophils also contain myeloperoxidase (Dunn *et al.*, 1968). Thus both the primary and tertiary granules are acceptable as primary lysosomes but the former are somewhat atypical because of their perioxidase content. The secondary granules of rabbits are known to contain alkaline phosphatase and various antibacterial substances. Thus secondary granules are not considered to be lysosomal in nature since they do not contain acid phosphatase or other typical lysosomal enzymes.

In the rabbit the primary granule presents as a single-membrane-bound electron-dense body approximately 0.8 μm in diameter. The smaller (0.3 μm) primary granule of man may be round or elliptical, and as it matures a crystalloid develops in its interior.

Plate 282

Fig. 1. In this osmium-fixed immature neutrophil from human bone marrow the large dense primary granules (P) are easily distinguished from the smaller, less dense secondary granules (S). The presence of a well defined Golgi complex (G) and very small granules (arrows) of a density similar to the secondary granules suggests that the cell is at a stage of development where secondary granule production is in progress. × 27 000

Fig. 2. In this glutaraldehyde-fixed neutrophil from the peripheral blood of man, the secondary granules (S) are markedly extracted and appear electron-lucent. A dense crystalloid (arrow) difficult to discern within the dense primary granules is present. × 28 000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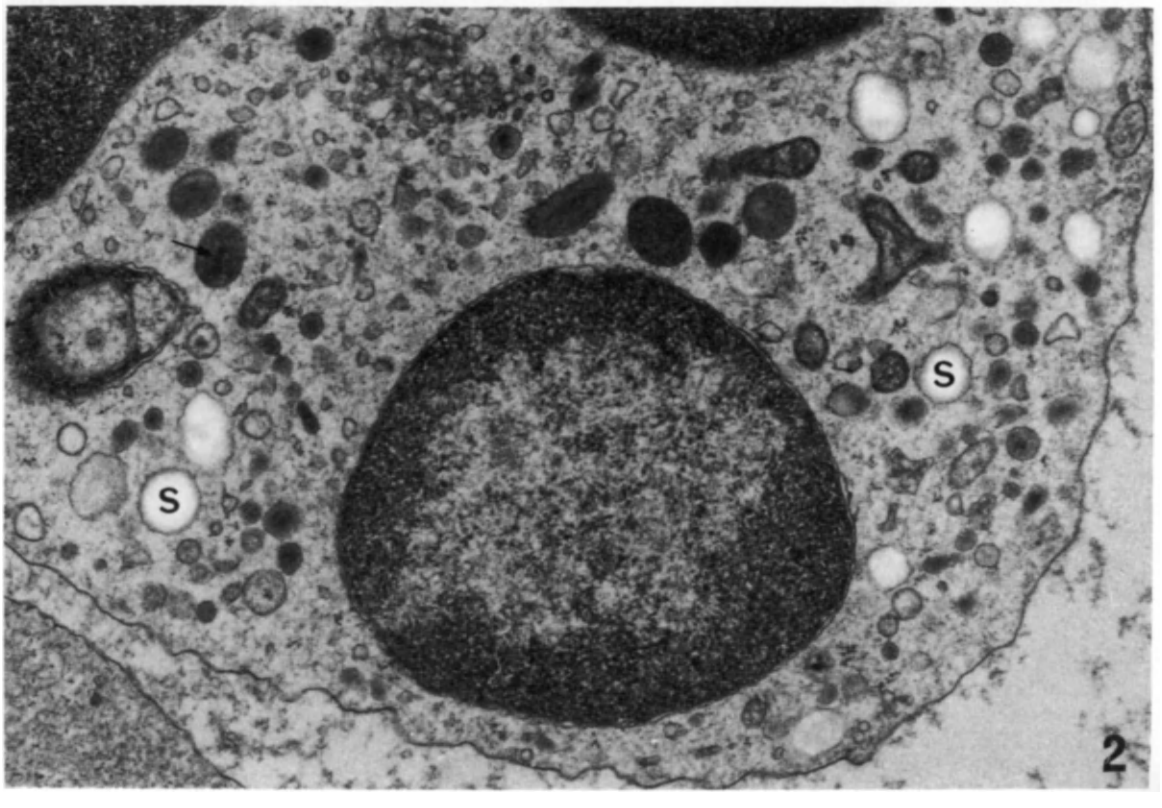
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The secondary granules in both rabbit and humans are less electron-dense than the primary granules. In glutaraldehyde-fixed preparation of buffy coat from human blood the secondary granules are often slightly or markedly extracted. In such instances they present as electron-lucent granules with or without a denser periphery (*Plate 282*). The tertiary granules are small pleomorphic bodies seen mainly in mature circulating polymorphs. In man they are moderately to markedly electron-dense and are said to vary in shape from round to quite elongated bacilliform bodies (*Plate 283*). The pleomorphism of this group suggests that a fourth type of granule may also exist in neutrophils (Daems, 1968).

The capacity of neutrophil leucocytes to phagocytose various materials such as bacteria, fungi, fibrin and also antigen-antibody complexes has been amply demonstrated (Riddle and Barnhart, 1964; Zucker-Franklin and Hirsch, 1964; Zucker-Franklin, 1968). It has already been noted that such material is taken in by a process of phagocytosis and comes to lie in a single-membrane-bound structure called a 'phagosome' or 'heterophagosome' (page 594). Fusion of neutrophil granules with the wall of the phagosome* and subsequent release of enzymes into the phagosome converts it into a heterolysosome, where the digestion of ingested material occurs. (Phagocytosis of bacteria by neutrophils is illustrated on *Plate 254*). Ultrastructural studies show that all three types of granules fuse with the phagosome, and that this leads to a degranulation of the neutrophil. No new granules are formed after such an event.

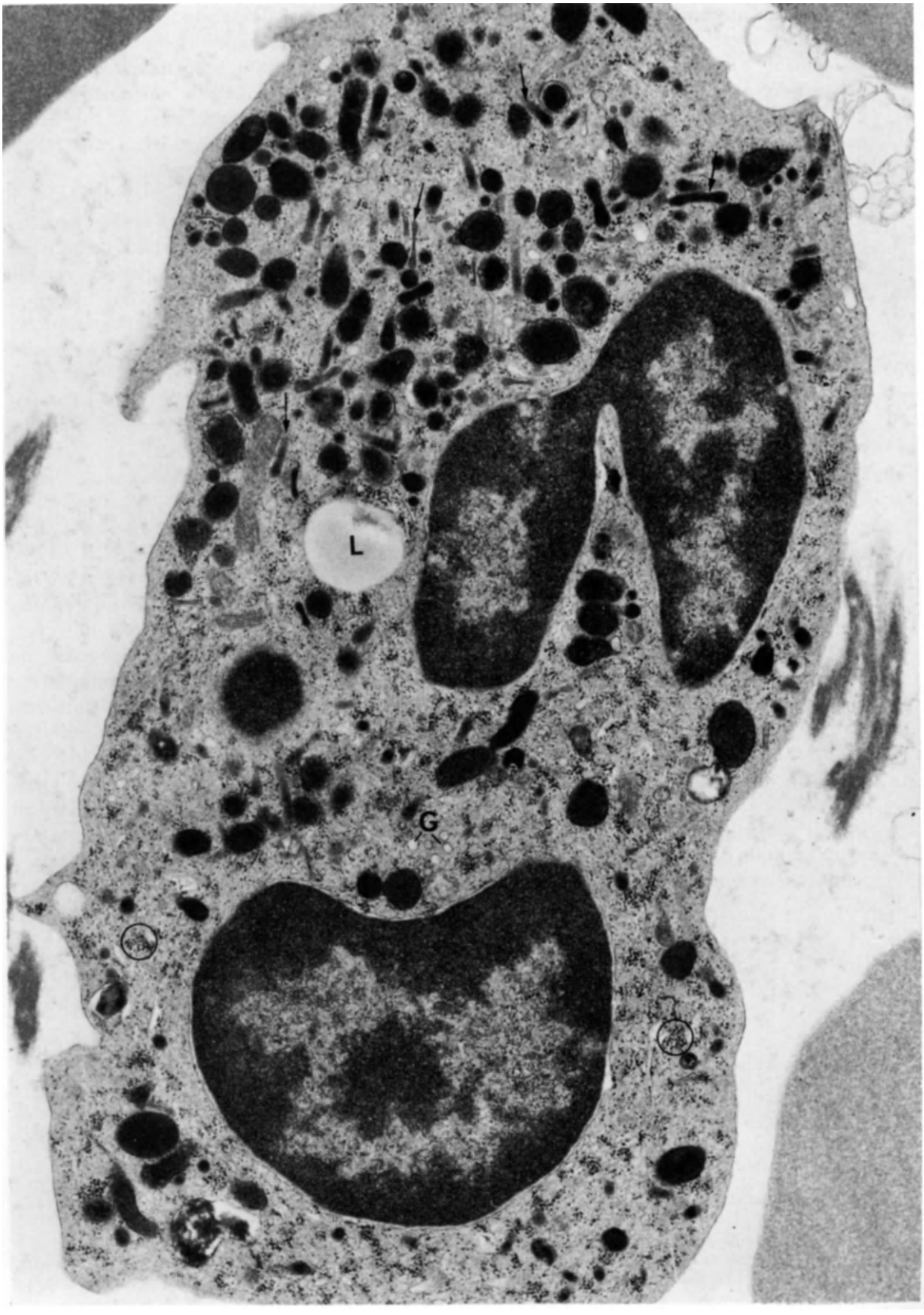
It is thought that microtubules facilitate the association of neutrophil granules with the phagosome, for agents such as colchicine and vinblastine which are known to disrupt microtubules also interfere with the process of degranulation and heterolysosome formation, but phagocytic activity (i.e. phagosome formation) is unimpaired. On the other hand, cytochalasin B, which is thought to interfere with the contractile action of myofilaments, impairs phagocytosis and the uptake of bacteria — many of which remain adhered to the cell surface (for references, *see* Malawista *et al.*, 1971).

The manner in which the energy requirements for the phagocytic act and the killing of ingested organisms are met has been the subject of many studies (for references, *see below*). These indicate that the energy required for the phagocytosis derives from glycolysis, but that the energy required for the events which follow is dependent upon oxidative metabolism and increased oxygen utilization. This marked increase in oxygen consumption is accompanied by oxidation of glucose via the hexose monophosphate shunt and hydrogen peroxide production, which is an important factor in the killing of certain organisms.

*The unqualified term 'phagosome' is commonly used to describe structures which could now be more fully and accurately described as 'heterophagosomes'. The term 'phagosome', meaning 'a body containing material which a cell had eaten', was in use long before (i.e. since Metschnikoff, 1884, 1892) the lysosome concept had developed and we became aware of two kinds of phagosomes, namely heterophagosomes and autophagosomes. Thus, historical precedence and the rules of scientific nomenclature make it quite legitimate (and convenient) to speak about 'phagosomes' rather than 'heterophagosomes', except of course in situations where confusion may arise or when one specifically wishes to distinguish between the two. To abolish the term 'phagosome' is hardly practical and brings in its wake other problems such as the need to abandon the time hallowed term 'phagocytosis' and speak only about 'heterophagocytosis' and 'autophagocytosis'. This would not only be impractical, but a little bit too pedantic.

Plate 283

In this osmium-fixed polymorphonuclear neutrophil from peripheral blood of man, the primary and secondary granules cannot be confidently identified. It is worth noting that this is not infrequently the case. However, numerous bacilliform tertiary granules (arrows) are evident in this cell. These, together with the segmented nucleus, numerous glycogen particles (in circles) and an atrophic Golgi complex (G) represented by a few vesicles, are indicative of maturity in this leucocyte. Also seen is a lipid droplet (L). × 22 000


L
G

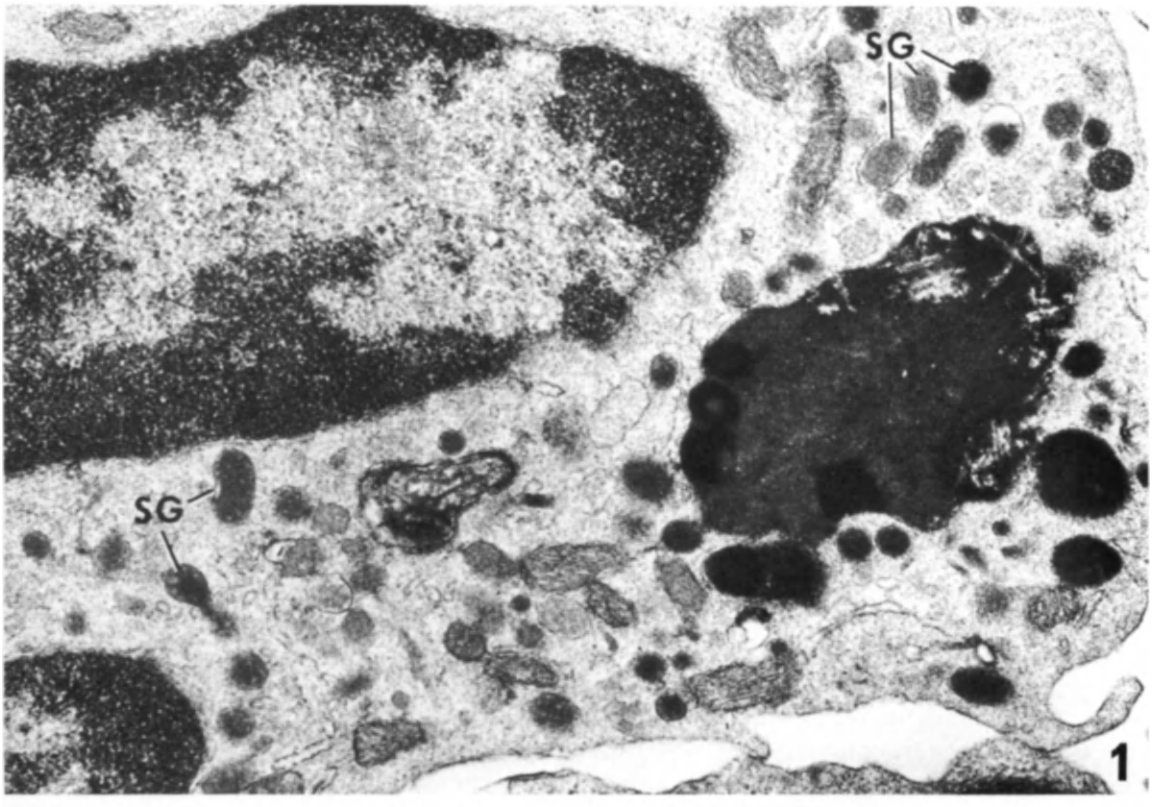
In certain inherited diseases, such as chronic granulomatous disease and Chediak–Higashi syndrome, there is a decreased ability to cope with some infections. There is no apparent abnormality of the immunological defence mechanism and it is thought that the defect resides in the neutrophil polymorph. In chronic granulomatous disease there is no impairment of phagocytosis, and according to most workers neither is there derangement of the fusion mechanism between granules and the phagosome, although according to Heyne *et al.* (1972) there may be 'a defect of the positive granule-taxis to the phagocytic vacuole'.

However, it has been shown that the neutrophils from these patients fail to show the burst of oxidative metabolism and hydrogen peroxide generation that follows the ingestion of particles by normal neutrophils. Several lines of investigation have indicated that it is this deficiency which is the bactericidal defect. Thus, for example, the neutrophils of these patients can kill fairly effectively organisms such as streptococci and pneumococci which generate hydrogen peroxide and thus contribute to their own destruction, but they are unable to cope with many Gram-negative pathogens and staphylococci that contain catalase which breaks down hydrogen peroxide. The many interesting aspects of phagocytic function and dysfunction and other defects such as myeloperoxidase deficiency are beyond the scope of this brief essay. For this information and support of statements made above the following papers and reviews should be consulted: Baehner and Nathan (1967, 1968), Holmes *et al.* (1966), MacFarlane *et al.* (1967), Quie *et al.* (1968), Klebanoff (1968, 1971), Klebanoff and White (1969), Elsbach *et al.* (1969), Spicer and Hardin (1969), Thompson *et al.* (1969), Lehrer (1971).

The Chediak–Higashi syndrome has been reported to occur in man, mink, mice, cats, cattle and killer whale (Beguez-Cesar, 1943; Chediak, 1952; Higashi, 1954; Kritzler *et al.*, 1964; Lutzner *et al.*, 1967; Padgett *et al.*, 1964; Padgett, 1967, 1968; Davis *et al.*, 1971; Taylor and Farrell, 1973; Hargis and Prieur, 1985). In all these species, large membrane-bound granules (acid-phosphatase-positive) occur in leucocytes and also in some other cell types. It is, however, worth noting that only a few granules in the neutrophils are so affected and that many others are of normal size. The significance and mechanism of production of the large granules in the neutrophil leucocyte have been interpreted in different ways by various workers. Thus they have been thought to be autophagic vacuoles, examples of toxic granulation, fused granules, or agglomerations of material liberated from normal granules and abnormally large granules produced by a failure of the mechanism which controls normal granule size. In mink neutrophils, Davis *et al.* (1971) found that pleomorphic primary granules, some smaller than normal and others larger than normal, were produced. However, the really large granules (giant granules or megagranules) were produced by the fusion of primary granules. Secondary granules were not involved in the fusion process and appeared normal in size and number (*Plate 284*).

Plate 284

Figs. 1 and 2. Neutrophils of mink with the homologue of the Chediak–Higashi trait of humans showing large atypical granules (megagranules). One of these appears to be formed by the fusion of numerous large primary granules, as indicated by the arrows. The secondary granules (SG) are not involved in the fusion process and appear normal in size and number. × 23 000; × 23 000 (*From Davis, Spicer, Greene and Padgett, 1971*)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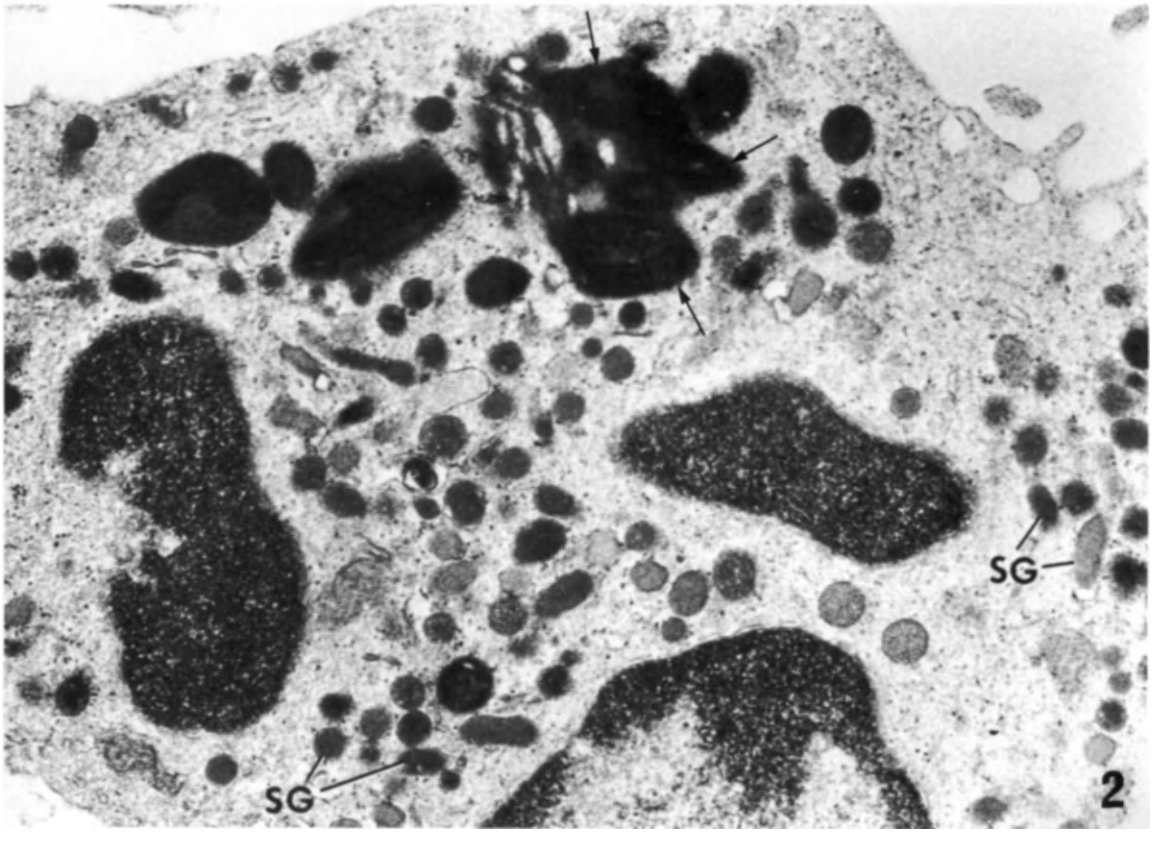
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Besides the giant primary granules there also occur quite massive inclusions in the neutrophils in Chediak–Higashi syndrome which are almost certainly secondary lysosomes. According to White and Clawson (1980) 'most of the giant primary granules undergo transformation or incorporation into huge secondary lysosomes, which are virtually unable to participate in the degranulation reaction after uptake of foreign particulates by CHS neutrophils'.

Abnormal granulogenesis is frequently seen in leukaemic cells, but giant granules are of rare occurrence. Giant granules* resembling those seen in Chediak–Higashi syndrome have been noted in: (1) eosinophils from a case of erythroleukaemia (Finkel and Grauer, 1966); (2) platelets in refractory anaemia (preleukaemia) and myelomonocytic leukaemia (Maldonado, 1975); and (3) acute leukaemic myeloblasts (Van Slyck and Rebuck, 1974; Parmley *et al.*, 1979; Ghadially and Cunningham, unpublished) (*Plate 285*). It would appear that as in the Chediak–Higashi syndrome the giant granules (up to about 3 μm in size) in leukaemic myeloblasts are derived by fusion of the primary azurophilic granules which as noted earlier are normally only about 0.3 μm in diameter.

Certain prominent basophilic granules seen in neutrophils in various infections and other pathological states are often referred to as 'toxic' granules. Ultrastructural studies have not as yet clearly defined the nature of these granules. Spicer and Hardin (1969) have interpreted such granules seen after the injection of endotoxin as mucopolysaccharide-rich primary granules. Zucker-Franklin (1968) observed such granulation in phagocytosis experiments conducted with rheumatoid factor complexed with aggregated γ-globulin, and interpreted toxic granules as heterophagosomes and heterolysosomes.

The Döhle body which is also seen in a variety of infections and other disorders appears to be quite different from toxic granules. According to Cawley and Hayhoe (1972), the characteristic basophilia and pyroninophilia of these bodies is due to focal aggregates of rough endoplasmic reticulum in the neutrophil leucocyte.

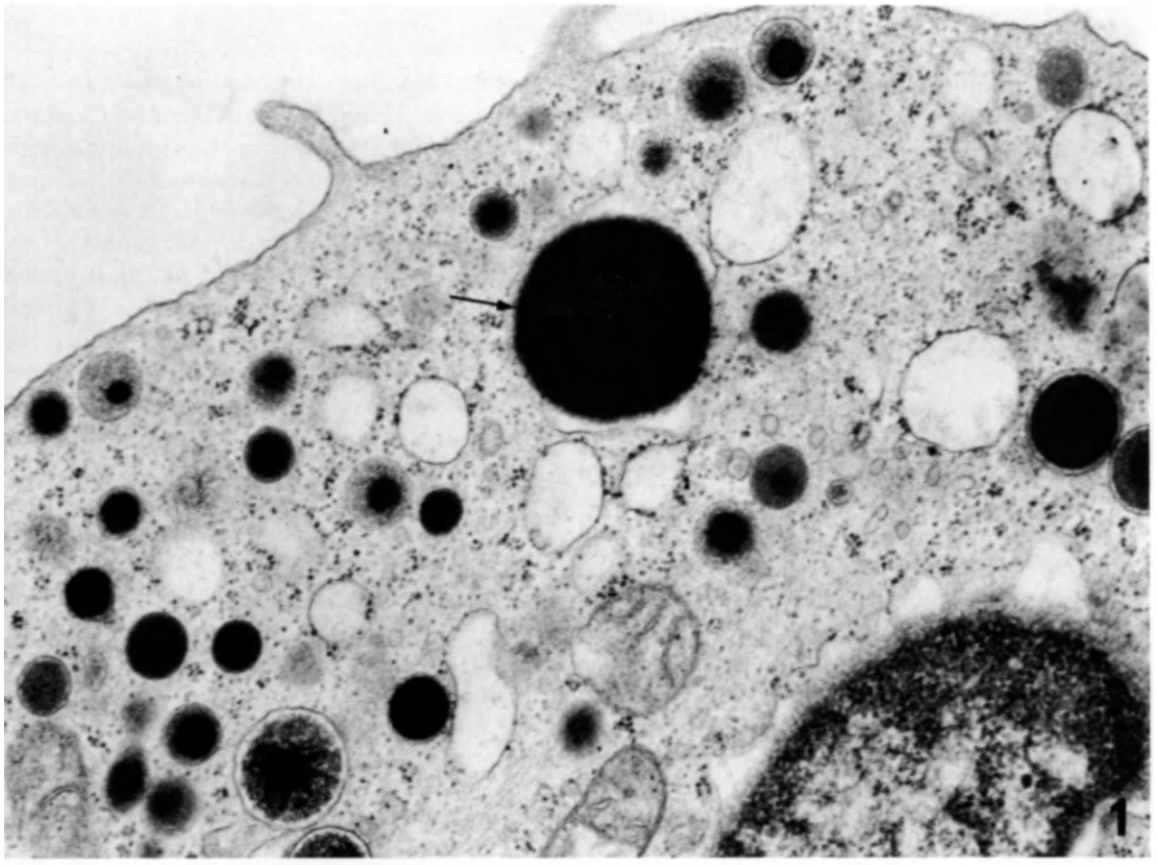
*At times referred to as 'pseudo-Chediak–Higashi granules'.

Plate 285

From the peripheral blood of a case of acute myelogenous leukaemia (*Ghadially and Cunningham, unpublished electron micrographs*)

Fig. 1. A rounded giant granule (arrow) in a leukaemic cell. × 34 000

Fig. 2. A leukaemic cell containing several primary granules, an irregular shaped giant granule (arrowhead) and an atypical granule (arrow). × 36 000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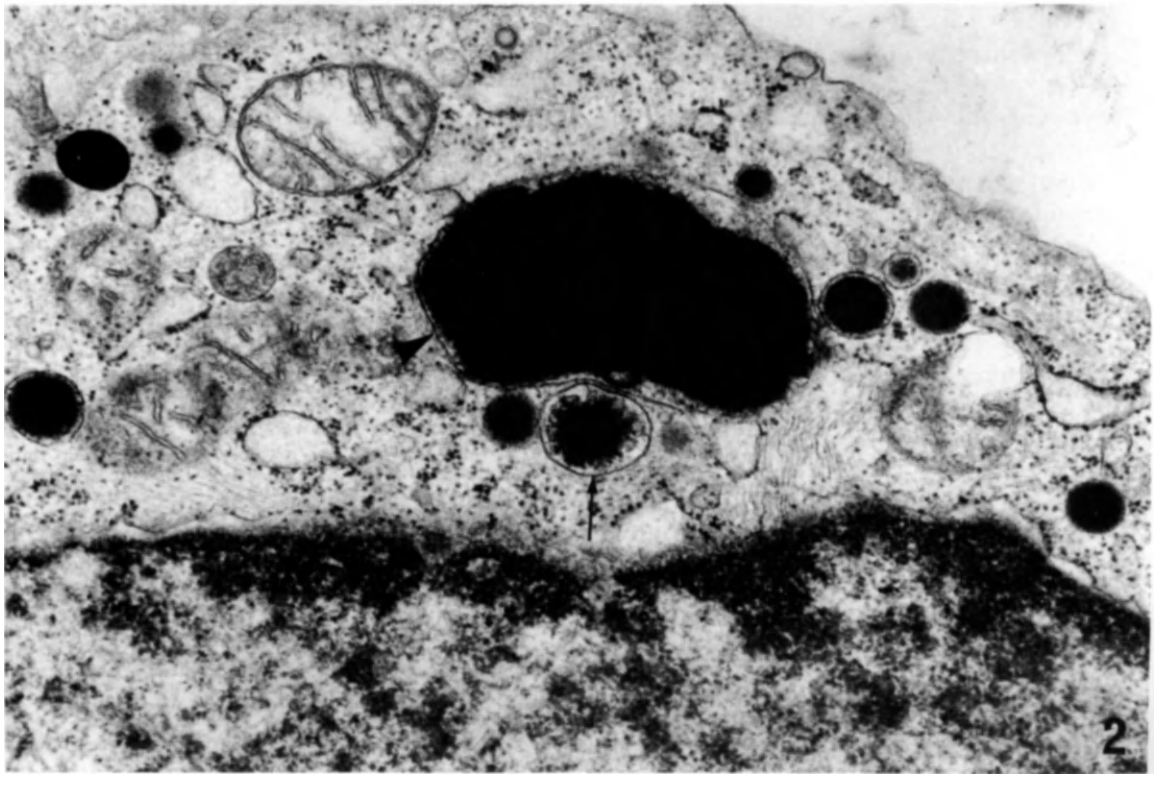
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Lysosomes in eosinophil leucocytes

Small numbers of eosinophils circulate in the peripheral blood but larger numbers accumulate in the vicinity of immediate hypersensitivity reactions, helminthic infestations and some neoplasms*. Like neutrophils, eosinophils when stimulated by appropriate agents respond with the release of superoxide and hydrogen peroxide (H_2O_2) (Baehner and Johnston, 1971). This is accompanied by the release of granules or granule contents into phagosomes (Cotran and Litt, 1969) or the extracellular milieu (Archer and Hirsch, 1963; McLaren *et al.*, 1977).

Since the eosinophilic granules of this leucocyte have now been shown to contain acid phosphatase (Ghidoni and Goldberg, 1966; Hudson, 1966) and also other hydrolases (Archer and Hirsch, 1963; Archer, 1963; Dunn *et al.*, 1968), they are considered to be a variety of lysosome. Functionally, the ability of the eosinophil leucocyte to phagocytose foreign material has been demonstrated, as has the fusion of eosinophil granules with heterophagosomes to form secondary lysosomes. Electron microscopic studies show that eosinophils can phagocytose: (1) ferritin-antiferritin complexes (Sabesin, 1963); (2) zymosan particles (Zucker-Franklin and Hirsch, 1964); (3) mycoplasma (Zucker-Franklin *et al.*, 1966); (4) *E. coli* (Cotran and Litt, 1969); (5) antigen-antibody complexes (Ishikawa *et al.*, 1971); and (6) *Candida albicans* (Ishikawa *et al.*, 1972).

The eosinophil granules of humans, guinea-pig, rabbit, rat, mouse, cat, orang-utan, chimpanzee, sheep, cattle, horse and mink have been studied and numerous variations of internal structure have been described (for references, *see* Yamada and Sonoda, 1970).

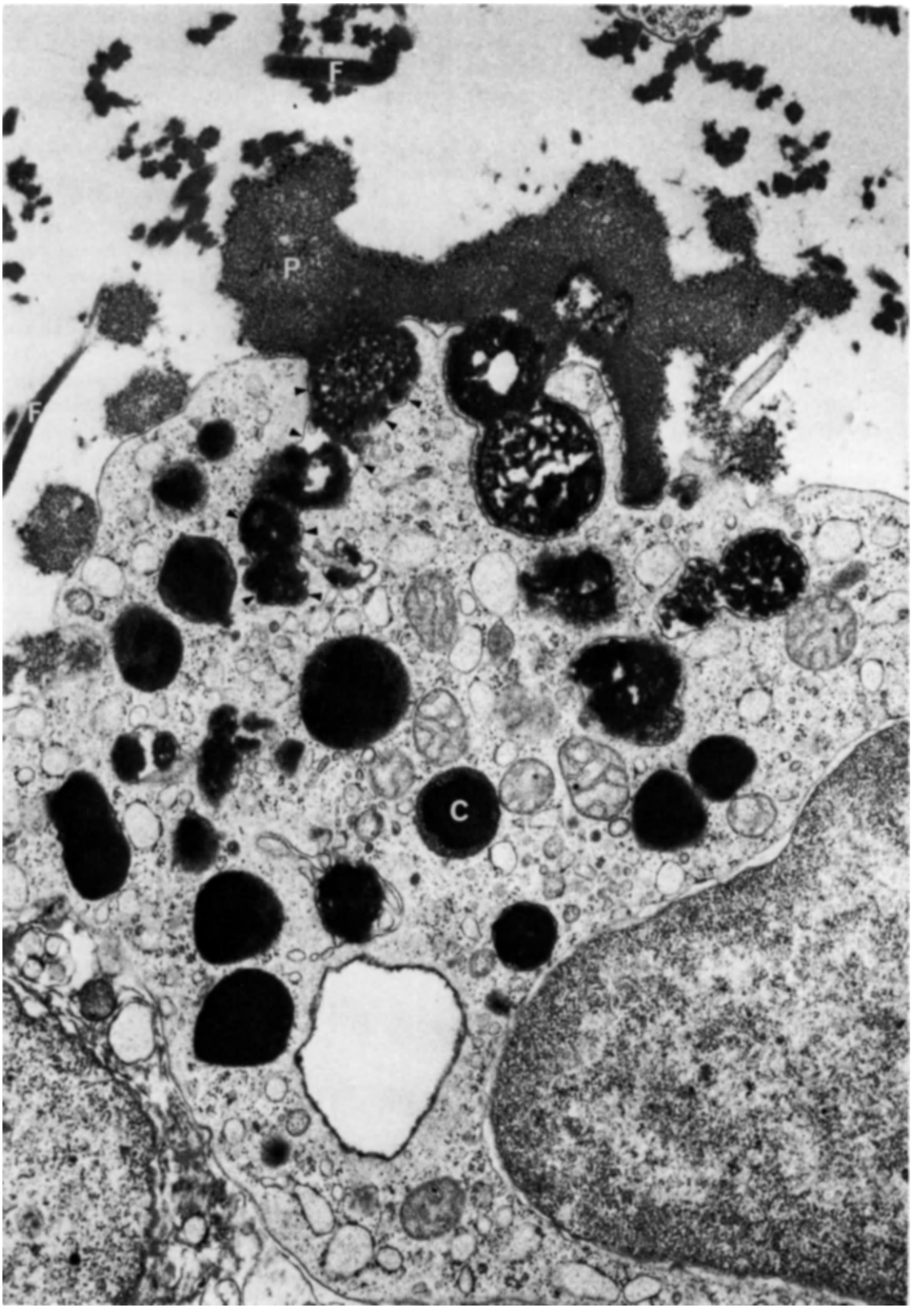
In most mammalian species the precursors of the eosinophil granules (in the eosinophilic myelocyte) present as dense homogeneous rounded bodies, and there is evidence that they are produced either directly from the rough endoplasmic reticulum or by rough endoplasmic reticulum and Golgi complex in the manner that secretory granules are usually produced. As the cell matures, part of the granule content crystallizes, forming one or more plate-like structures which alter the form of the granule to an ellipsoid, discoid or biconvex lens-like form. Details of the successive stages of maturation of the human eosinophil leucocyte and its granule have been described by Hardin and Spicer (1970).

According to Scott and Horn (1970b), not all granules of human eosinophil leucocytes mature in this fashion, for some immature granules are secreted by the eosinophil. A somewhat similar sentiment has been expressed by Barnhart and Riddle (1963), who found, with fluorescent antibody staining techniques, that profibrinolysin occurs in immature but not mature eosinophil granules of rabbit bone marrow. They therefore suggested that profibrinolysin was released during maturation. Unequivocal evidence of granule discharge by eosinophils has been seen by us (Skinnider and Ghadially, 1974) in the bone marrow of a child thought to have chronic active hepatitis and in which there were episodes of disseminated intravascular coagulation. There was an increase in the population of eosinophils and many of them appeared to have discharged their granule content into the intercellular space which contained much fibrin (*Plate 286*). These observations support the idea that the eosinophil leucocyte may have a role in fibrinolysis.

*Tumours where accumulations of eosinophils are at times prominent include carcinomas, particularly those of the lung and cervix, some lymphomas and malignant fibrous histiocytomas. Eosinophilotactic factor has been demonstrated in anaplastic squamous cell carcinoma, histiocytic lymphoma and malignant fibrous histiocytoma (for references *see* Isoda and Yasumoto, 1986).

Plate 286

An immature eosinophil from human marrow where crystalloid formation has commenced in only a few granules (C). Some of the granules are seen being discharged via deep channels (delineated by arrowheads) extending to the cell surface. Material presumed to be secretory product (P) and fibrin (F) are seen in the extracellular matrix. × 27 000 (*From Skinnider and Ghadially, 1974*)


F
P
F
C

There is, however, little evidence to support the idea that entire crystal-containing granules of the eosinophil are discharged (i.e. secreted) to the exterior. If this does occur it must be a singularly rare event. The occasional sightings of eosinophil granules lying in tissue matrix or in macrophages are better explained on the basis of necrosis of tissue eosinophils and subsequent release of granules.

The dense core or plate of the mature granule has been examined by a number of workers and in some (but not all) species a crystalline structure has been demonstrated (Osako, 1959). In the mouse the crystal is described as a lamellated structure of alternating light and dense lines with a periodicity of about 5 nm (Sheldon and Zetterquist, 1955). In the cat it is said to be a cylindrical structure composed of concentric lamellae (Bargmann and Knoop, 1958). In humans and laboratory rodents, the crystal is described (Miller *et al.*, 1966) as having a cubic lattice with a repeat of approximately 4 nm for humans and 3 nm for rodents. Like many other protein crystals, in longitudinal section the crystal presents as a series of evenly spaced parallel dense lines, and in transverse sections as a square array of dots.

We (Ghadially and Parry, 1965b) have drawn attention to the fact that examination of electron micrographs of eosinophils published in the literature reveals two distinct appearances. In some eosinophils the granules have a dense matrix and a pale core or crystalloid, while in others these densities are reversed (*Plate 287*). We have not yet encountered an indubitable instance where both varieties of granules occurred in one eosinophil, but we have on a few occasions seen eosinophils, some containing one variety and some the other variety of granule in a single ultrathin section. The significance of this is not apparent from available data. That such appearances can be produced by different methods of handling the tissue is not doubted but this can hardly explain the phenomenon noted by us or by G. Kelenyi (personal communication) who has observed both varieties in a single eosinophil.

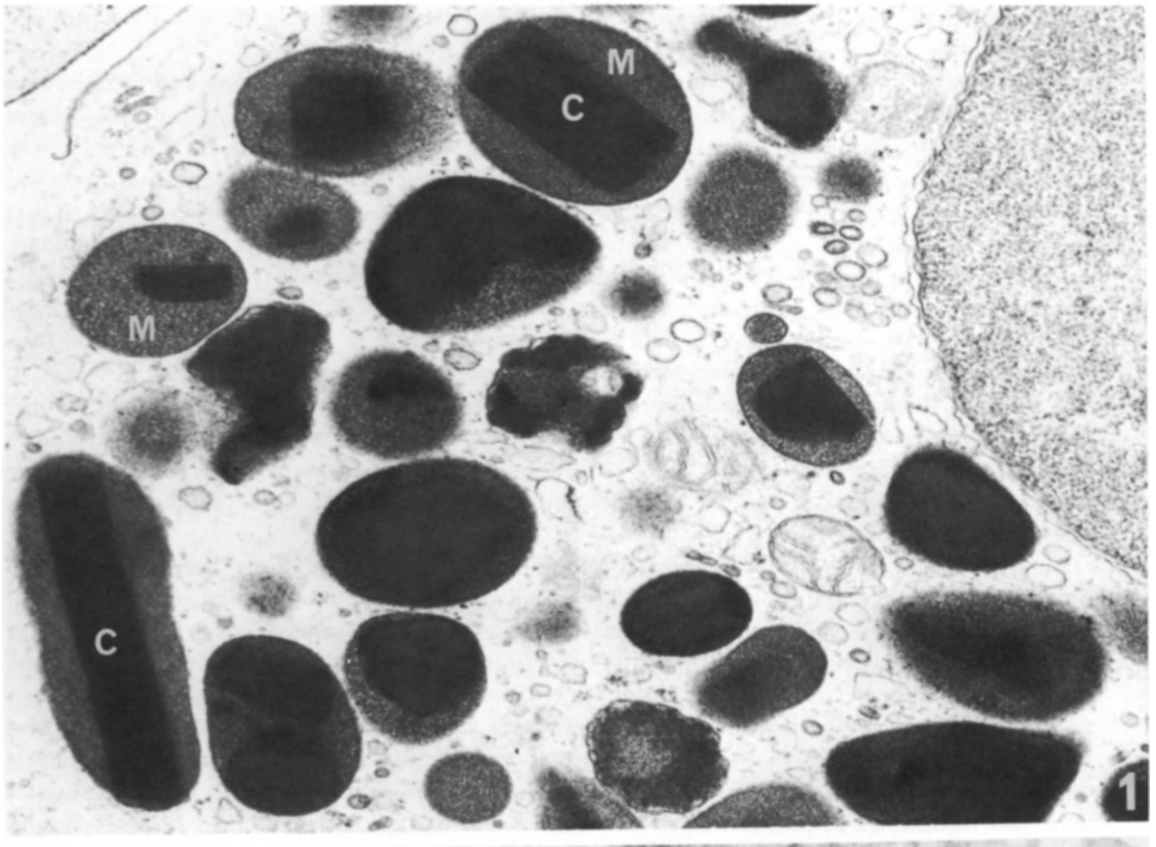
Somewhat different in appearance are eosinophil granules where there is a ragged loss of the crystalline core so that in this instance also, granules with more or less electron-lucent cores occur in company with granules with dense cores (*Plate 288*). Here again one can argue that this may be an extraction phenomenon dependent on methods of tissue processing, but the constancy with which this change is seen in certain conditions and the fact that dense-core granules and lucent-core granules occur in the same eosinophil argue against the idea that this is an *in vitro* artefact.

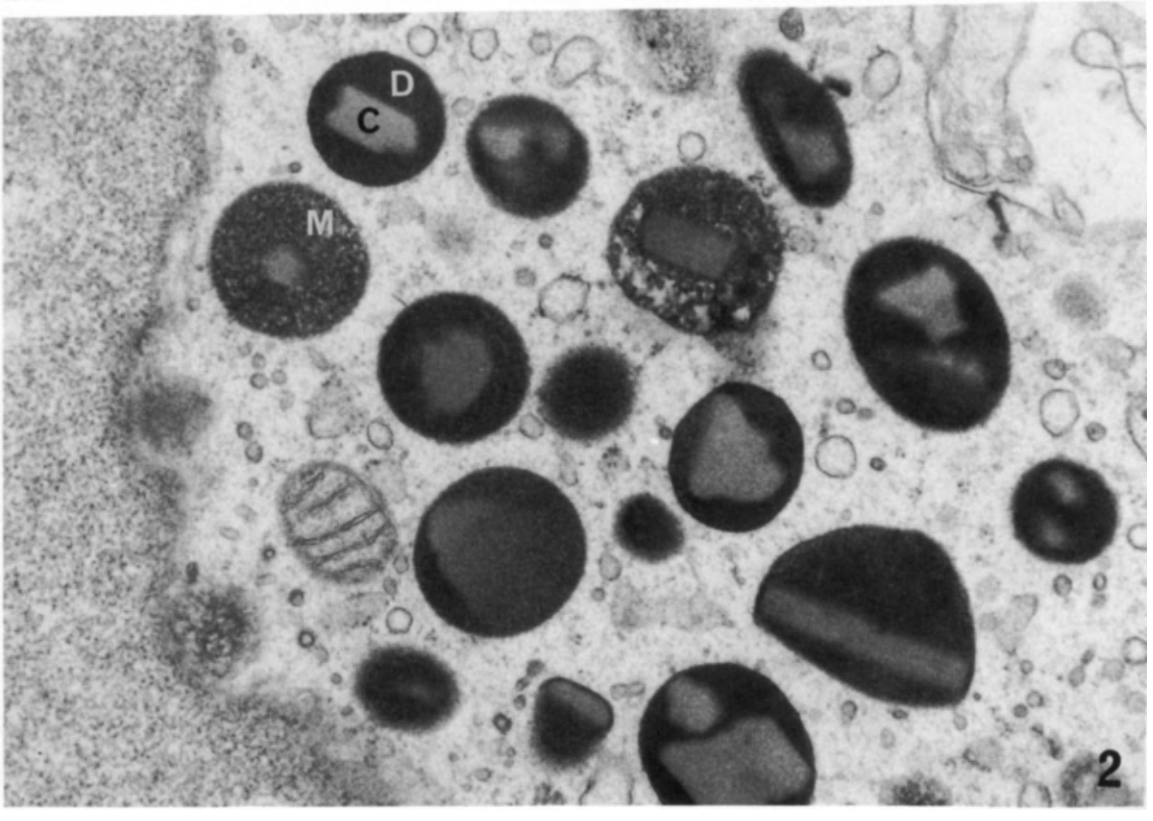
Parmley and Spicer (1975) have noted a variety of structural alterations in the crystalloid cores of eosinophil granules in Hodgkin's disease, while Dvorak (1980) has noted similar changes in the eosinophils from the ileum of patients with Crohn's disease, the most interesting alteration being a ragged loss of granule cores. We (Ghadially *et al.*, 1982) have seen a large

Plate 287

Fig. 1. The granules of this eosinophil leucocyte show an electron-dense crystalloid (C) set in a paler granular matrix (M). From human bone marrow fixed in osmium and stained with uranium and lead. × 34 000

Fig. 2. The granules of this eosinophil leucocyte show pale crystalloids (C) set in a granular matrix (M) (similar to the matrix around some of the crystalloids in *Fig. 1*) or a highly electron-dense matrix (D). From another specimen of human bone marrow prepared in the same way as *Fig. 1*. × 30 000


M
C
M
C
1


D
C
M
2

number of eosinophils with similarly altered granules (*Plate 288*) adjacent to necrotic zones in a fibrous histiocytoma.

According to Dvorak (1980) 'this represents an *in vivo* release of eosinophil cores in human disease'. Since the major basic protein purified from the crystalline core of eosinophils induces cytotoxicity in a variety of mammalian cells (Lewis *et al.*, 1978; Gleich *et al.*, 1979), Dvorak (1980) suggests that the necrosis of cells in the autonomic nervous system, smooth muscle and vascular endothelium regularly observed in tissues of patients with Crohn's disease may in part be due to the discharge of this material from the crystalline core of the eosinophil granule.

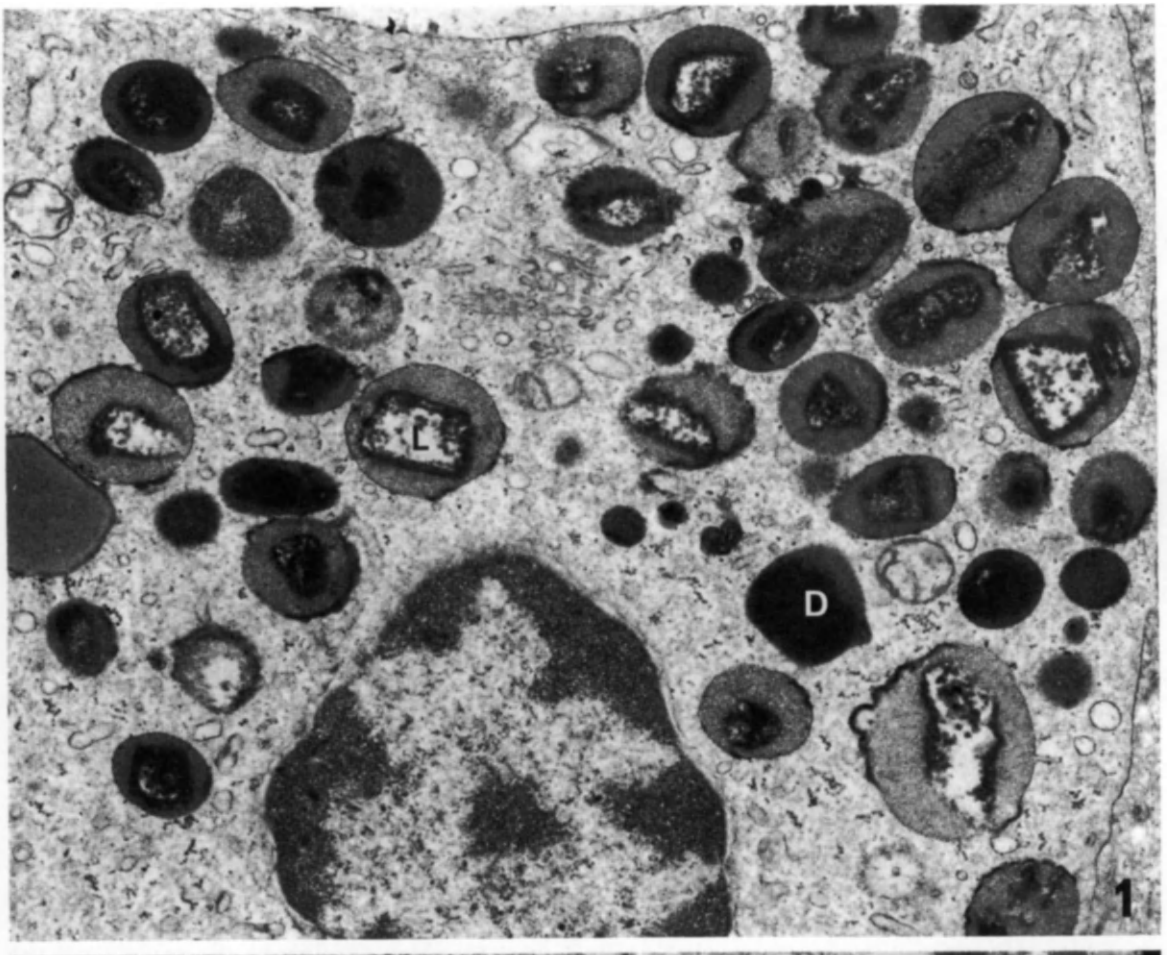
Some interesting older studies also recall the toxic nature of eosinophil granules. In 1933 Gordon reported that homogenates of Hodgkin's lymph nodes caused a paralytic disorder when injected into laboratory animals. He thought that this indicated the presence of a pathogenic agent and that this procedure could have diagnostic application. Later, however, it was shown (McNaught, 1938; Turner *et al.*, 1938; Seiler *et al.*, 1969) that this 'diagnostic test for Hodgkin's disease' could be correlated with the presence of eosinophils while further studies (Lewis *et al.*, 1978; Gleich *et al.*, 1979) have demonstrated that the cytotoxic and neurotoxic substance responsible for this phenomenon is the basic protein in the crystalline core of the eosinophil granule.

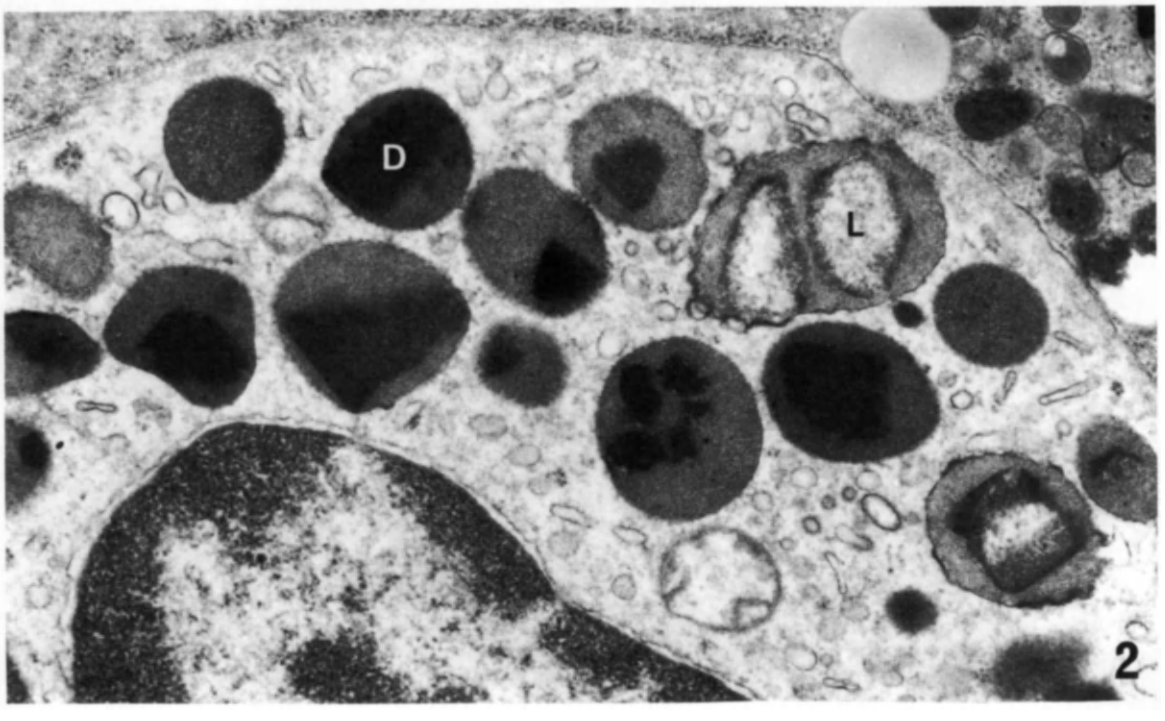
Charcot-Leyden crystals and their association with focal accumulations of eosinophils have long been known to pathologists. They have been seen in the sputa of patients with bronchial asthma, pulmonary ascariasis and tropical eosinophilia. They have also been found in granulomas associated with tissue invasion by helminths, in the stools of patients with amoebic dysentery and indeed in many other instances where disintegrating eosinophils occur. They can also be produced *in vitro* by lysing eosinophils with a surface-active agent (Aerosol OT), and ultrastructural studies (El-Hashimi, 1971) show that these crystals are derived from the granule of the eosinophil leucocyte. The fact that Charcot-Leyden crystals occur in primates such as man and monkey, but not in a variety of other species studied, once more stresses species differences in these granules.

The chemical composition of the core and cortex of the eosinophil granule has not been elucidated. According to Miller *et al.* (1966), the available evidence suggests that the crystalline core comprises a specific peroxidase known to occur in the eosinophil granule, but Cotran and Litt (1969) believe that the peroxidase resides in the matrix rather than the crystalline core. The granule is also said to be rich in phospholipids and various lysosomal enzymes, such as cathepsin, ribonuclease, aryl sulphatase, β-glucuronidase and acid phosphatase. Antibacterial agents such as phagocytin and lysozyme were not found in eosinophil granule preparations by Archer and Hirsch (1963). The eosinophilia of the granules is said to be due to the presence of a high concentration of basic proteins. (For further references and details, *see* Miller *et al.*, 1966; Hardin and Spicer, 1970; Scott and Horn, 1970b; Bainton and Farquhar, 1970.)

Plate 288

Figs. 1 and 2. Eosinophils found in a fibrous histiocytoma. Note the normal-looking granules with a dense core (D) and granules where a ragged loss of cores (L) has occurred. × 23 000, × 28 000 (*Ghadially, Fergus Murphy and Lalonde 1982*)


L
D
1


D
L
2

Lysosomes in monocytes and macrophages

The terms 'macrophage' (big eater) and 'microphage' (small eater) were coined by Metschnikoff (1884, 1892) to distinguish large mononuclear phagocytic cells capable of ingesting large particles (e.g. entire erythrocytes and leucocytes, ten or 20 of which may be found in a single macrophage) from the much smaller polymorphonuclear leucocytes which are able to ingest only smaller particles (e.g. bacteria). He demonstrated the existence of macrophages in various animals ranging from starfish to mammals. He also recognized that such cells exist in the spleen, lymph nodes, bone marrow and connective tissues and proposed that they should be considered members of the 'macrophage system'.

However, it must not be construed that macrophages take up only large particles, for they can also endocytose (i.e. ingest) small particles such as: (1) bacteria (Whitby and Rowley, 1959; Mackaness, 1960); (2) viruses (Friend *et al.*, 1969); (3) antigen-antibody complexes (Sorkin and Boyden, 1959) and a variety of inorganic materials such as various metallic compounds of iron, beryllium, gold, platinum and plutonium (*see* Policard, 1962 and pages 726–737).

The ability of phagocytic cells to take up injected vital dyes became a tool for recognizing such cells, and on the basis of this Aschoff (1913, 1924) developed his well known concept of the reticuloendothelial system which, however, included several cell types besides the mononuclear phagocytes★. The reticuloendothelial system is thought to be composed of three categories of cells: (1) reticulum (or reticular) cells; (2) mobile tissue cells called histiocytes†; and (3) blood cells called small and large monocytes (Bessis, 1973). The concept of the reticuloendothelial system has been the subject of long standing criticisms (Maximow, 1927) and several modifications and terms to alter or replace it have been proposed. The most recent attempt of this kind places all highly phagocytic mononuclear cells and their precursors into one system called the 'mononuclear phagocytic system' (Langevoort *et al.*, 1970). This is justified 'on the basis of their common origin, morphology and function' (Van Furth *et al.*, 1975).

According to Van Furth *et al.* (1975) the mononuclear phagocytic cells include precursor cells in the bone marrow (monoblasts and promonocytes) monocytes in the blood and tissue macrophages which derive from them (*Plates 289–291*). The latter include‡: (1) macrophages or histiocytes in connective tissue: (2) Kupffer cells in the liver; (3) alveolar macrophages in the lung; (4) free and fixed macrophages in lymph nodes; (5) pleural and peritoneal macrophages; (6) microglial cells in the nervous system; and (7) perhaps also the osteoclasts in bone.

Perhaps the most familiar member of this system is the monocyte in the circulating blood (*Plate 289*). Electron microscopy shows that these cells have an indented nucleus, a well

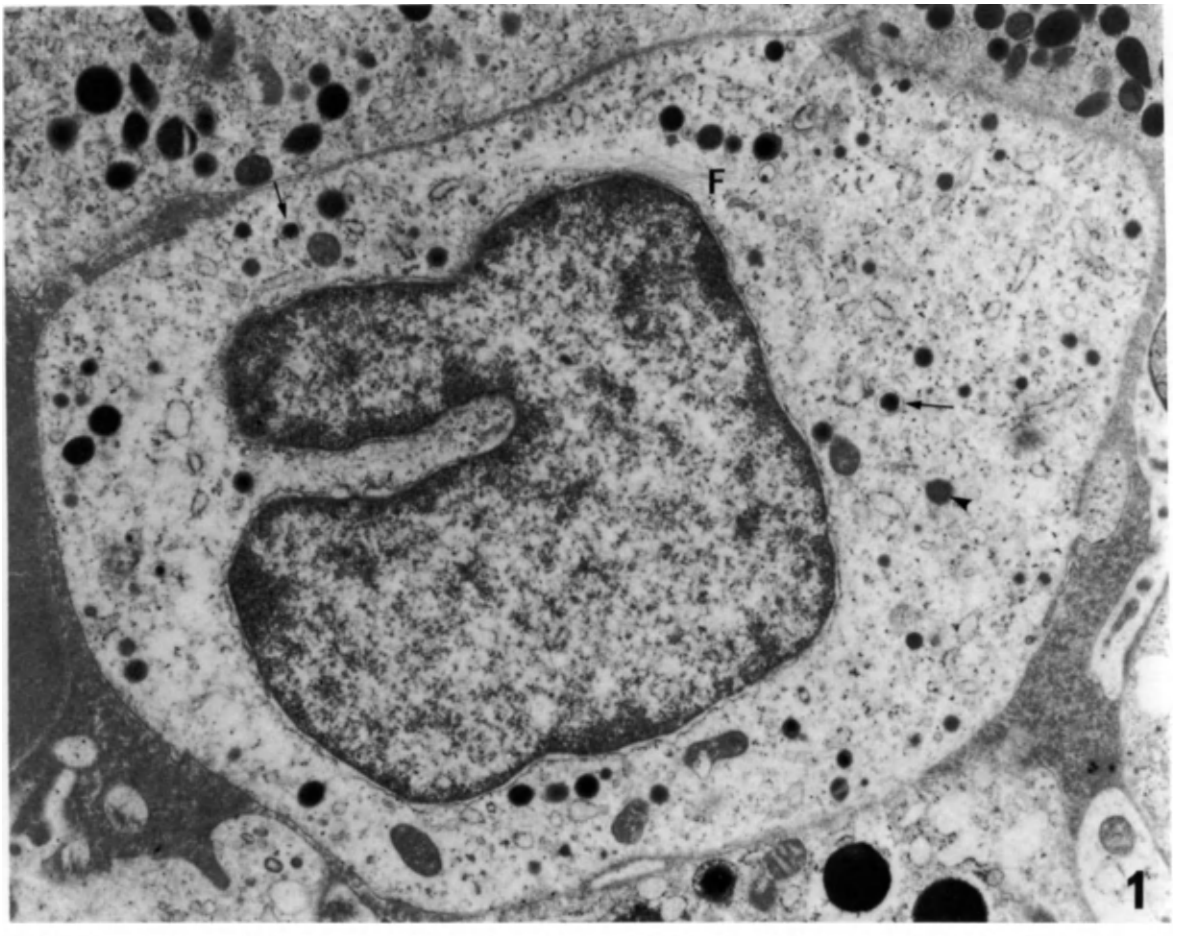
★The uptake of injected dyes is not too reliable a criterion for unequivocally distinguishing the highly phagocytic mononuclear cells from all others. Whether such material is picked up depends not only on the cell in question but also on the nature of local vasculation and escape of dye from the the circulation and hence its availability for endocytosis. Some not too actively phagocytic cells may pick up the dye particularly if quite large doses are employed. Further one may argue that this is hardly a test of the power to 'avidly ingest large particles' as the mononuclear phagocytes do. It must also be remembered that virtually all cells have some power to endocytosis, via micropinocytotic vesicles.

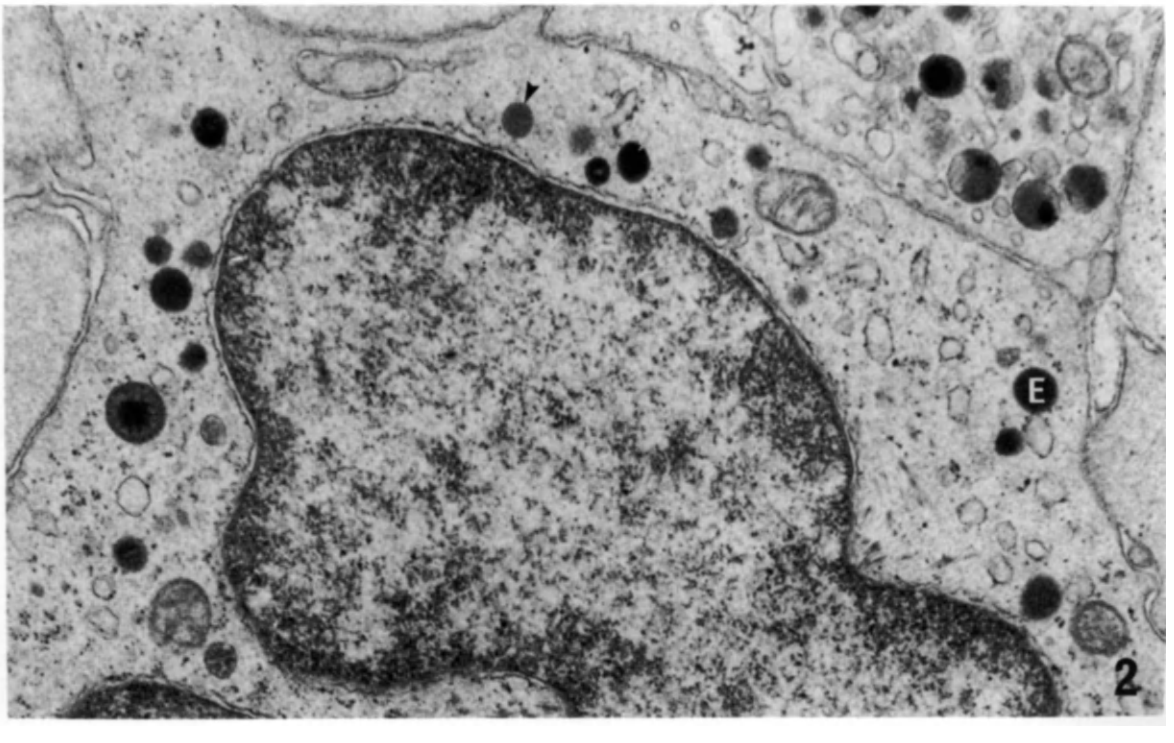
†A histiocyte is a macrophage of connective tissue, just as the Kupffer cell is the macrophage of the liver. Some authors now avoid the term histiocyte and call these cells 'macrophages'.

‡One should perhaps add the synovial intimal cells to this list because their phagocytic powers easily match those of any macrophage. However there is no reason to believe that they originate from a bone marrow precursor cell.These cells also qualify for membership in the reticuloendothelial system (mononuclear phagocytic system?) because of their ability to trap injected dyes (for a review *see* Ghadially, 1980).

Plate 289

Figs. 1 and 2. Monocytes from human bone marrow (*Fig. 1*) and peripheral blood (*Fig. 2*) showing granules of variable size and morphology. Note the presence of electron-dense granules (E), medium density granules (arrowheads) and granules showing a halo (arrows). Other characteristic features of this cell type include an indented nucleus and a few intermediate filaments (F) in the juxtanuclear region. × 11 500; × 34 000


F
1


E
2

developed Golgi complex, small amounts of glycogen and rough endoplasmic reticulum, a few rounded or elongated mitochondria and numerous quite small (100–450 nm, average diameter 190 nm) round, oval or elongated electron-dense granules limited by a single membrane. In some of the round granules a clear halo separates the granule contents from the limiting membrane (Low and Freeman, 1958; Sutton and Weiss, 1966; Watanabe *et al.*, 1967; Zucker-Franklin, 1968; Fedorko and Hirsch, 1970; Nichols *et al.*, 1971; Tanaka and Goodman, 1972; Nichols and Bainton, 1975; Van der Rhee *et al.*, 1979).

Nichols *et al.* (1971) and Nichols and Bainton (1975) studied granule production in various species with emphasis on rabbit and man. Their results indicate that two distinct populations of granules (morphologically indistinguishable) exist in the human monocyte. The first type of granule (primary granule) is formed during the promonocyte stage in the bone marrow. Primary granules contain acid phosphatase and arylsulphatase and peroxidase. They can be regarded as primary lysosomes or storage granules comparable to the azurophilic granules of neutrophil leucocytes. The enzymes are produced and packaged by the well-known pathway through the rough endoplasmic reticulum and Golgi complex. Although in some species (e.g. man, guinea-pig and rat) they contain peroxidase, in others (e.g. rabbit) they do not.

After the first stage of granulogenesis is over, a second type of granule (secondary granule) is produced in more mature bone marrow monocytes and in the circulating monocytes. The contents of such granules are not known. Technical problems have militated against demonstrating acid hydrolases, but it is said* that they lack both peroxidase and alkaline phosphatase (Nichols and Bainton, 1975).

Because of their easy availability, macrophages in the peritoneal cavity (*Plate 290*) have been studied more often than macrophages in 'solid' tissues (*Plate 291*), but it would appear that in both instances transformation from monocyte to macrophage is essentially similar. In response to characteristic stimuli large numbers of monocytes migrate from the blood into the peritoneal cavity. Predictably during the early stages of this process the morphology of the cells recovered from the peritoneal cavity is similar to that of the circulating blood monocyte, but after phagocytosis the storage granules are discharged into the phagocytic vacuole (heterophagosome) thus forming a heterolysosome. Once the quota of storage granules is expended no new storage granules are formed by the macrophage (in this it resembles the neutrophil leucocyte), but large quantities of acid hydrolases are produced (in this it differs from the neutrophil leucocyte) which depart from the Golgi complex in coated vesicles or simple vesicles which then fuse with heterophagosomes to give heterolysosomes. Thus the primary lysosome of what might be called the mature macrophage is a Golgi derived simple or coated vesicle† similar to

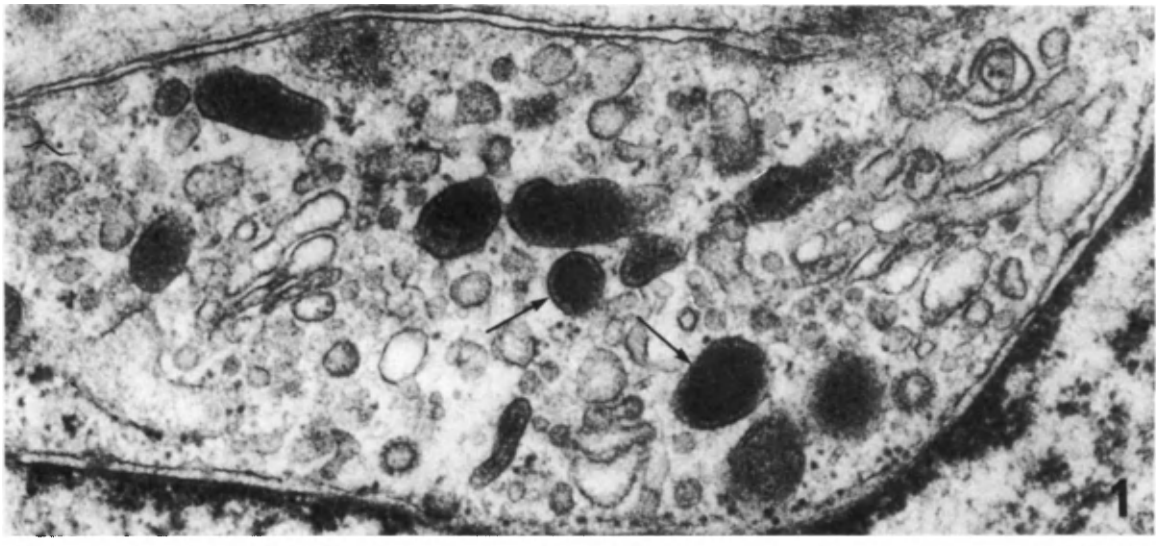
*Under certain chemical conditions both the primary and secondary granules show peroxidatic activity (Van der Rhee *et al.*, 1977).

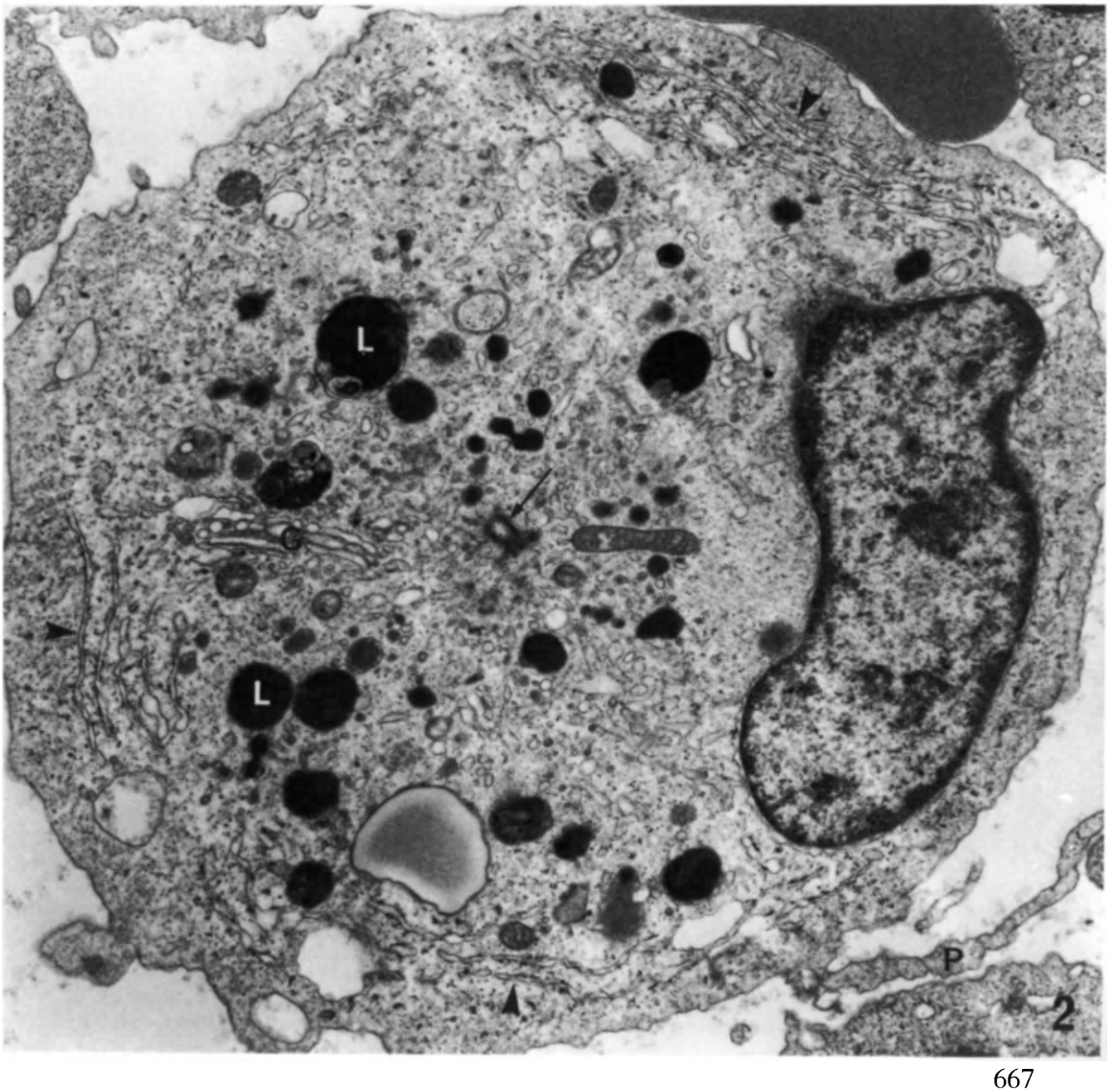
†Coated vesicles or simple vesicles without a coat can be derived from the cell membrane by the process known as micropinocytosis. Pinocytosis and micropinocytosis imply cell drinking, whereby the cell impounds fluid (simple vesicles) or fluids with proteins (coated vesicles) from its environment (*see* pages 1134–1139). Vesicles with or without a coat can also be derived from the Golgi complex. Here they transport enzymes (e.g. primary lysosomes) and secretory products. It would be absurd to call these 'micropinocytotic vesicles' because they are not involved in 'cell drinking'. One must not confuse such vesicles with quite different origins and functions as do Carr and Wright (1979) when they state that in the mature macrophage 'the primary lysosome is a micropinocytic vesicle'. Incidentally, the correct term is 'micropinocytotic' not 'micropinocytic'.

Plate 290

Fig. 1. Macrophage from a fibrosarcoma. Note the granules, with a halo (arrows) which are thought to be primary lysosomes. No secondary lysosomes were present in this cell. × 58 000 (*From Ghadially, 1980a*)

Fig. 2. An activated macrophage from a haemorrhagic malignant ascites showing numerous secondary lysosomes (L) containing membranous and electron-dense material (some of which at higher magnifications showed the particulate structure of haemosiderin), a few stacks of rough endoplasmic reticulum (arrowheads) poorly populated by ribosomes, a Golgi complex (G), centriole (arrow) and some cell processes (P). × 15 000 (*From Ghadially, 1980a*)


1


L
L
P
2

the primary lysosomes of a majority of cells and not a storage granule like that found in neutrophils and monocytes.

However, in the macrophage another kind of granule (*Plate 290*) with an average diameter of about 280 nm also occurs*. It has a homogeneous medium density content separated from the limiting membrane by a halo (*Plate 291*). It seems likely that this too is a primary lysosome but whether this is a new type of granule produced by the macrophage deserving to be called a 'macrophage granule' as Carr (1968) has proposed or whether this is a slightly modified monocyte granule (slightly enlarged and not quite so electron-dense as primary and secondary granules of the monocyte) is difficult to say. The distinction between the so-called 'macrophage granule' and some secondary granules of the monocyte can be quite difficult for the latter also at times have a halo and are not always overtly electron-dense.

In any case such so-called 'macrophage granules' and primary and secondary granules are rare or absent in macrophages that have engaged in substantial phagocytic activity. The cytoplasm of such cells contains solitary or multiple heterolysosomes, the contents of which reflect what the cell has ingested.

Macrophages actively engaged in phagocytic acts (called activated macrophages) show more or better developed cell processes (presumably to impound material) and rough endoplasmic reticulum (presumably needed for the synthesis of acid hydrolases) than resting macrophages. However, by no stretch of the imagination can the rough endoplasmic reticulum in these cells be regarded as abundant or prominent†.

Macrophages as a class are poorly endowed with rough endoplasmic reticulum and this is one of the features which helps distinguish these cells from fibroblasts which are well endowed with rough endoplasmic reticulum. A further point of distinction is that the profiles of rough endoplasmic reticulum in the macrophage appear to be poorly populated by ribosomes, that is to say quite large segments of membrane appear to bear no ribosomes. This is explainable by *en face* views of the membranes which show that the attached polyribosomes comprise aggregates of only four or five ribosomes. In contrast to this, the rough endoplamic reticulum in the fibroblast appears thickly populated by polyribosomes comprising as many as 30 ribosomes (Leibovich and Ross, 1975).

A vexatious question about which there is little agreement is whether macrophages secrete lysosomal enzymes and other materials (*see below*). Metschnikoff (1884) firmly believed that,

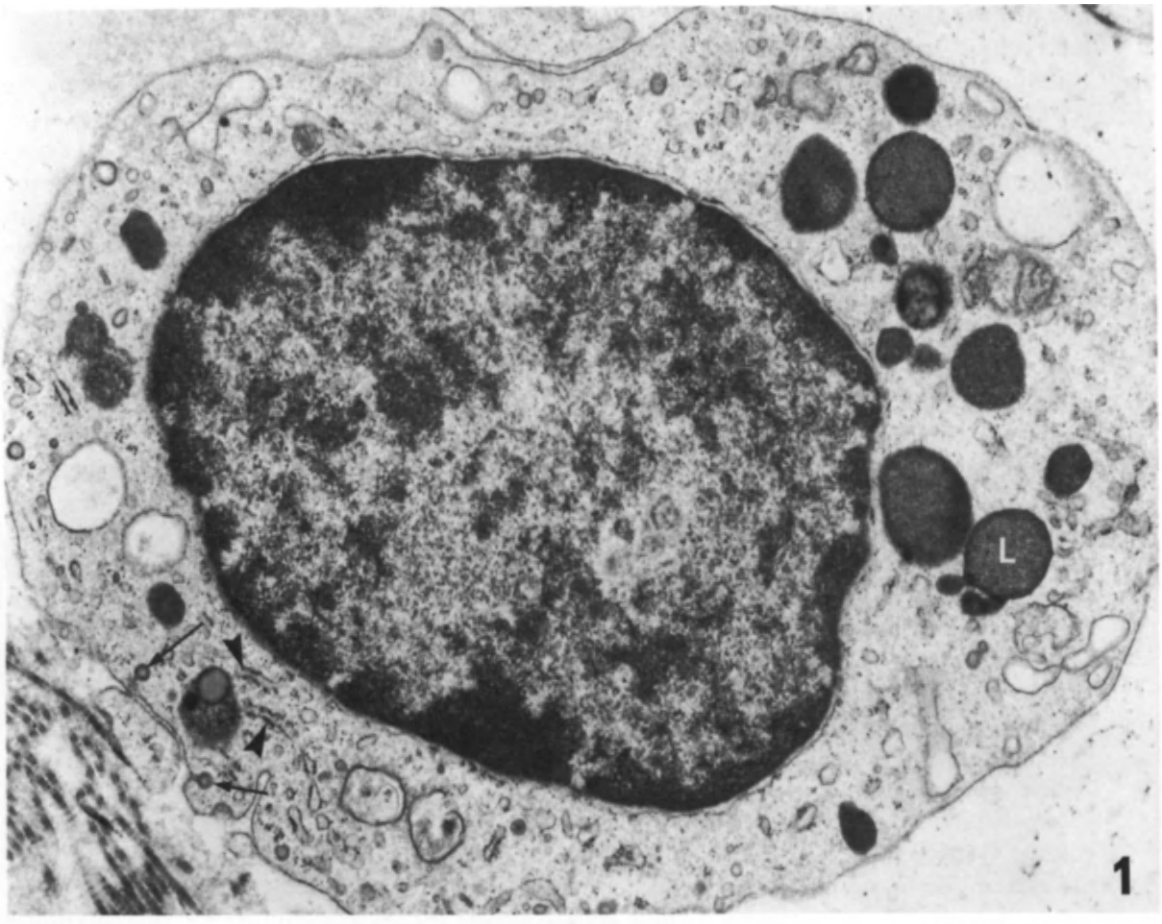
*In granulomas a sequence of changes are seen suggesting that blood monocytes undergo differentiation into exudate monocytes, immature macrophages, epithelioid cells, Langerhans-type giant cells and foreign-body-type giant cells. Epithelioid cells are said to have characteristic granules somewhat larger than macrophage granules. The two types of giant cells are formed by fusion of macrophages. The granules in giant cells (*Plate 292*) are described as 'heterogeneous'. (For references and details *see* Van der Rhee *et al.*, 1979.)

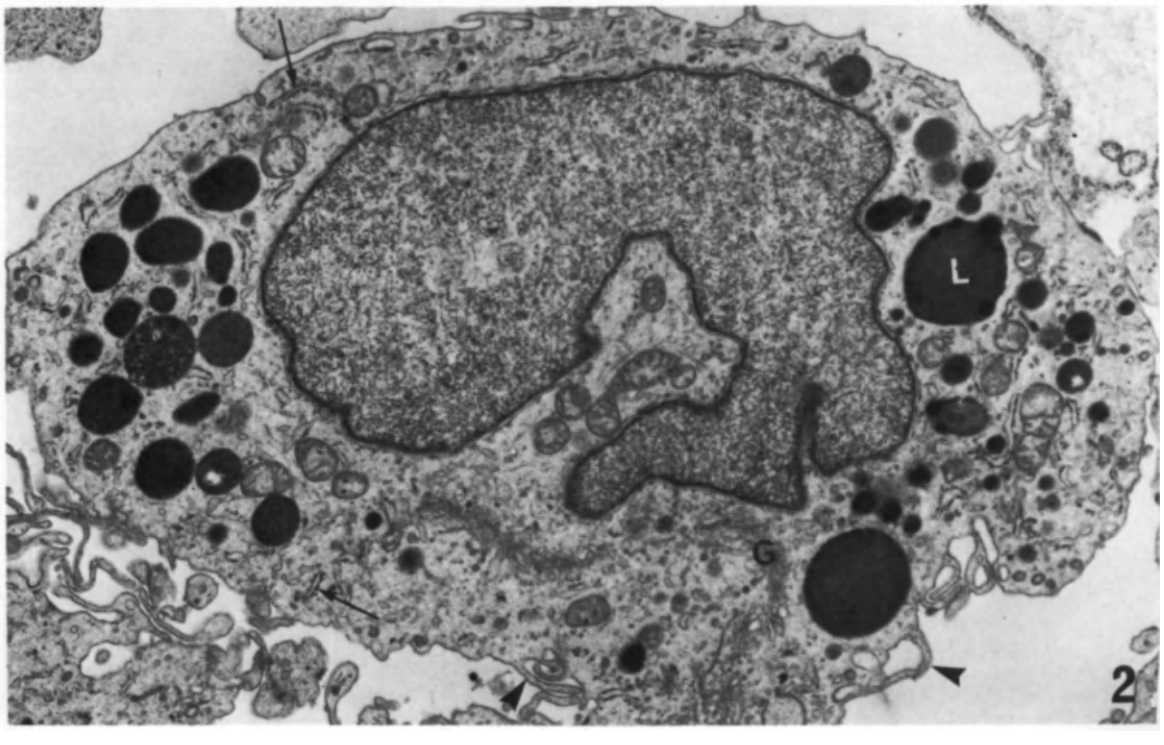
†*See* footnote on page 718.

Plate 291

Fig. 1. Macrophage from a fibroadenoma of the breast. Although this cell contains some lysosomal bodies (L) with a particulate content (morphologically acceptable as siderosomes) it is not noticeably activated, for the rough endoplasmic reticulum (arrowheads) is scant, the Golgi complex is not evident (at least in this plane of sectioning) nor are the cell processes. One could interpret this as a resting macrophage that is no longer engaged in active phagocytosis. The cell however does seem to be picking up some proteinaceous material, as suggested by the presence of coated vesicles (arrows). × 20 000 (*From Ghadially, 1980a*)

Fig. 2. Macrophage from a hamartoma of lung. Most of the heterolysosomes (L) seen here are derived from phagocytosis of erythrocyte fragments or haemoglobin. Note the cell processes (arrowheads), small but numerous profiles of rough endoplasmic reticulum (arrows) and the prominent Golgi complex. × 12 000 (*From Ghadially, 1980a*)


L
1


L
G
2

like protozoa, macrophages destroy their prey by intracellular digestion and that their 'cytases' are released to the extracellular environment only when the cells are suffering or dying; a condition he called 'phagolysis'. Perhaps the first suggestion that macrophages may be secretory cells was put forward in 1907 by Renaut who named them 'rhagiocrine' cells.

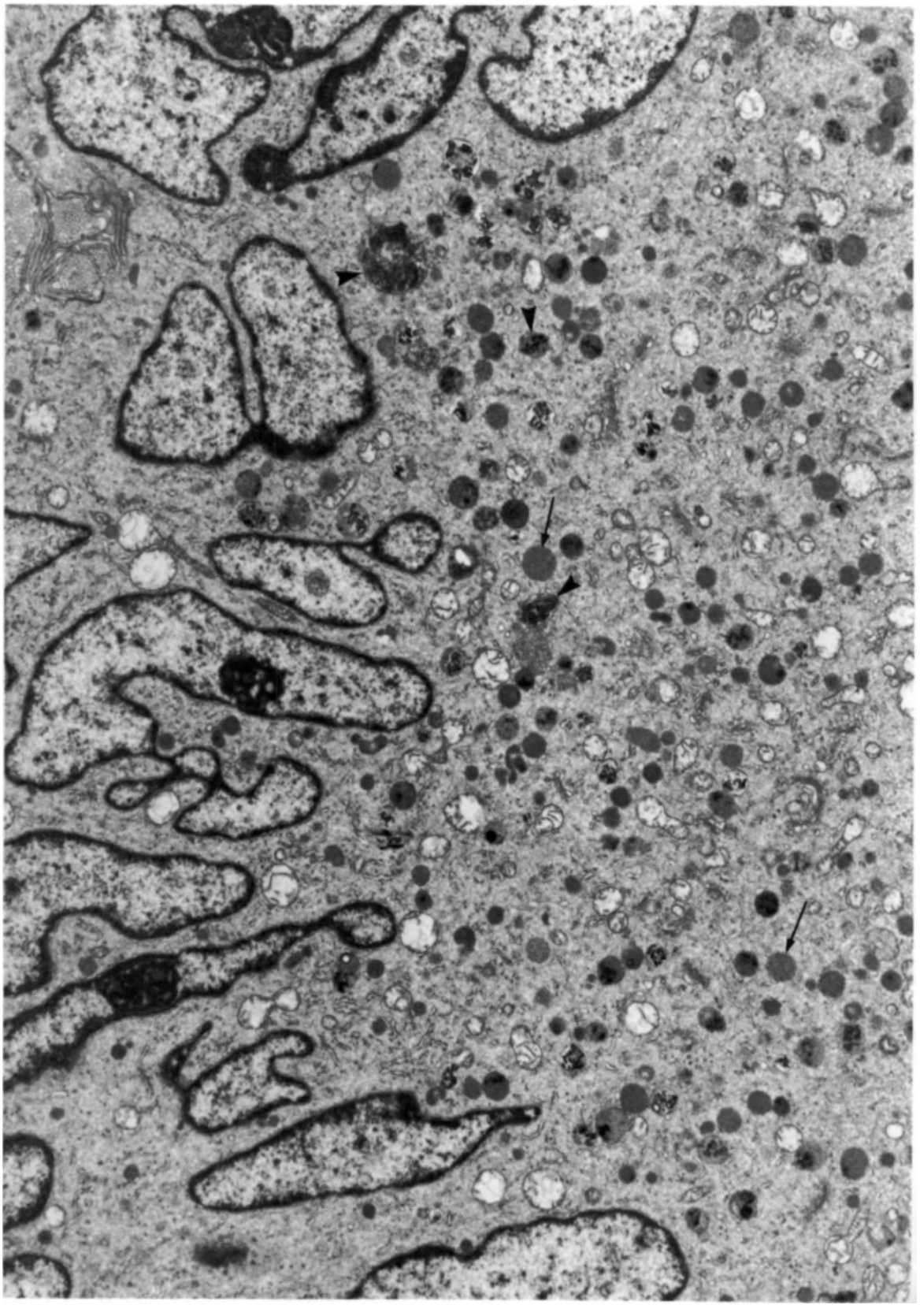
It would appear that macrophages produce a variety of biologically active substances besides acid hydrolases, such as neutral proteases (elastases and collagenases) and lysozyme and *in vitro* studies show that such materials are released into the medium (Unanue, 1976) but this of course is no proof of secretion, particularly when one considers that in the innumerable published electron micrographs one does not see secretory granules discharging on the cell surface. It is for reasons such as this that one has grave reservations about the rare illustration (and not very convincing at that) purporting to show discharge of granule contents on to the surface (*see* for example *Fig. 5* in Carr and Wright, 1979). Clearly, the rarity of such images should make one suspect that they might be artefactual or fortuitous in nature. It would seem unwise on the basis of such evidence to assert as Lobo *et al.* (1978) do that 'macrophages are secretory cells' or be carried away and call a granuloma an 'endocrine gland'. However, the possibility that macrophages may secrete various substances is not refuted by such arguments, only that the mechanisms involved are likely to be more subtle.

Granuloma macrophages have been shown to contain lysozyme by immunocytochemical techniques at the light microscopic level (Klockars and Selroos, 1977). An attempt has also been made to localize lysozyme in macrophage giant cells in sarcoidosis at the ultrastructural level (Lobo *et al.*, 1978). They claim that their findings 'identify for the first time lysozyme in macrophage granules immunocytochemically at the electron microscopic level' but the illustration presented to support this (their *Fig. 2*) is not convincing. With immunocytochemical techniques one expects that only the site(s) of localization of the enzyme will be 'stained' (i.e. appear electron-dense) but in this illustration (*Fig. 2* in Lobo *et al.*, 1978) the nuclear heterochromatin and nucleolus appear every bit as electron-dense as the granules alleged to contain lysozyme. One could therefore contend that lysozyme occurs in all these sites (i.e. nucleus, nucleolus and granules) or accept the more plausible explanation, namely, that one is witnessing non-specific 'staining'. These authors ignore the nuclear staining and state, 'staining was never seen outside dense bodies'.

A repeat performance of the same or similar experiment (i.e. macrophage giant cells in sarcoidosis) is also available (Carr, 1980) but this time a control section which shows an overall lack of staining and contrast is presented for comparison with an experimental section (i.e. one stained to demonstrate lysozyme) where just about everything except the cytoplasmic matrix is more or less electron-dense (including the nucleus). We are told that the electron microscope and photographic conditions were identical for these two electron micrographs. One wonders how or why this was done for this might lead to improperly exposed negatives. Be that as it may, despite such alleged identical electron microscopic and photographic conditions one illustration ends up at × 12 000 and the other at × 13 800! Alarming also is the statement made by Carr (1980) about the immunocytochemical technique employed by him. He states 'this technique is capricious; a considerable number of experiments have to be discarded because of non-specific staining and other artefacts'. This could be translated to mean that appearances and experiments that fit preconceived notions (i.e. that there is lysozyme in the granules) are acceptable and others (nuclear staining?) may be safely ignored. It is for reasons such as these that one has reservations about accepting the claims of Lobo *et al.* (1978) and Carr (1980).

Plate 292

A giant cell found in a sarcoid nodule removed from the lung. Note the multiple nuclear profiles and the pleomorphic granules. Those with a homogeneous content (arrows) are probably primary lysosomes while those with a heterogeneous content are likely to be secondary lysosomes containing fibrin (arrowheads). × 8000



Lysosomes in melanosis coli and some other melanoses

The abnormal brown or black pigmentation of the colonic mucosa, which is now called melanosis coli, was first described by Cruveilhier in 1829. The term 'melanosis coli'* was first used by Virchow (1857). Melanosis coli is dismissed rather briefly as a curiosity in most standard works on medicine and pathology for, although the condition is tinctorially spectacular, it is not thought to produce severe or specific symptomatology on its own. Symptoms which may be manifest are attributed to the accompanying conditions and these range from 'idiopathic' constipation to carcinoma of the colon.

Much interest has, however, existed for a long time regarding the nature of the pigment, the aetiology of the condition and its alleged association with carcinoma of the colon. Numerous excellent clinical, pathological, histochemical, and experimental studies have been reported (McFarland, 1917; Stewart and Hickman, 1931; Bockus *et al.*, 1933; Roden 1940; Speare, 1951; Cabanne and Couderc, 1963), and the ultrastructure of the pigment granules and the colon (*Plates 293* and *294*) in this condition have been reported and reviewed by us (Ghadially and Parry, 1966b, 1967).

Many of the earlier theories regarding the causation of melanosis coli have long been abandoned. For instance, the view that ingestion of heavy metals, with their deposition in the colon, was responsible for the pigmentation has not been supported by chemical analyses of affected tissue, and the belief that the colonic pigment may be of haematogenous origin as suggested by Virchow was discarded when attempts to produce the condition experimentally by introducing blood into the colon of animals failed. The more recent ideas regarding the aetiology of this condition centre around two factors; firstly, disturbance of bowel function resulting in chronic constipation, and secondly, the chronic ingestion of cathartics of the anthracene group such as cascara, senna, aloes and rhubarb. The role of such purgatives (particularly cascara) in the production of this condition has been unequivocally demonstrated by experimental studies both in monkey and man (Bockus *et al.*, 1933; Roden, 1940; Speare, 1951), for, by sigmoidoscopy and biopsy, the colonic pigment has been observed to wax and wane with cycles of administration and withdrawal of the drug.

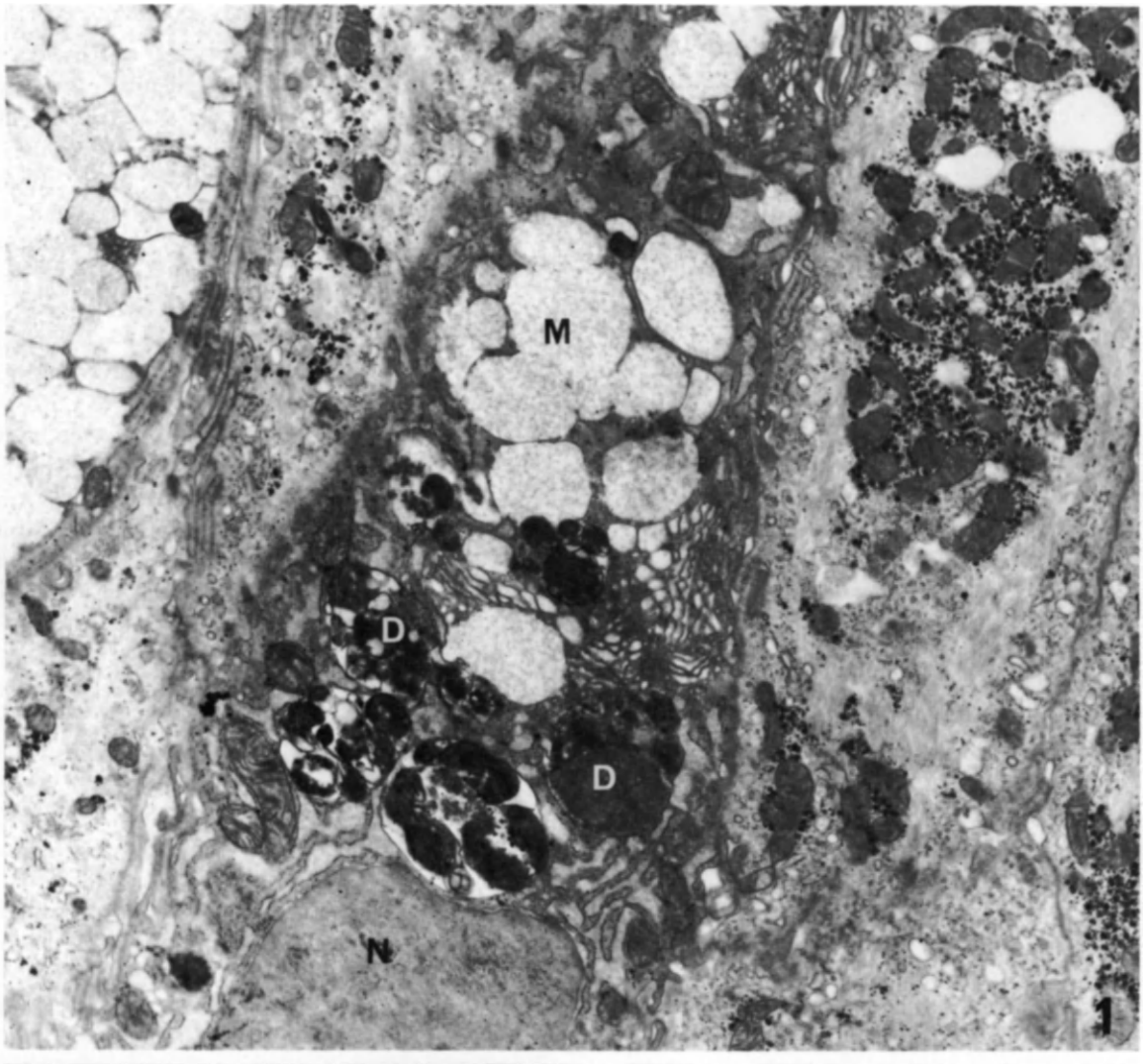
The theory that the pigment might be melanin or a melanin-like substance has been popular for a long time. McFarland (1917) classified the pigment as intermediate between true melanin and the wear and tear pigment, lipofuscin. Cabanne and Couderc (1963) believe that both lipofuscin and melanin occur in the pigment granules of melanosis coli. The histochemical studies conducted by us (Ghadially and Parry, 1966b) also showed that the pigment gives many of the reactions for both lipofuscin and melanin. In our hands, Lillie's Nile blue sulphate reaction, however, supports the idea that the pigment is lipofuscin.

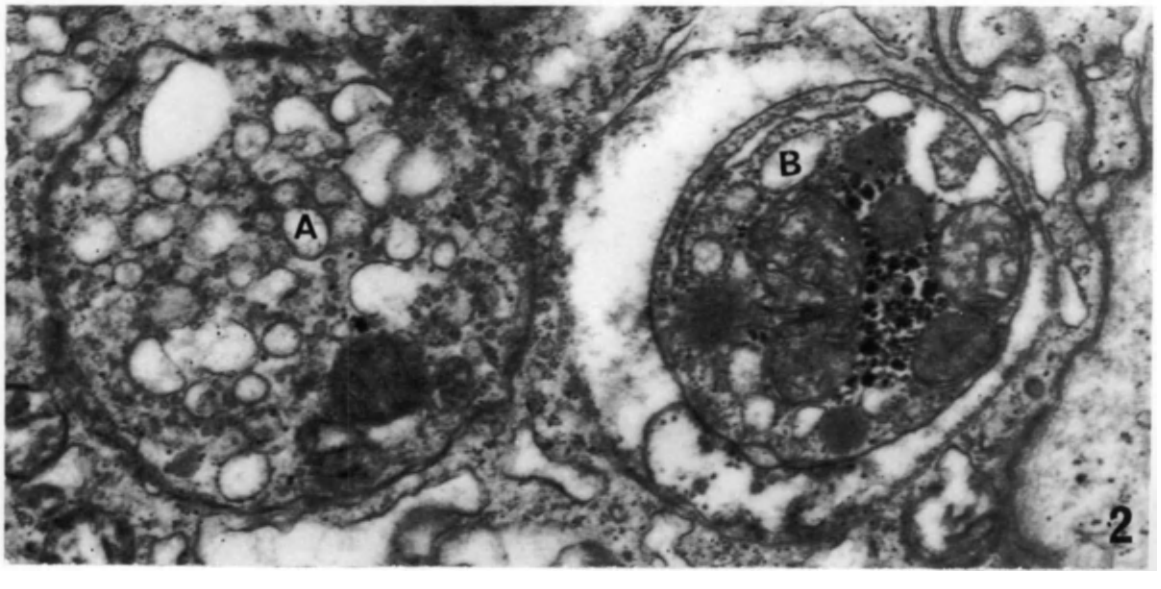
*In view of what is now known about the nature of the pigment it would be more apt to call this condition 'lipofuscinosis coli' (Ghadially, 1975b).

Plate 293

Fig. 1. Colonic mucosa from a case of melanosis coli, showing numerous single-membrane-bound dense bodies (D) lying between the nucleus (N) and some mucus droplets (M). Besides electron-dense particles and granules, these bodies also contain some membranous structures. They are thus interpreted as autophagic vacuoles (autolysosomes). × 12500 (*From Ghadially and Parry, 1966b*).

Fig. 2. Two autolysosomes found in a colonic epithelial cell; one of them (A) contains numerous vesicles and a mitochondrion, while the other (B) contains endoplasmic reticulum, mitochondria and glycogen. From the same case as *Fig 1. See also Plate 319,* which shows glycogen-containing autolysosomes found in submucosal cells of the same patient. × 24000 (*From Ghadially and Parry, 1966b*)


M
D
D
N
1


A
B
2

A strong argument against the pigment being melanin is that no melanocytes are found in this region. If one accepts the now well proven fact that melanin is synthesized in melanocytes and melanosomes which contain the enzyme tyrosinase, then it is clear that melanin derived by enzymic oxidation, as found in the skin or the eye, cannot be produced in sites where these cells are absent. Nevertheless, it is known that oxidation of many cyclic compounds such as phenol, pyrogallol, indole and skatole may produce melanin-like compounds without the intervention of melanocytes (Thomson, 1962). Therefore, one may argue that the pigment in melanosis coli is produced in such a fashion. This, however, is pure speculation, as there is not a shred of evidence that this actually happens.

Be that as it may, ultrastructural studies (Ghadially and Parry, 1966b, 1967; Kermarec *et al.*, 1972) on melanosis coli have now clearly shown that the pigment is lipofuscin (pages 608-613) that is to say residual bodies derived from material sequestrated and digested in autolysosomes. Autolysosomes containing mitochondria, endoplasmic reticulum and glycogen were seen in the mucosal cells (*Plate 293*) even though in the past light microscopists have repeatedly reported that no pigment is seen in the mucosa but only in the macrophages in the submucosa. However, it is true that most of the pigment granules do lie in this region (submucosa), for the electron microscope shows huge phagocytic cells filled with innumerable single-membrane-bound bodies with electron-dense, granular and amorphous material and also occasionally lipid droplets so characteristic of lipofuscin (*Plate 294*). Such bodies are akin to residual bodies containing lipofuscin known to be derived by lysosomal activity (pages 608-613).

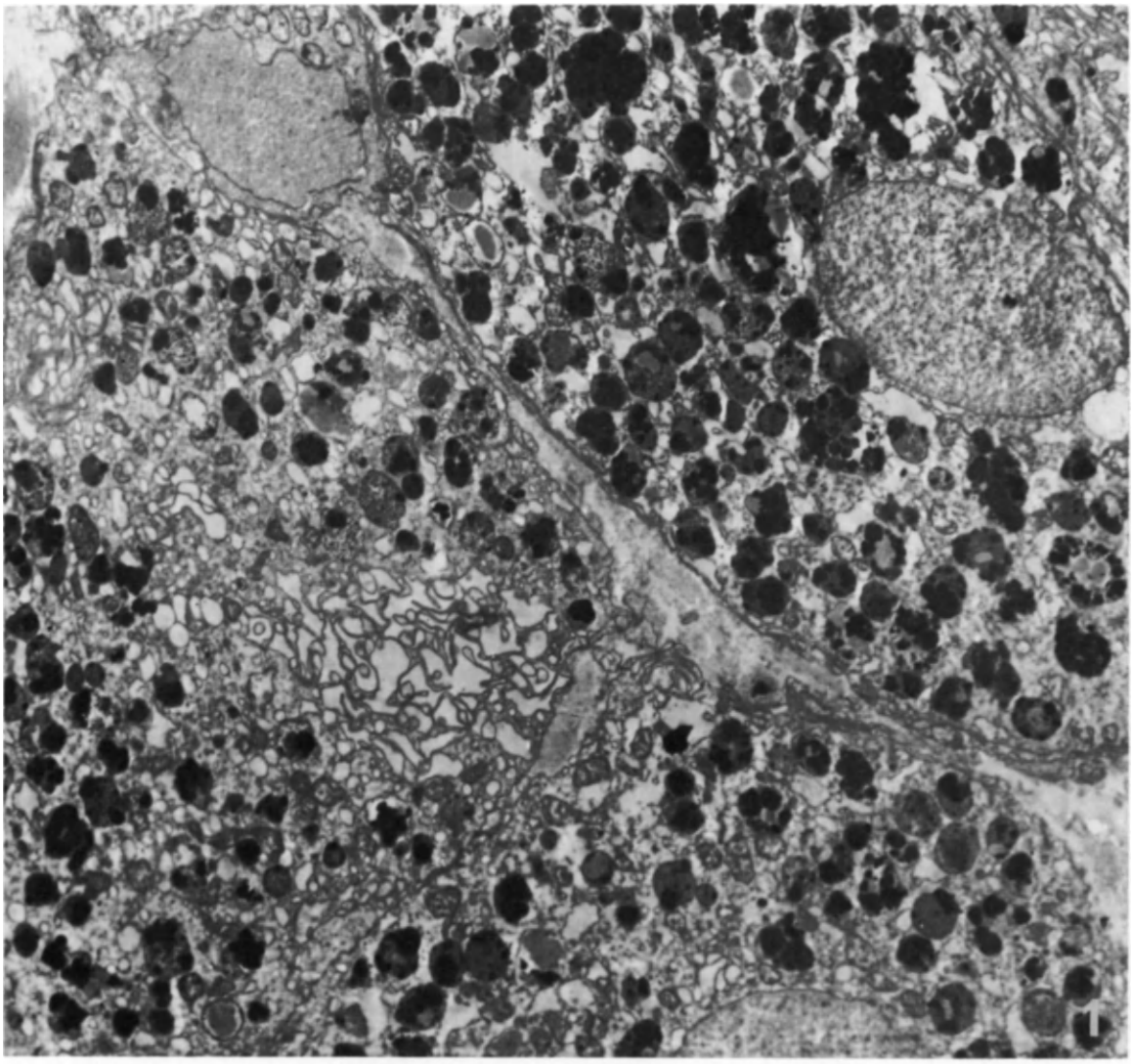
It has already been noted that autolysosomes are commonly produced as a result of organelle injury by many drugs and other noxious influences (page 598). It would therefore appear that melanosis coli is a condition produced by the damaging effect of some constituent in the anthracene group of purgatives, which leads to the formation of autolysosomes and lipofuscin-containing residual bodies. Whether such bodies also contain some pigmented material produced in the gut or present in the purgative, which is phagocytosed by cells, remains a matter of conjecture. Certainly there is as yet no evidence to support such an idea.

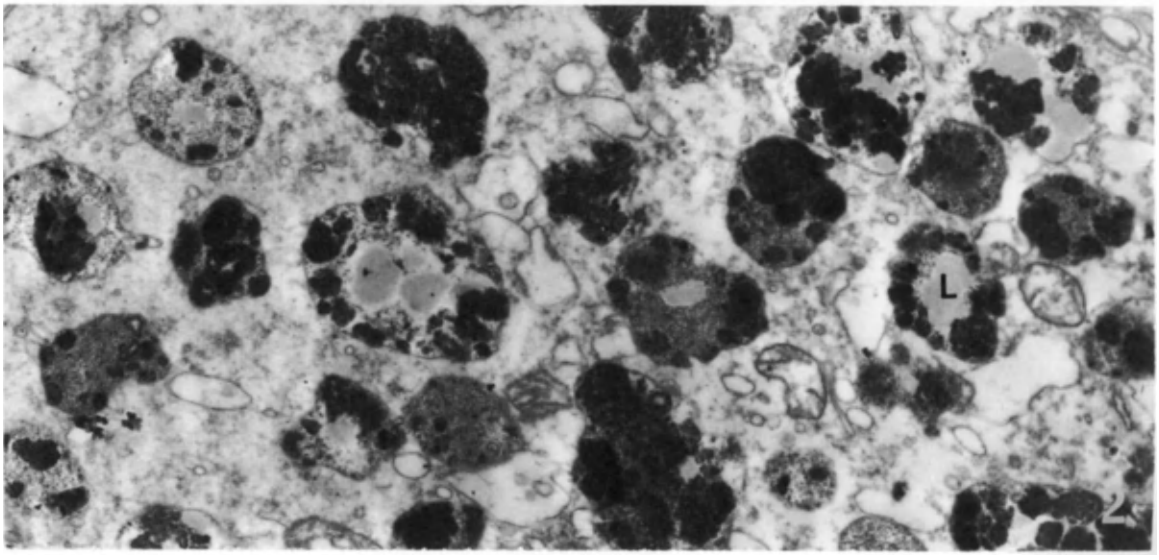
There seem to be no reports on the production of melanosis coli in the common laboratory rodents, except for the reports by Russell *et al.* (1980, 1982) who produced melanosis coli by repeated oral administration of Danthron (1,8-dihydroxyanthraquinone) to guinea pigs. Dark brown coloration of the colon and caecum was seen after 10 days. Pigment was found in the macrophages in the lamina propria. Electron micrographs are not presented but the authors state that ultrastructurally the pigment resembled 'lipofuscin and myelinoid bodies'.

Plate 294

Fig. 1. Typical pigment-bearing macrophages found in the colonic submucosa from a case of melanosis coli. The pigment granules have the morphology of lipofuscin-containing residual bodies. × 5000 (*From Ghadially and Parry, 1966b*)

Fig. 2. Higher-power view of some of the residual bodies shown in *Fig. 1.* Note the presence of electron-dense particles and granules and also the occasional medium-density lipid droplets (L) so characteristic of lipofuscin. Compare with other lipofuscin-containing residual bodies shown in *Plates 261–263.* × 21 000 (*From Ghadially and Parry, 1966b*)




L

It will be noted from the above that ultrastructural studies have now clearly demonstrated that the pigment in melanosis coli is not melanin or melanin-like material but lipofuscin. However, this is by no means a unique situation for there are several other sites and situations where brownish-black pigmentation is probably produced by, or certainly produced by, the accumulation of lipofuscin.

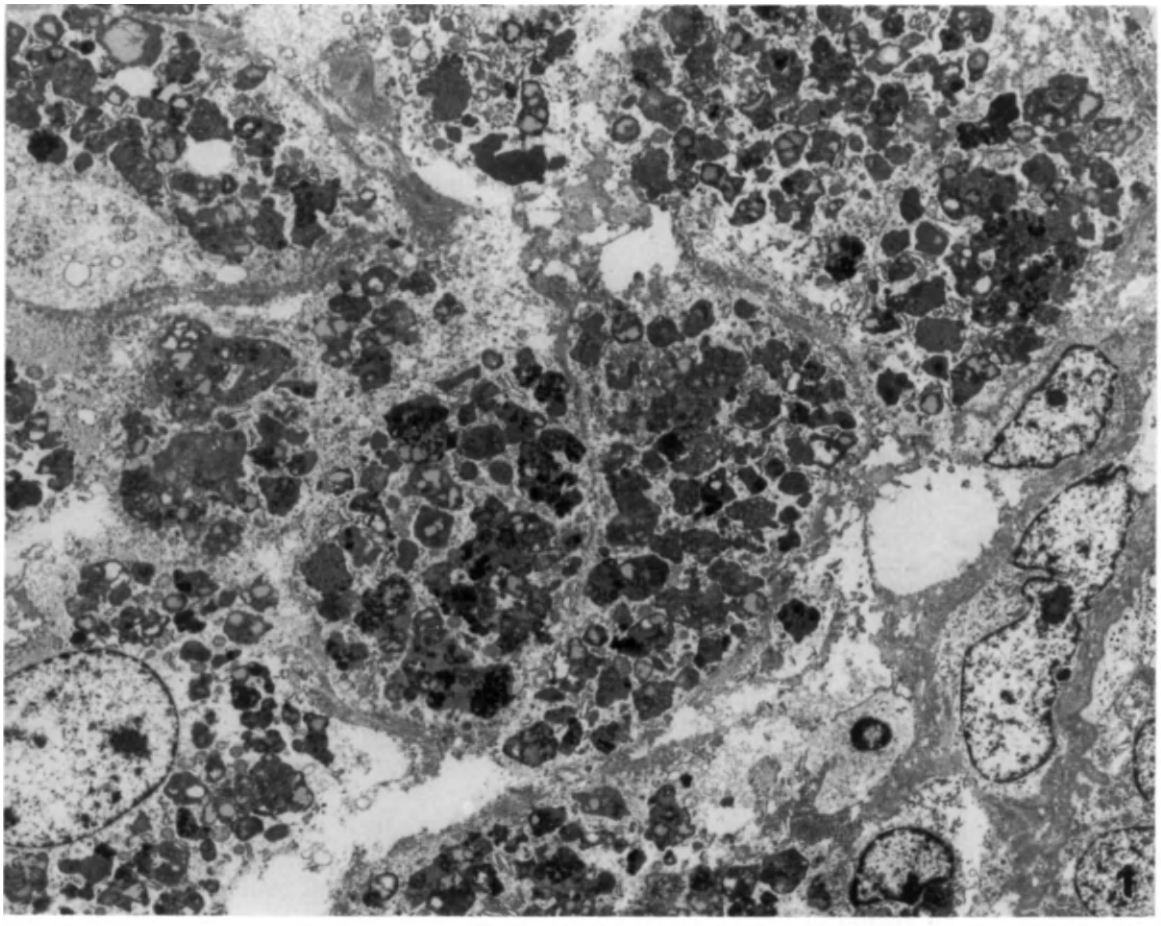
For example, such brownish-black pigmentation has been reported to occur in: (1) the rectum, appendix and mesenteric lymph nodes (often associated with melanosis coli) due to accumulation of lipofuscin in macrophages, although some authors still persist in calling it 'melanin or a closely related compound' (Morson and Dawson, 1972; Hall and Eusebi, 1978; Badiali *et al.*, 1985); (2) a rare case described as 'oesophagitis with melanosis' where the nature of the pigment is not known (Andrejauskas, 1937); (3) the brown bowel syndrome, where the deep brown pigmentation of the bowel and stomach is due to massive deposits of lipofuscin in the smooth muscle cells of the tunica muscularis, but the mucosa appears normal (Foster, 1979; Gallager, 1980; Lambert *et al.*, 1980; Horn *et al.*, 1985); (4) a case of melanosis of the gall bladder where electron microscopy showed that the pigment is lipofuscin and that it lies predominantly in the mucosal epithelial cells. Interestingly enough, this patient consumed large quantities of rhubarb daily, but it is not known whether the patient had melanosis coli (Weedon *et al.*, 1980); (5) a new entity called 'pigmentosis tubae' where large accumulations of lipofuscin-laden macrophages were seen under the epithelium of the fallopian tubes in a patient with carcinoma of the cervix (Herrera *et al.*, 1983) (*Plate 295*). After careful study of possible aetiological agents, the authors conclude that the pigment most likely resulted from cellular damage caused by radiotherapy; and (6) the well known black adenoma of the adrenal where the tumour cells are loaded with lipofuscin granules (Mackay, 1969; Fisher and Danowski, 1973; Garret and Ames, 1973; Bahu *et al.*, 1974; O'Leary *et al.*, 1982).

Plate 295

Pigmentosis tubae (*From a block of tissue supplied by Drs G. A. Herrera and B. E. F. Reimann*)

Fig. 1. Low-power view showing a cluster of pigment-laden macrophages found under the epithelium of the fallopian tube. × 3700

Fig. 2. Higher-power view shows that the pigment granules have the characteristic morphology of lipofuscin granules. Compare with lipofuscin granules shown in *Plates 261–263.* × 16000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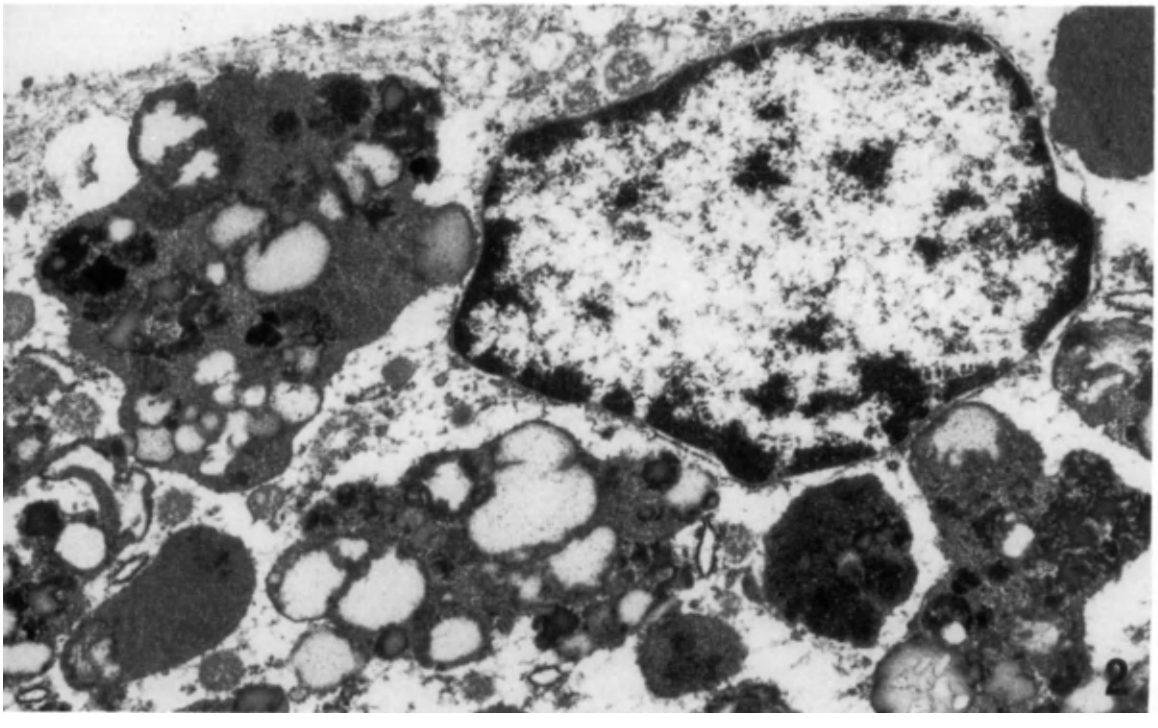

2

Lysosomes in melanosis duodeni

Melanosis duodeni was first described in 1976 by Bisordi and Kleinman. Later four further cases of this condition were described (Breslaw, 1980; Cowen and Humphries, 1980; Ganju *et al.*, 1980; Sharp *et al.*, 1980). On the basis of histochemical studies, in four out of five cases the verdict was that the pigment is melanin or a melanin-like substance, while in one paper (Sharp *et al.*, 1980) we find the odd statement that 'The pigment is similar to that observed in melanosis coli and histochemically does not stain like melanin or lipofuscin'.

In melanosis duodeni, the duodenal mucosa has a speckled black appearance. Histologically the epithelium on the villi appears normal, but the lamina propria (i.e. core of the villi) of some, but not all villi, contains numerous pigment-laden macrophages. In our hands (Pounder *et al.*, 1982), the pigment was positive for the periodic acid Schiff reaction, negative for Perl's Prussian blue reaction and Turnbull blue reaction except that at times the margins of a few pigment granules were Prussian blue positive*, and uninterpretable with the Fontana Masson stain because the pigment was quite dark to begin with.

Our ultrastructural studies (Pounder *et al.*, 1982) showed that the pigment granules in the macrophages bore no resemblance to melanin, lipofuscin or anything else that we had seen before (*Plate 296*). They presented as single-membrane-bound, presumably lysosomal bodies, containing rounded and angular masses of high electron-density. Our electron-probe x-ray analytical studies (including assessment of atomic ratios of elements present) showed that the electron-dense material in these granules is essentially ferrous sulphide (FeS). We also found traces of calcium and potassium. Calcium has been found in company with other metallic deposits in lysosomes, for example gold (Ghadially *et al.*, 1976) and uranium (Ghadially *et al.*, 1982a, b). However, the significance of this phenomenon is obscure. Traces of aluminium, magnesium and silicon were also detected in the pigment granules. This we attribute to antacid medications (e.g. aluminium hydroxide, magnesium trisilicate, hydrated magnesium aluminate). A surprising finding was the occurrence of minute traces of silver in the pigment granules. We wonder whether this came from dental fillings in this patient.

A review of the histories of cases of melanosis duodeni shows that this condition is associated with bleeding in the GI tract from peptic ulcer or from some other cause. Therefore one may surmise that the iron in the iron sulphide deposits derives from the shed blood. The sulphur presumably derives from ingested foods and/or drugs. However, we were unable to elicit any unusual dietary or drug history. In any case it is difficult to understand why iron occurs as a sulphide in the lysosomes of the macrophages in melanosis duodeni, while common haemosiderin deposits in the siderosomes are composed entirely or almost entirely of iron hydroxide oxide and only occasionally are traces of sulphur found.

*We believe this is due to autoxidation of FeS at the periphery of the pigment granules. This is in keeping with our observation that sticks of FeS are Prussian blue and Turnbull blue negative, but the rusty coat which develops on their surface is Prussian blue positive.

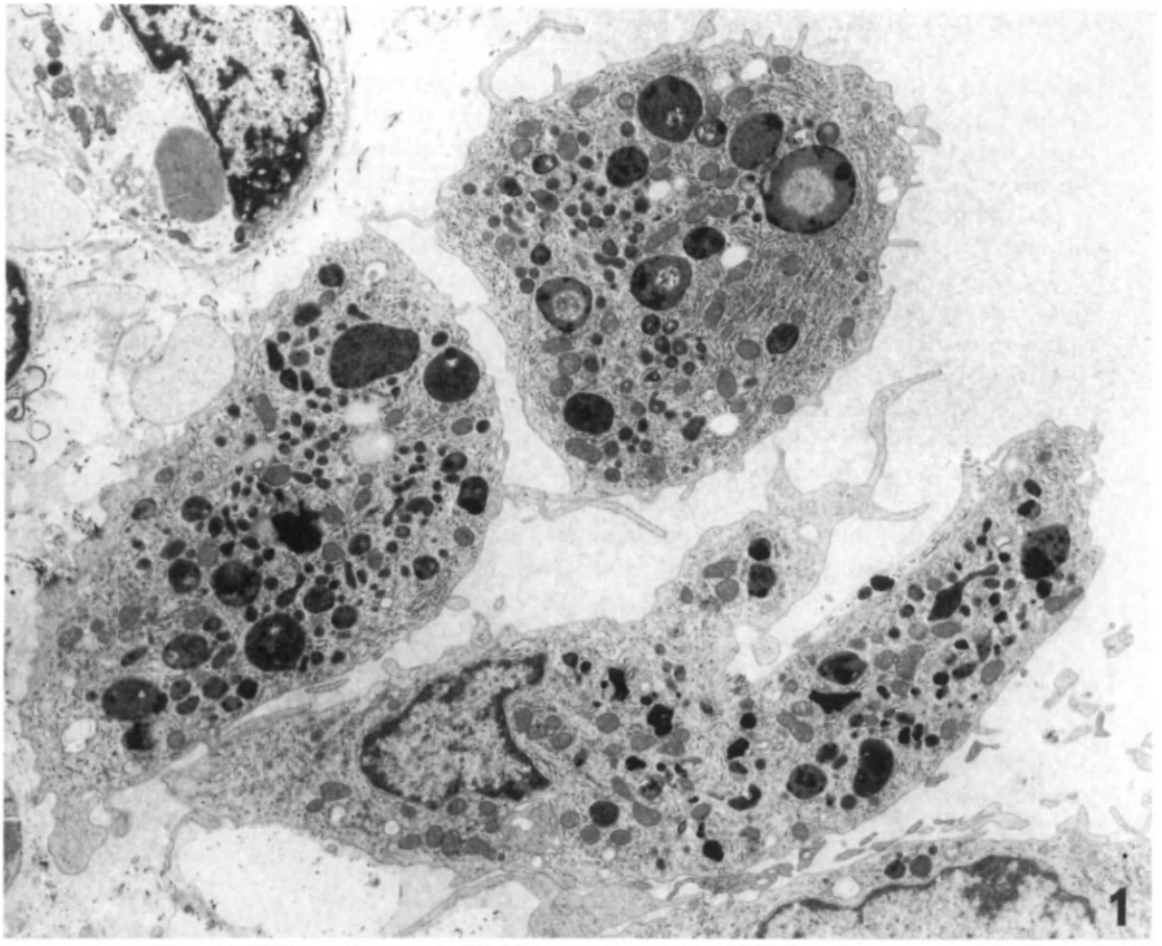
Plate 296

Duodenum from a case of melanosis duodeni (*From Pounder, Ghadially, Mukherjee, Hecker, Rowland, Dixon and Lalonde, 1982*)

Fig. 1. A group of macrophages found in the core of a villus. The cells contain numerous pleomorphic electron-dense granules. × 6900

Fig. 2. Pigment granules in a macrophage. Note the irregular and angular profiles (arrows) of these electron-dense granules. They are acceptable as angulate lysosomes (*see* page 706). Lipid droplets (arrowheads) and electron-dense particles are present in some of these granules. × 15 000

Fig. 3. Higher-power view of a pigment granule. It is evident that this is a single-membrane-bound (arrows) lysosomal structure containing angulated and rounded electron-dense bodies, and smaller electron-dense granules and particles. Note also the lipid droplets (arrowheads) peppered with electron-dense particles. × 30 000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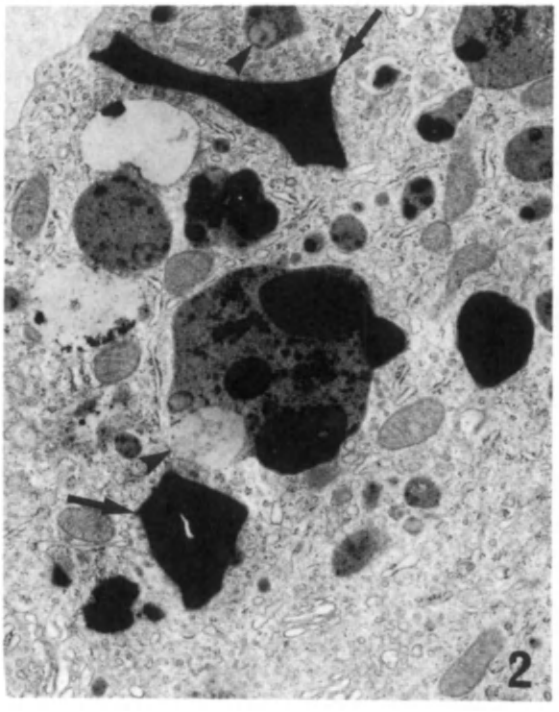
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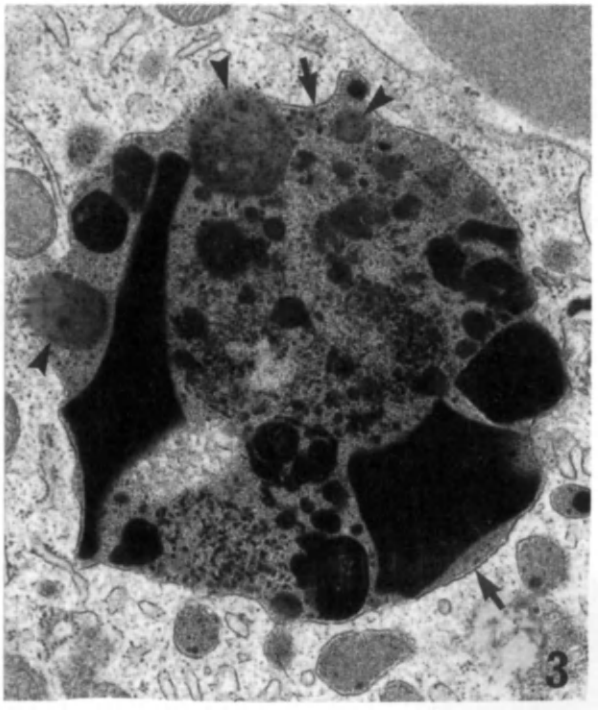
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Lysosomes in malakoplakia

Malakoplakia (Greek, soft plaque) is a rare granulomatous lesion characterized by the accumulation of macrophages (histiocytes) called 'von Hansemann's macrophages' or 'von Hansemann's cells'. These cells contain diastase resistant, PAS positive inclusions and calcified bodies or spherules called 'Michaelis–Gutmann bodies'.

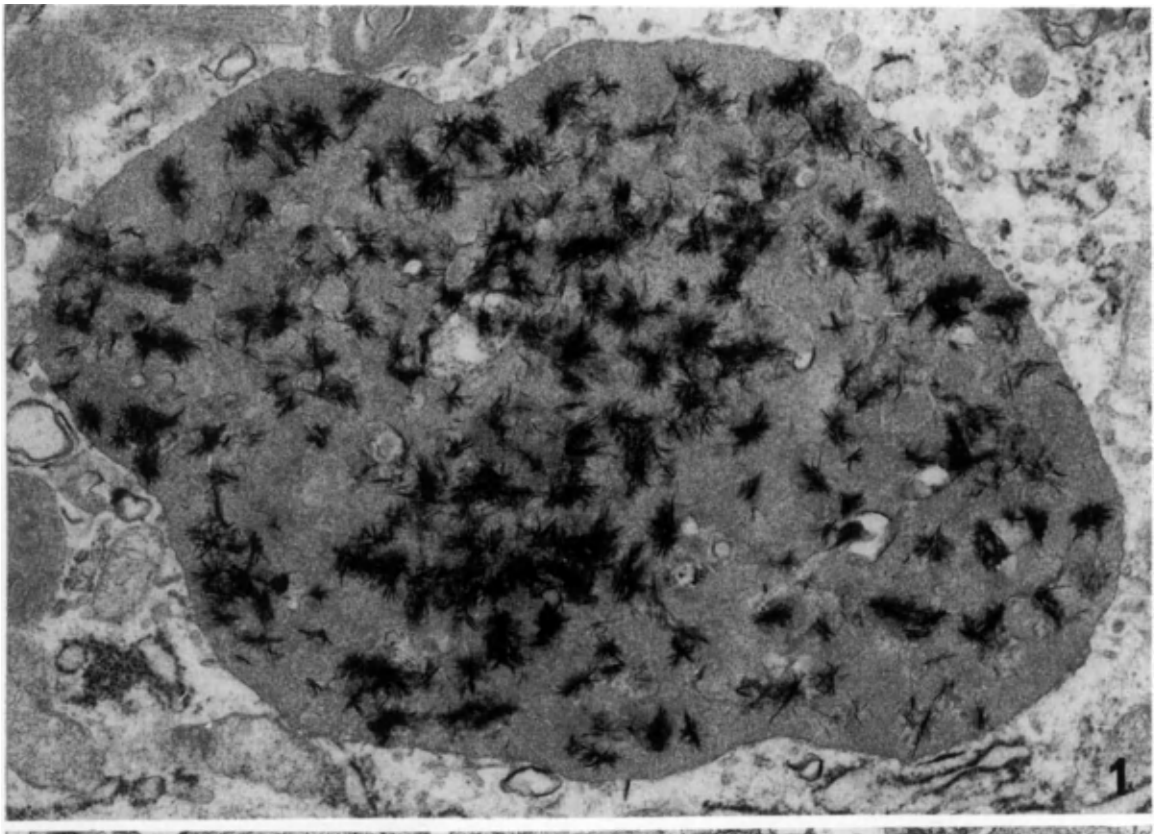
Malakoplakia has been recorded to occur in: (1) the urinary tract (principally in the urinary bladder) (Bleisch and Konikov, 1952; Yunis *et al.*, 1967; Csapó *et al.*, 1973; LeCharpentier *et al.*, 1973; Thorning and Vracko, 1975; Damjanov and Katz, 1981; Moller and Gerdes, 1981); (2) female genital tract (Chalvardjian *et al.*, 1980; Willén *et al.*, 1983; Paquin *et al.*, 1986); (3) testis, epididymis and prostate (Yang and Marathe, 1969; Rhodes and Wittmann, 1977; Rinaudo *et al.*, 1977; Rubenstein and Bucy, 1977); (4) gastrointestinal tract (Yunis *et al.*, 1967; Ranchod and Kahn, 1972; Lou and Teplitz, 1974; Miranda *et al.*, 1979); (5) retroperitoneum (Povýšil, 1974); (6) conjunctiva (Destombes *et al.*, 1975); (7) skin (Price *et al.*, 1973; Sencer *et al.*, 1979); (8) middle ear and mastoid cavity (Azadeh and Ardehali, 1983); (9) lymph nodes (Gonzáles-Angulo *et al.*, 1965; Yunis *et al.*, 1967); (10) brain (Chandra and Kapur, 1979; Chang *et al.*, 1980); (11) adrenal (Sinclair-Smith *et al.*, 1975; Ghadially, unpublished); (12) colon and supraclavicular region of one patient (Chaudhry *et al.*, 1980); and (13) urinary bladder, lungs and bones of one patient (Gupta *et al.*, 1972).

Ultrastructural studies have shown that the Michaelis–Gutmann bodies, which are considered to be indispensible for the diagnosis of malakoplakia, are in fact modified lysosomes. These bodies are von Kossa positive and calcium and phosphorus (probably $CaHPO_4$) have been demonstrated in them by electron-probe x-ray analysis (Kuthy and Ormos, 1978). Histochemical studies show that at times they also contain some iron. The calcium deposits in these lysosomes (i.e. Michaelis–Gutmann bodies) may present as amorphous or needle-shaped crystalline (calcium apatite) electron-dense structures set in a medium-density organic matrix (*Plate 297, Fig. 1*). They may also present as calcified spherules

Plate 297

Fig. 1. Malakoplakia of kidney. A Michaelis–Gutmann body containing focal deposits (sometimes called 'hedgehogs') of needle-shaped calcium apatite crystals. × 26000 (*From a block of tissue supplied by Drs J. H. Martin and P. B. Marcus*)

Fig. 2. Malakoplakia of adrenal gland. A Michaelis–Gutmann body presenting as a multilaminated calcified spherule. × 14500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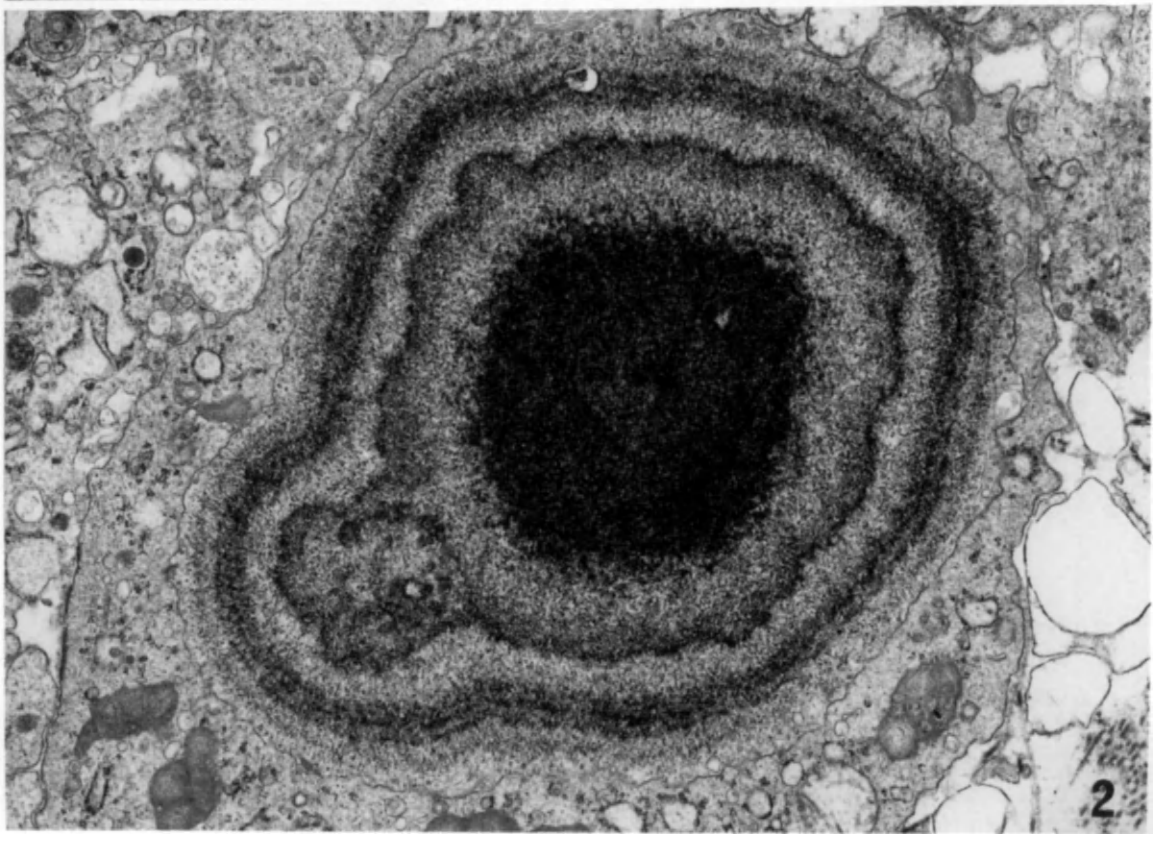
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of various morphologies including multilaminated (*Plate 297, Fig. 2*) or targetoid forms, or a unilaminated annular structure (*Plate 298*).

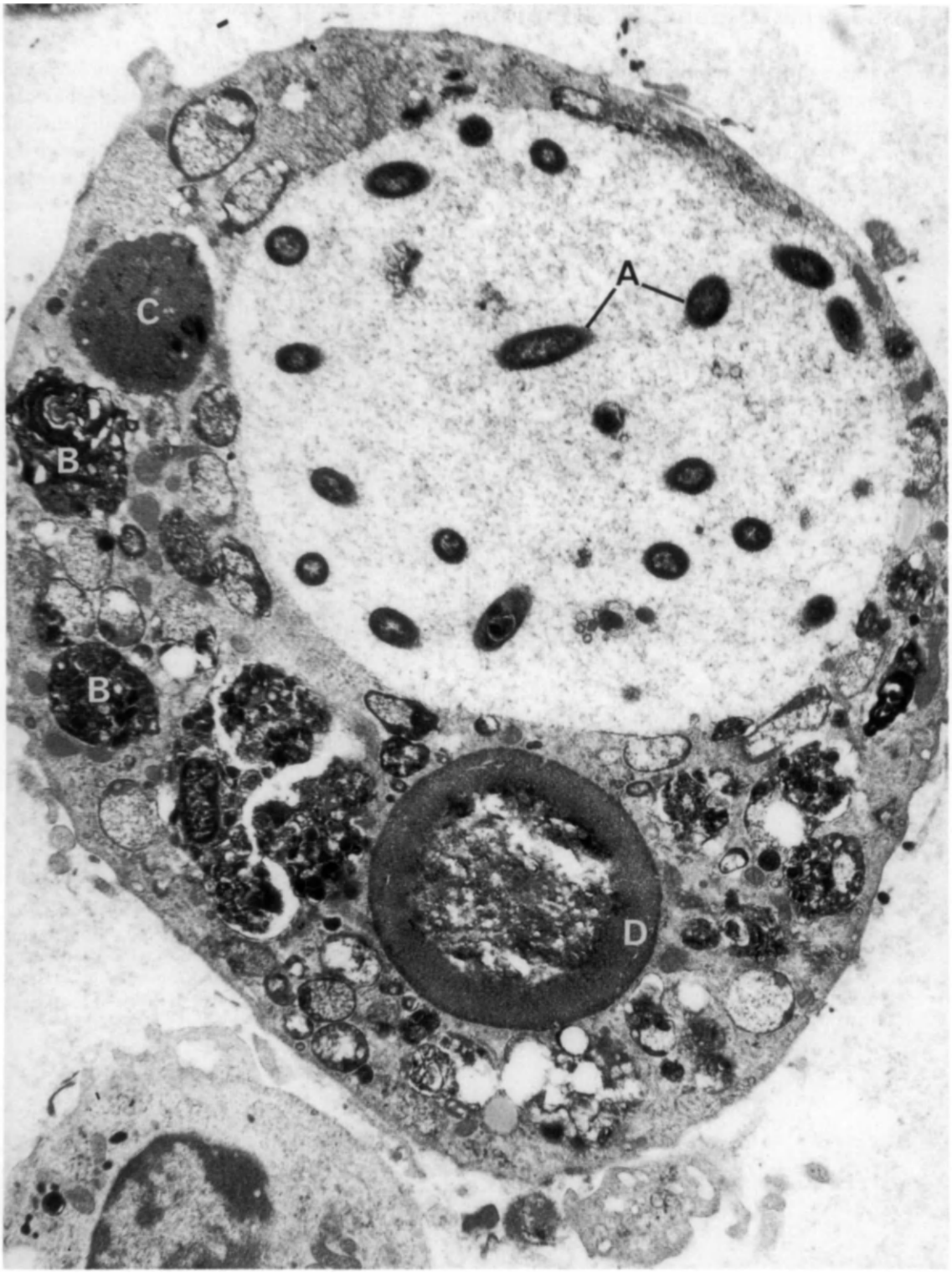
Some of the details of the pathogenesis of malakoplakia are not clearly established, but it would now appear that in a majority of instances this is an atypical granulomatous response to bacteria. In about 75 per cent of cases *E. coli*★ have been found in the urine of patients with malakoplakia of the urinary tract. Bacteria are also found in vacuoles (i.e. heterophagosomes) in von Hansemann's cells. It is thought that the bacteria are not adequately digested in the heterolysosomes that develop when primary lysosomes fuse with the heterophagosomes. It is postulated that there may be a defect in the lysosome and that calcification of the partially digested residues in the lysosome leads to the formation of the Michaelis–Gutmann body.

The exact nature of the acquired lysosomal defect is not clear, but one may suggest that: (1) pH in lysosomes may be too high for acid hydrolases to operate but ideal for calcium deposition; and/or (2) there may be an overwhelming of the lysosomal apparatus by bacteria and other substrates, and in time the necrotic debris in the lysosome calcifies (as does necrotic debris elsewhere. *See* pages 1278–1289).

★The role of *E. coli* in the pathogenesis of malakoplakia is supported by the fact that injection of crude endotoxin-antigen complex of *E. coli* into the kidney and testis of normal rats produces malakoplakia in the organs (Csapó *et al.*, 1975). However, *E. coli* is not the only organism associated with malakoplakia. For example, staphylococci have been found in malakoplakia of the skin.

Plate 298

Malakoplakia of the vagina of an 84-year-old woman. The von Hansemann's cell shown in this illustration is somewhat remarkable in that it shows just about everything needed to support the ideas expressed in the text. Thus we see: a phagocytic vacuole containing bacteria (A); a heterolysosome containing partially digested bacteria (B); a Michaelis–Gutmann body containing amorphous calcium deposit in a lysosome (C); and another Michaelis–Gutmann body containing a calcified structure which presents a unilaminated or annular profile (D). × 9600 (*From Chalvardjian, Picard, Shaw, Davey and Cairns, 1980*)


A
B
B
C
D

Lysosomes in granular cell tumours

Large eosinophilic granular cells occurring in normal and neoplastic tissues have long been the subject of interest and speculation amongst histologists and pathologists. By and large they can be divided into two main groups: (1) those where the granularity is due to an abundance of mitochondria (oncocytes and oncocytomas); and (2) those where the granularity is due to an abundance of lysosomes*. Oncocytes and oncocytomas have already been dealt with (pages 260-265), in this section of the text we shall consider only those tumours (*Plates 299–301*) where cytoplasmic granularity stems from an unusually large number of lysosomes.

Such tumours include: (1) granular cell myoblastoma (*Plate 299*) (Fisher and Wechsler, 1962; Moscovic and Azar, 1967; Aparicio and Lumsden, 1969; Garancis *et al.*, 1970; Sobel *et al.*, 1971, 1973); (2) granular cell ameloblastoma† (*Plate 300*) (Navarrete and Smith, 1971; Mincer and McGinnis, 1972; Brocheriou *et al.*, 1975; Tandler and Rossi, 1977; Geisinger *et al.*, 1985; Mori *et al.*, 1985); (3) congenital epulis, also known as granular cell tumour of the gingiva of infants (Kay *et al.*, 1971; Kameyama *et al.*, 1983; Lifshitz *et al.*, 1984); (4) ameloblastic fibroma (White *et al.*, 1977; Takeda, 1986); (5) three cases of oligodendroglioma (out of ten cases studied) described by Takei *et al.* (1976); (6) a rare granular cell variant of basal cell carcinoma (Barr and Graham, 1979); (7) a unique ultrastructural variant of Wilm's tumour (Kurtz, 1979); (8) a unique case of granular cell angiosarcoma (McWilliam and Harris, 1985); (9) transplantable granular cell tumour of the uterine cervix of the oestrogen-treated mouse; and (10) granular cell tumour of leptomeninges in the aged rat (Aiuchi *et al.*, 1986).

Granular cell myoblastoma (*Plate 299* and *Plate 301, Fig. 1*) is characterized by cells which contain large numbers of lysosomes in their cytoplasm. The nature and origin of these cells and the lysosomes they contain have been subjects of much past dispute.

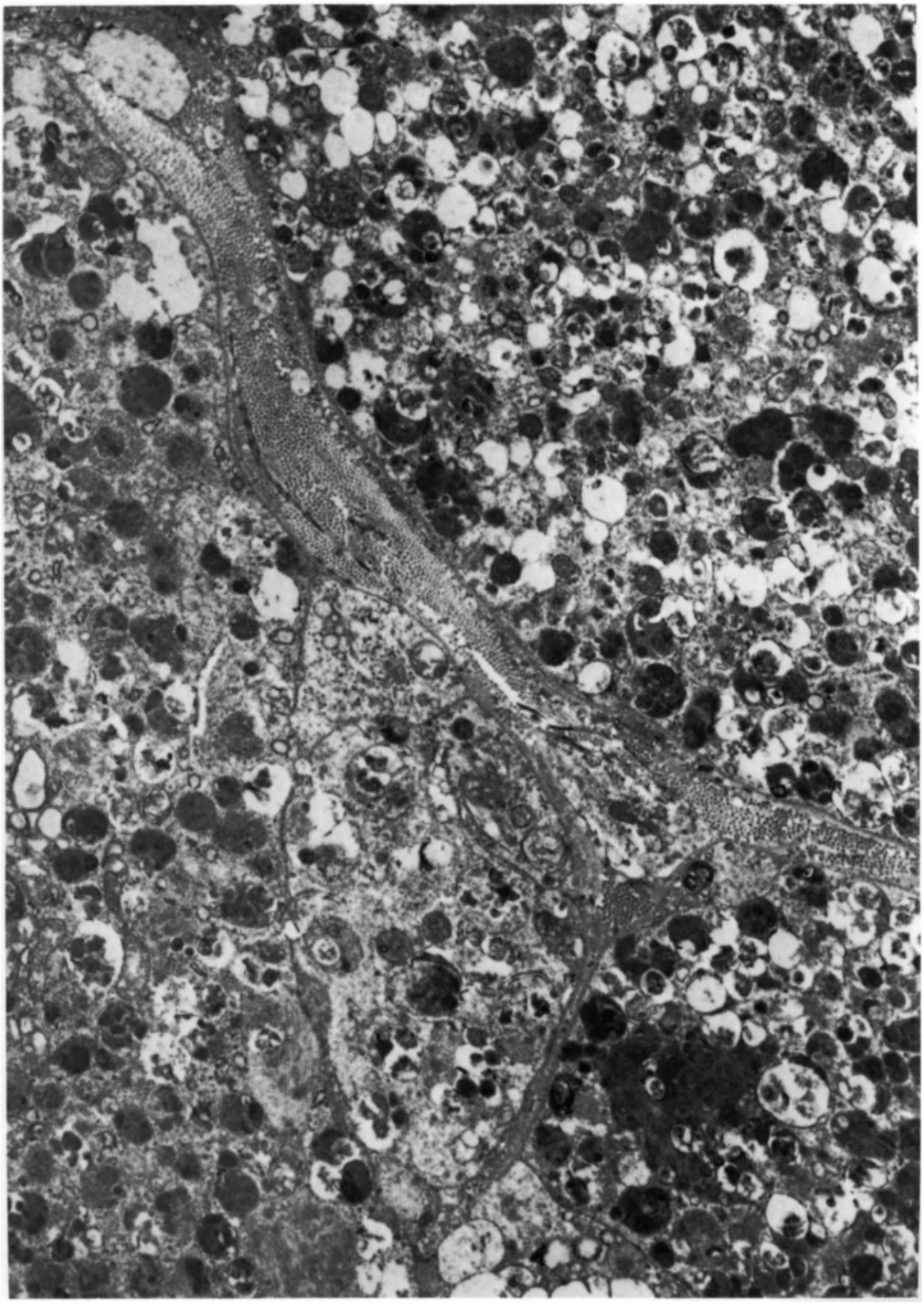
Various previous suggestions regarding the histogenesis of this lesion include: (1) a neoplastic or non-neoplastic derivation from adult or embryonic muscle tissue (Abrikossoff, 1926; Klemperer, 1934; Gray and Gruenfeld, 1937); (2) a non-neoplastic accumulation of histiocytic cells (Leroux and Delarue, 1939); (3) a granuloma probably due to parasitic infestation (Gullino, 1946); (4) a histocytic storage phenomenon (Lauche, 1944); (5) a lipoid thesaurus: stored material probably myelin (Azzopardi, 1956); (6) neural origin (Fust and Custer, 1949; Bangle, 1952); (7) a degeneration of perineurial and/or endoneurial fibroblasts (Pearse, 1950); (8) derivation from Schwann cells or undifferentiated mesenchymal cells (for references *see* Sobel and Marquet, 1974).

*Cytoplasmic granularity in tumours may be due to various other reasons such as: (1) abundant mitochondria (oncocytomas); (2) microcrystals and a fair number of mitochondria in alveolar soft part sarcoma; (3) abundant smooth endoplasmic reticulum occurring focally or clumped due to preparative procedures; and (4) abundant secretory granules.

†Only about 5 per cent of ameloblastomas contain granular cells and only about 1 per cent of all odontogenic tumours are ameloblastomas, so the granular cell ameloblastoma is a rather rare tumour.

Plate 299

Granular cell myoblastoma of the orbit. The cytoplasm of the tumour cell is packed with innumerable lysosomes. × 9000 (*From Ghadially, 1980a*) (Block of tissue supplied by Dr A. H. Cameron)



Most of the views mentioned above (i.e. items 1–7) are of historic interest only. There is now overwhelming evidence that granular cell myoblastoma (but not all granular cell tumours, *see below*) is a variety of schwannoma and one may suggest that this tumour should now be called 'granular cell schwannoma'. Support for this concept comes from: (1) the well known close association of these tumours with nerves; (2) histochemical studies showing products of myelin degeneration in the tumour cells (Bangle, 1952); (3) immunohistochemical studies showing myelin basic protein (Penneys *et al.*, 1983); and S-100 protein in tumour cells (for references *see* Willén *et al.*, 1984 and Smolle *et al.*, 1985); (4) ultrastructural studies (*see below*); and (5) the reported (Finkel and Lane, 1982) occurrence of typical granular cells in neurofibromas from a case of neurofibromatosis. A malignant granular cell tumour metastasizing into lymph nodes was also present in this patient.

The neoplastic nature of granular cell myoblastoma is attested by the rare but well documented occurrence of malignant versions of this tumour (for references *see* Cadotte, 1974; Kindblom and Olsson, 1981; Shimamura *et al.*, 1984) and the transplantable granular cell tumour of the uterine cervix of the oestrogen-treated mouse (Dunn and Green, 1963, 1965; Mazzarella and DeBenedictis, 1967).

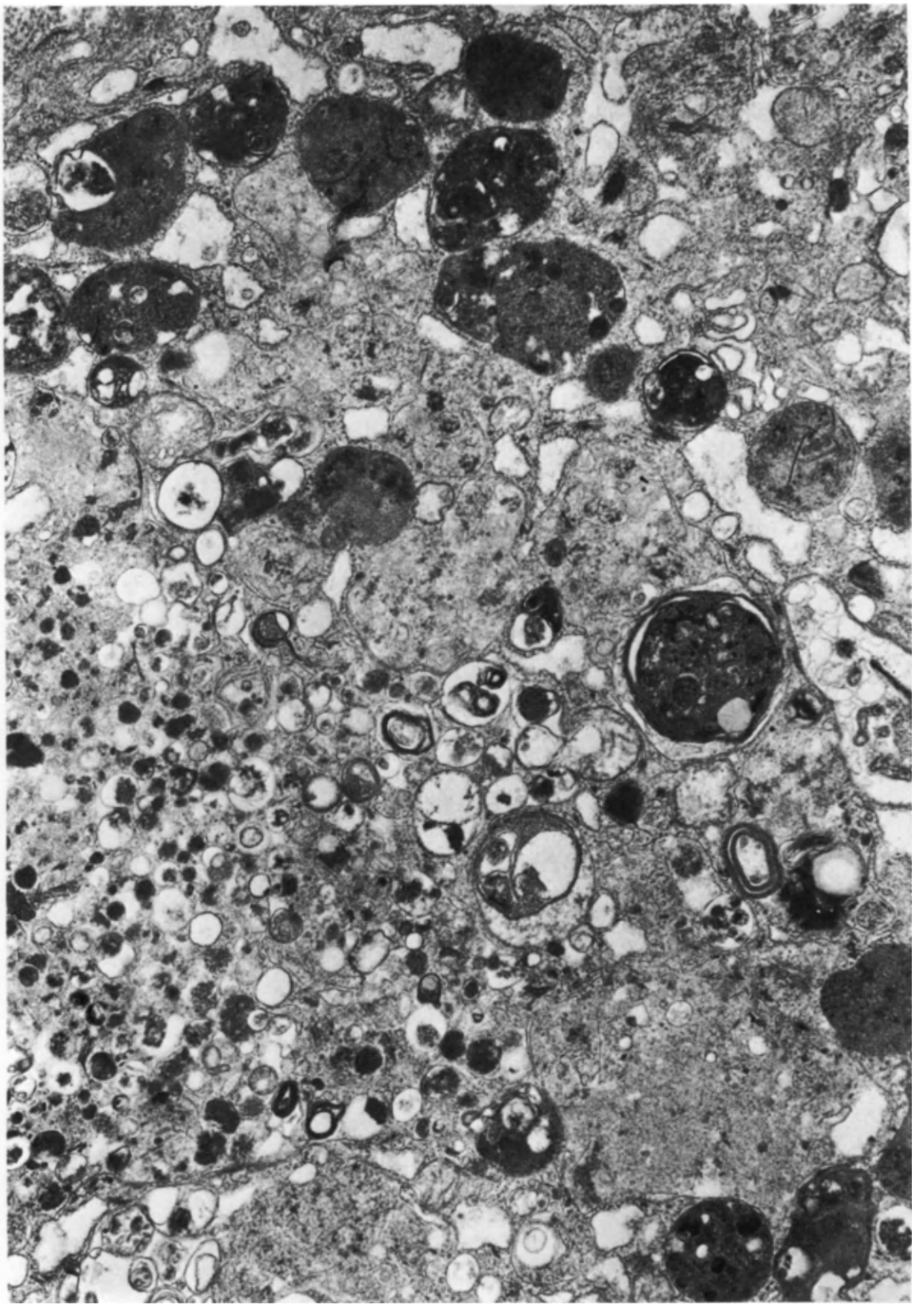
Numerous ultrastructural studies on granular cell myoblastoma have been published (Moscovic and Azar, 1967; Aparicio and Lumsden, 1969; Garancis *et al.*, 1970; Sobel *et al.*, 1971, 1973; Fisher and Wechsler, 1972; Weiser, 1978; Chomette *et al.*, 1980; Bedetti *et al.*, 1983; Seo *et al.*, 1984; Willén *et al.*, 1984). These studies have shown that there are quite a few ultrastructural similarities between schwannomas and granular cell myoblastomas. For example: (1) a few or not so few lysosomes (probably containing altered myelin) are seen in schwannomas (*see* illustration in Ghadially, 1985) but many more occur in granular cell myoblastomas; (2) a prominent or reduplicated external lamina or basal lamina is a feature of schwannomas and granular cell myoblastomas (*see* illustration in Ghadially, 1985); (3) Luse bodies (fibrous long-spacing collagen) are of frequent occurrence in the stroma of schwannomas and they have also been seen at times in granular cell myoblastoma; and (4) angulate bodies or angulate lysosomes (pages 698–707) have been found in both granular cell myoblastomas and schwannomas*.

Let us now probe the significance of the massive accumulation of lysosomes seen in granular cell tumours. We will first deal with granular cell myoblastoma and then with other granular cell tumours. There is no reason to doubt that the single-membrane-bound bodies with heterogeneous electron-dense contents seen in the granular cell tumours are in fact lysosomes. Lysosomal enzymes such as acid phosphatase and esterase have been demonstrated in the granular cells of granular cell myoblastoma at the light microscopic level (Fisher and Wechsler, 1962; Sobel, 1969), but I have not found a report where this has been demonstrated at the ultrastructural level.

*In both these tumours angulate lysosomes occur in the histiocytes within these tumours and not in the tumour cells themselves.

Plate 300

Granular cell ameloblastoma. Note the abundant lysosomes in the tumour cells. × 10 500 (*Tandler, unpublished electron micrograph*)



There has been much debate in the literature as to whether the lysosomes in granular cell myoblastoma are autolysosomes or heterolysosomes. The virtual absence of identifiable disintegrating cytoplasmic structures (such as mitochondria or rough endoplasmic reticulum) within these lysosomes argues against the idea that they are autolysosomes. To say they are heterolysosomes raises the question: what have these cells been endocytosing? If we accept that the cells in these tumours are modified Schwann cells then the most likely material within the lysosome would be myelin. Since myelin is modified Schwann cell membrane, one might conclude that these lysosomes are probably autolysosomes, however, some myelin could also be endocytosed myelin produced by another Schwann cell, in which case the lysosomes would be heterolysosomes.

Be that as it may, the electron-dense lipidic material and the membranous structures seen in the lysosomes could easily be derived from myelin and the granular material seen in some of the lysosomes could be looked upon as a stage of disintegration leading ultimately to lysosomal forms resembling lipofuscin granules (i.e. residual bodies). Such notions are supported by the fact that lysosomes similar to the ones seen in granular cell myoblastoma are seen in Schwann cells phagocytosing myelin in injured nerves. The question now arises as to why such large numbers of lysosomes accumulate in these Schwann cells to turn them into granular cells? Two possibilities exist: (1) that the defect engendered by the neoplastic process is a paucity of a certain lysosomal enzyme or enzymes, so that myelin is not digested and accumulates in the lysosomes; or (2) that the neoplastic cells synthesize excessive amounts of myelin sheath material, and that this is sequestrated in lysosomes where it is not readily or completely digested because of an overwhelming of the lysosomal apparatus with more material than it can handle.

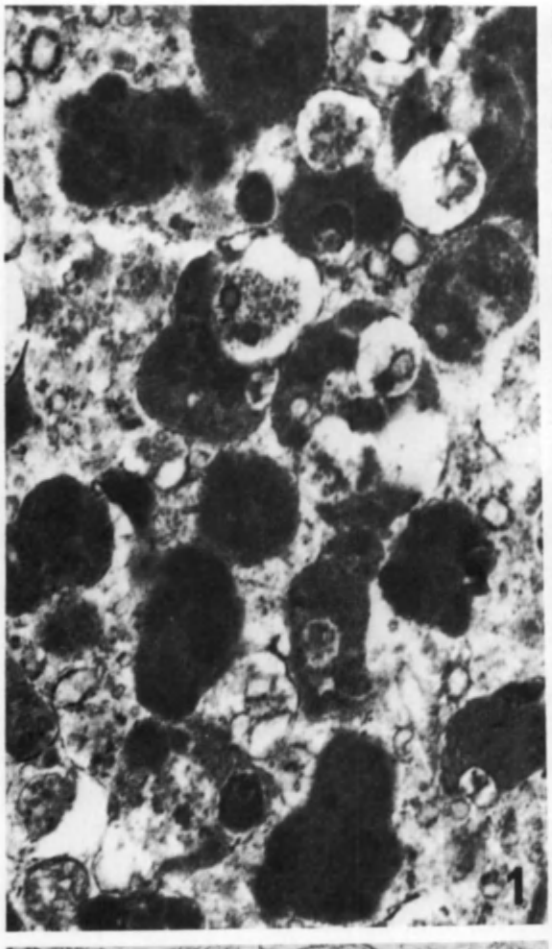
From the above it will be evident that granular cell myoblastoma is of schwannocytic lineage and deserves to be called a granular cell schwannoma. However, there is no reason to believe that all lysosome-containing granular cell tumours are of Schwann cell lineage. Indeed, the current view based on ultrastructural and immunohistochemical studies is that the granular cells in ameloblastic fibroma and granular cell epulis derive from mesenchymal cells, probably fibroblasts (Kameyama *et al.*, 1983; Takeda, 1986), while the granular cells seen in some ameloblastomas and basal cell carcinomas (Barr and Graham, 1979) are probably of epithelial origin (Slootweg *et al.*, 1983). The histogenic diversity of granular cell tumours (where the granularity is due to the presence of numerous lysosomes) is underlined by a subcutaneous granular cell tumour of presumed histiocytic origin (positive for alpha-l-antichymotrypsin but negative for neuron specific enolase and S 100 protein) described by Nathrath and Remberger (1986), and the interesting angiosarcoma reported by McWilliam and Harris (1985) where the neoplastic endothelial cells in about 60 per cent of the tumour mass were typical granular cells loaded with secondary lysosomes. I am in agreement with McWilliam and Harris (1985) who suggest that the common so-called 'granular cell myoblastoma' should be called 'granular cell schwannoma' and that other granular cell tumours should be recognized and named by histogenetically specific labels where possible.

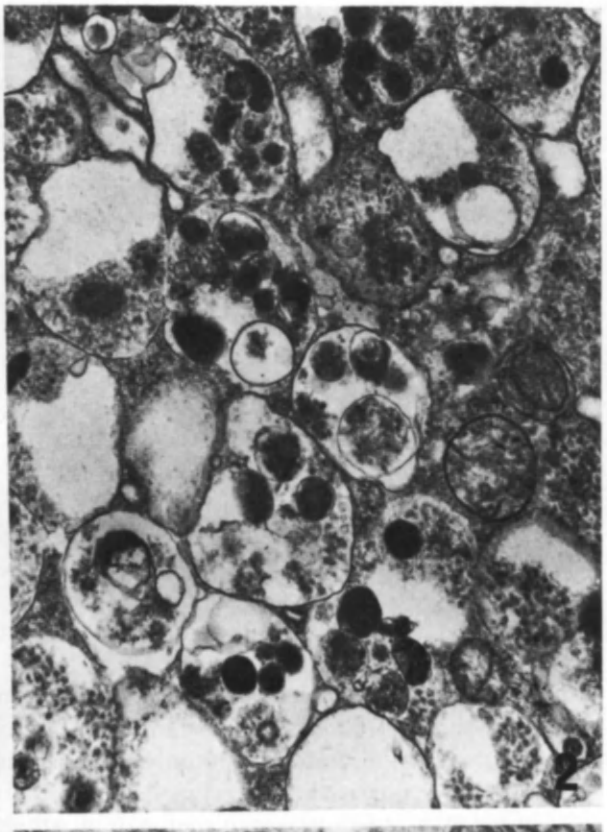
Plate 301

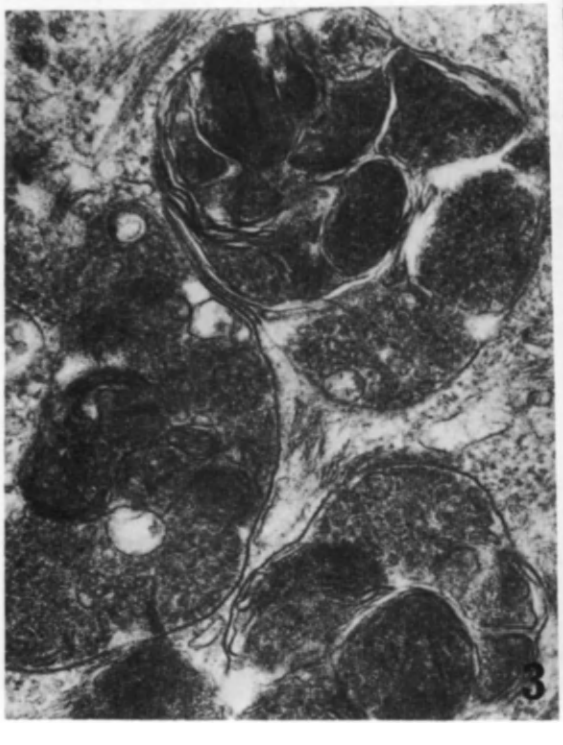
Fig. 1. Granular cell myoblastoma. Same case as *Plate 299*. Note that most of the lysosomes resemble residual bodies containing lipofuscin, except that the characteristic lipid droplet is missing. × 64 000

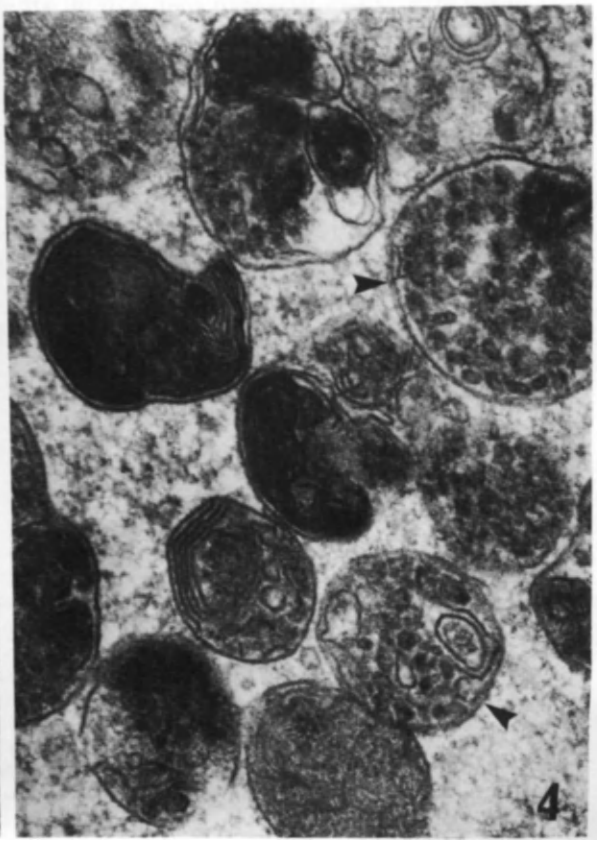
Figs 2 and 3. Granular cell ameloblastoma. Same case as *Plate 300*. The lysosomes contain electron-dense particles and granules and membranous material (*From Tandler and Rossi, 1977*) × 15 500

Fig. 4. Granular cell ameloblastoma. Same case as *Plate 300*. Some of the lysosomes are acceptable as multivesicular bodies (arrowheads). × 45 000 (*Electron micrograph supplied by Dr B. Tandler*)









Lysosomes in rheumatoid arthritis

Numerous studies attest to the fact that lysosomes and their enzymes play an important role in inflammatory processes (de Duve and Wattiaux, 1966; Weissmann, 1966, 1967; Houck, 1968; Fell, 1969). It would appear that some of the morphological changes seen in chronic inflammation are due to the release of hydrolytic enzymes from lysosomes that develop in the cells of the inflamed tissues and/or from the neutrophils and macrophages which invade the tissue. In order to illustrate this point the following discussion will be limited to one well known and much studied example of chronic inflammation, namely rheumatoid arthritis. A striking increase in the number of lysosomes (*Plates 253, 302, 303* and *536*) has been observed in the synovial membrane in rheumatoid arthritis by many workers (Barland *et al.*, 1964; Wyllie *et al.*, 1966; Norton and Ziff, 1966; Ghadially and Roy, 1967a, 1969), but it must be noted that this change is not specific and that modest increases in lysosomes are common enough in many pathological synovial membranes.

The manner in which the initial increase in lysosomes occurs is not clear. One theory is that the lysosomes are derived from phagocytosis of the interaction product of γ-globulin and the rheumatoid factor (Hollander *et al.*, 1965; Robinson, 1966). On the other hand, Hamerman (1966) has suggested that phagocytosis of bacteria or cell fragments may be the exciting factor. It is also possible that the noxious aetiological agent, whatever it might be, primarily damages cell organelles and leads to the formation of an excessive number of autolysosomes. Our morphological studies reveal that fibrin, cell fragments and erythrocytes are avidly phagocytosed by synovial cells (*Plate 302*). At times electron-dense iron-containing particles are also found in these lysosomes and cytoplasmic matrix. Such particles have been regarded as ferritin by other workers (Muirden, 1966; Muirden and Senator, 1968). Acid phosphatase has been demonstrated in the lysosomes of synovial cells, and also of leucocytes occurring in the synovial fluid from cases of rheumatoid arthritis (*Plate 253*) (Barland *et al.*, 1964; Coimbra and Lopes-Vaz, 1967).

Results of biochemical studies are in keeping with morphological observations, for an increase in the amount of lysosomal enzymes has been demonstrated in the synovial membrane (Luscombe, 1963; Hendry and Carr, 1963) and in the synovial fluid (Jacox and Feldmahn, 1955; Lehman *et al.*, 1964; Caygill and Pitkeathly, 1966). The bulk of the hydrolytic enzymes are probably derived from the synovial cell lysosome and not from the lysosomes of polymorphonuclear leucocytes, as there is no concurrent increase in the amount of alkaline phosphatase (Lehman *et al.*, 1964), an enzyme found in the secondary granules of this leucocyte.

Thus biochemical findings clearly show that hydrolytic enzymes are liberated from lysosomes, and many theories have been proposed to explain this phenomenon. Regarding the rupture of lysosomes in other situations, de Duve (1963) has suggested that cellular over-feeding, especially with non-consumable diets, may be responsible and he has also

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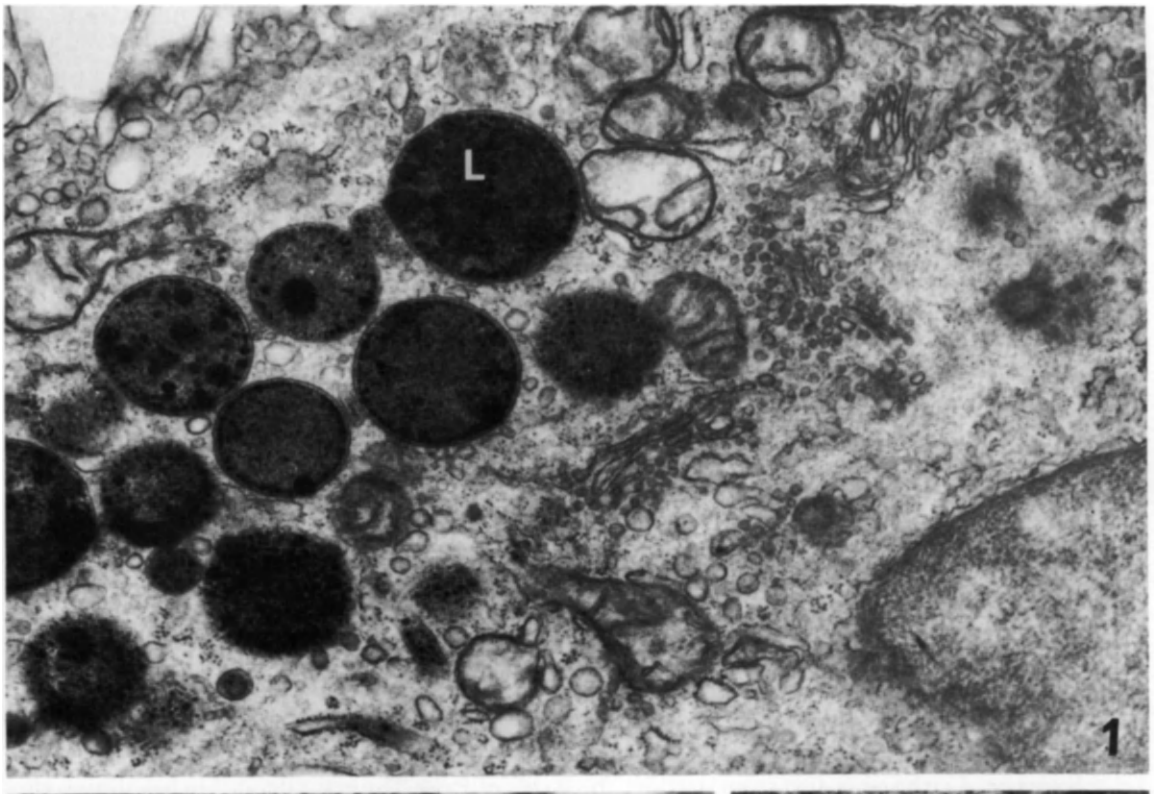
Plate 302

Synovial intimal cells from cases of rheumatoid arthritis. (*From Ghadially and Roy, 1967a*)

Fig. 1. Type A synovial cell containing numerous lysosomes (L). × 34 000

Fig. 2. The lysosome containing membranous formations may be interpreted as an autolysosome (C) for there is little to suggest that it is a phagolysosome derived from an ingested cell fragment. Other lysosomes containing medium-density material are present. The limiting membrane of one of these organelles is ruptured and the contents appear to have extruded into the cytoplasm (arrow). Whether this is an artefact of tissue collection or a meaningful *in vivo* event is debatable. × 30 000

Fig. 3. Lysosomal pleomorphism is depicted in this electron micrograph. × 32 000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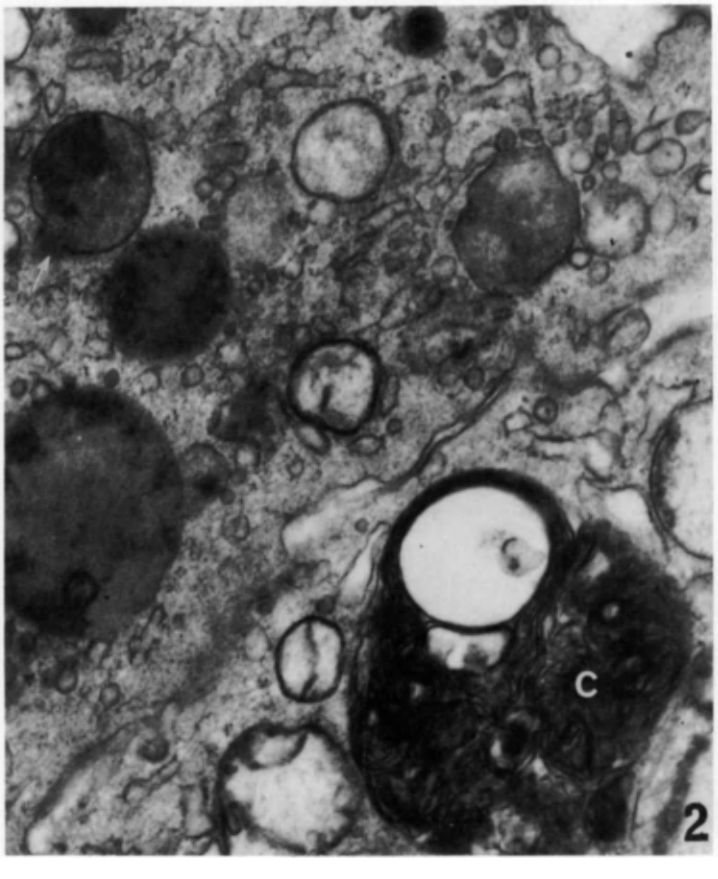
1


C
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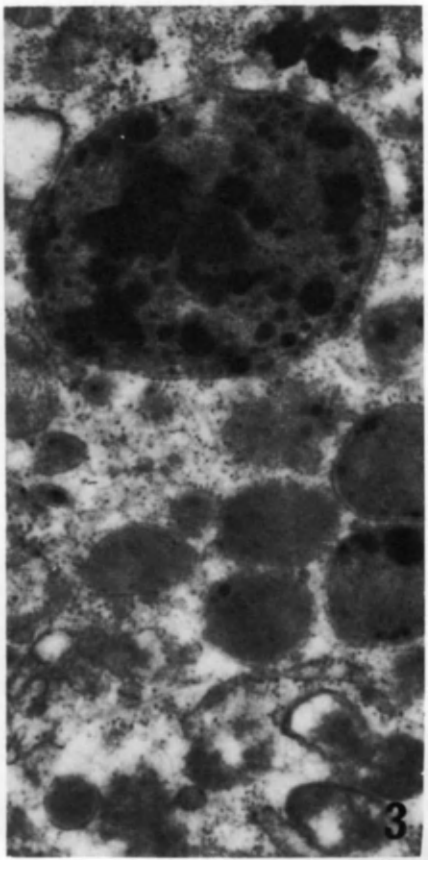
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suggested that newly formed lysosomes may be unstable and rupture easily. An extension of this concept was proposed by Page Thomas (1969) who coined the term 'intraphagosomal dyslysis' to describe this phenomenon. Here it is visualized that there is a relative lack of lysosomal enzymes, in the presence of an appropriate substrate (i.e. basically digestible) due to excessive phagocytosis, and that such a situation leads to an increased production of primary lysosomes (via a feedback mechanism), some of which discharge their contents extracellularly. Besides the above-mentioned mechanisms there is also the obvious one whereby lysosomal enzymes are liberated when synovial cells die and suffer autolysis.

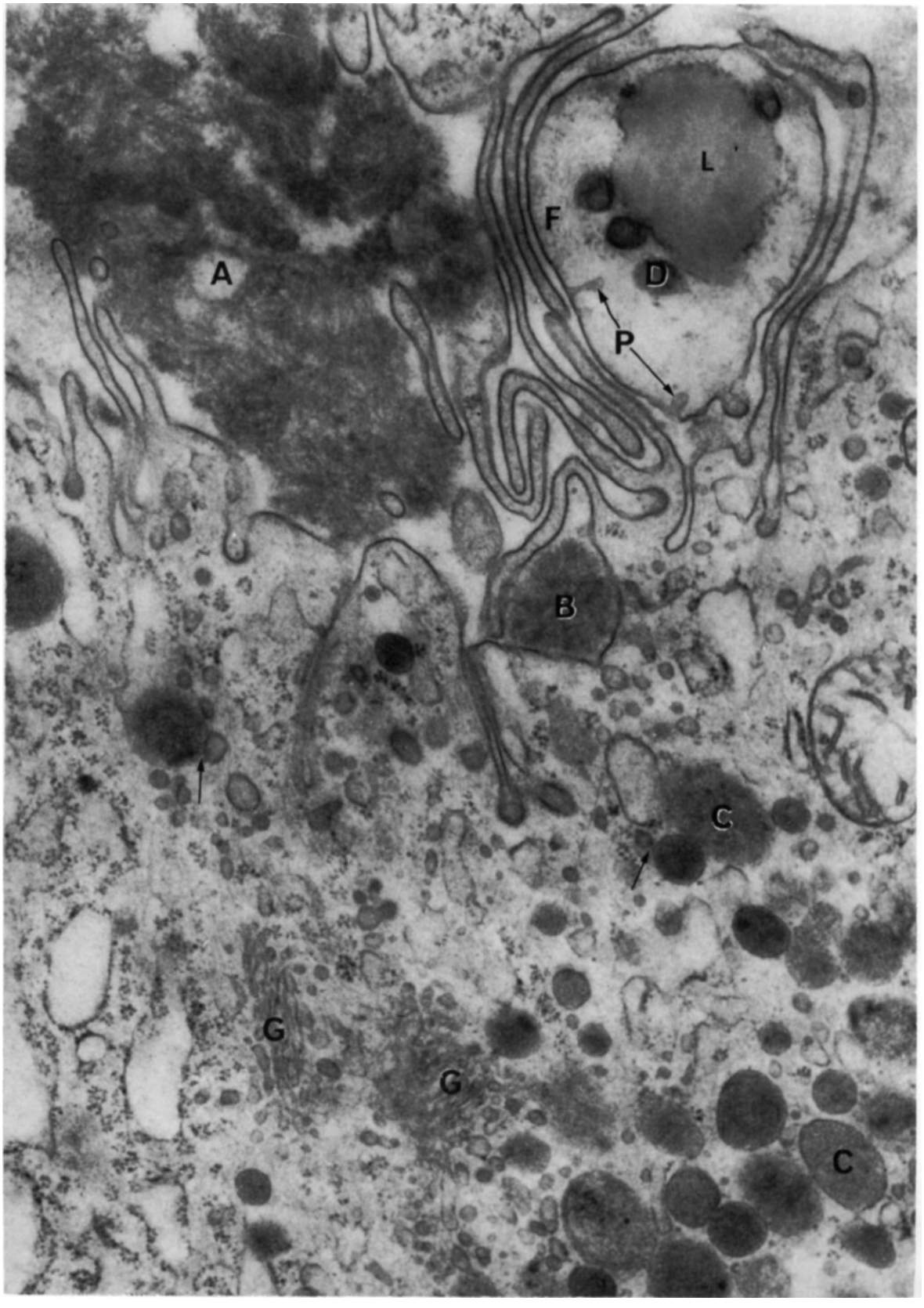
Whatever the mechanisms involved may be, it is clear that a release of lysosomal enzymes does occur and it is not difficult to visualize that this can lead to tissue damage and inflammation; indeed it has been shown that inflammation of subcutaneous tissues or joints can be produced by injections of purified extracts of leucocyte lysosomes (Weissmann, 1966). The inflammation and cell damage engendered by the release of hydrolytic enzymes may in turn lead to further phagocytic activity by the synovial cells and the production of more lysosomes. One could speculate that ultimately a situation might well be reached in which the joint tissues proceed to destroy themselves without any further assistance from the noxious agent which initiated the process.

It is interesting to note that agents which bind and inactivate lysosomal enzymes (e.g. gold salts; *see also* pages 730–735) or agents which stabilize lysosomal membranes (e.g. glucocorticoids, chloroquine and colchicine) are agents of value in the treatment of joint diseases while agents which labilize lysosomal membranes, such as streptolysin S and filipin, produce inflammation, arthritis and destruction of cartilage when injected into the joint (Weissmann and Thomas, 1964; Weissmann *et al.*, 1965; Pras and Weissmann, 1966).

The idea that lysosomal dysfunction and release of hydrolytic enzymes may be operative in rheumatoid arthritis is also supported by the work of Page Thomas (1969). He has shown that intra-articular injections of dyes such as Congo red which inhibit lysosomal hydrolases, or the injection of polysaccharides such as chitin which presumably do not suffer rapid degradation in synovial lysosomes, lead to a morphological picture (light microscope studies) reminiscent of rheumatoid arthritis. Such changes include synovial hyperplasia, villus formation, focal round cell aggregation and plasma cell infiltration.

Plate 303

This electron micrograph illustrates the manner in which synovial cells phagocytose fibrinoid material and cellular detritus from the joint space. Fibrinoid material can be seen lying in the joint space at A. This material can also be seen trapped between the cell wall and a filopodium (actually a lamellar, fold or ruffle and not a thread-like structure, *see* pages 1131 and 1132) at B. Furthermore, morphologically similar material can be demonstrated in single-membrane-bound structures (heterophagosomes and heterolysosomes) within the cell (C). These appearances are compatible with the idea that the fibrinoid material is being phagocytosed by the synovial cell to form heterophagosomes. Heterophagosomes are known to acquire hydrolytic enzymes by fusing with primary lysosomes arising from the Golgi complex and thus become converted into heterolysosomes. It is interesting therefore, to observe that many small vesicles probably arising from the Golgi complex (G), are seen adjacent to or in contact with (arrows) the heterophagosomes and heterolysosomes. An early stage of phagocytosis of a cell fragment is also probably depicted in this picture. In the joint space is seen a cell fragment (identified as such by micropinocytotic vesicles (P) along its plasma membrane), intracytoplasmic filaments (F), lipid droplet (L) and electron-dense bodies (D), (probably lysosomal in nature) ensheathed by a somewhat complex array of synovial cell filopodia. × 43000 (*From Ghadially and Roy, 1967a*)


L
F
A
D
P
B
C
G
G
C

Lysosomes in the liver of the tumour-bearing host

A marked increase in numbers and also to some extent in the size of peribiliary dense bodies, occurs in rats bearing a variety of tumours (*Plate 304*). This phenomenon, noted by us (Ghadially and Parry, 1965a) during ultrastructural studies on the liver of rats bearing carcinogen-induced subcutaneous sarcomas in their flank, has since been extended by us and others to other tumour-host systems. It should be noted that such changes occur in the liver free of metastatic growth. Before this interesting phenomenon is discussed it is worth recalling some aspects of tumour-host relationships.

Many striking morphological and biochemical changes occur in animals bearing tumours. One such constant phenomenon is an increase in liver weight (Medigreceanu, 1910; Abels *et al.*, 1942; Annau *et al.*, 1951), associated with a decreased liver catalase activity (Greenstein, 1954). This increase in liver weight is particularly remarkable, for it occurs in an animal that is wasting away to satisfy the metabolic needs of the growing tumour (*see* the review by Wiseman and Ghadially, 1958). It is now clear that the increase in liver weight is due to an increase in liver nitrogen (Yeakel, 1948; Sherman *et al.*, 1950, Yeakel and Tobias, 1951) as well as in liver water content (McEwen and Haven, 1941). Thus, protein is diverted not only to the growing tumour mass but also to the liver of the cachectic host.

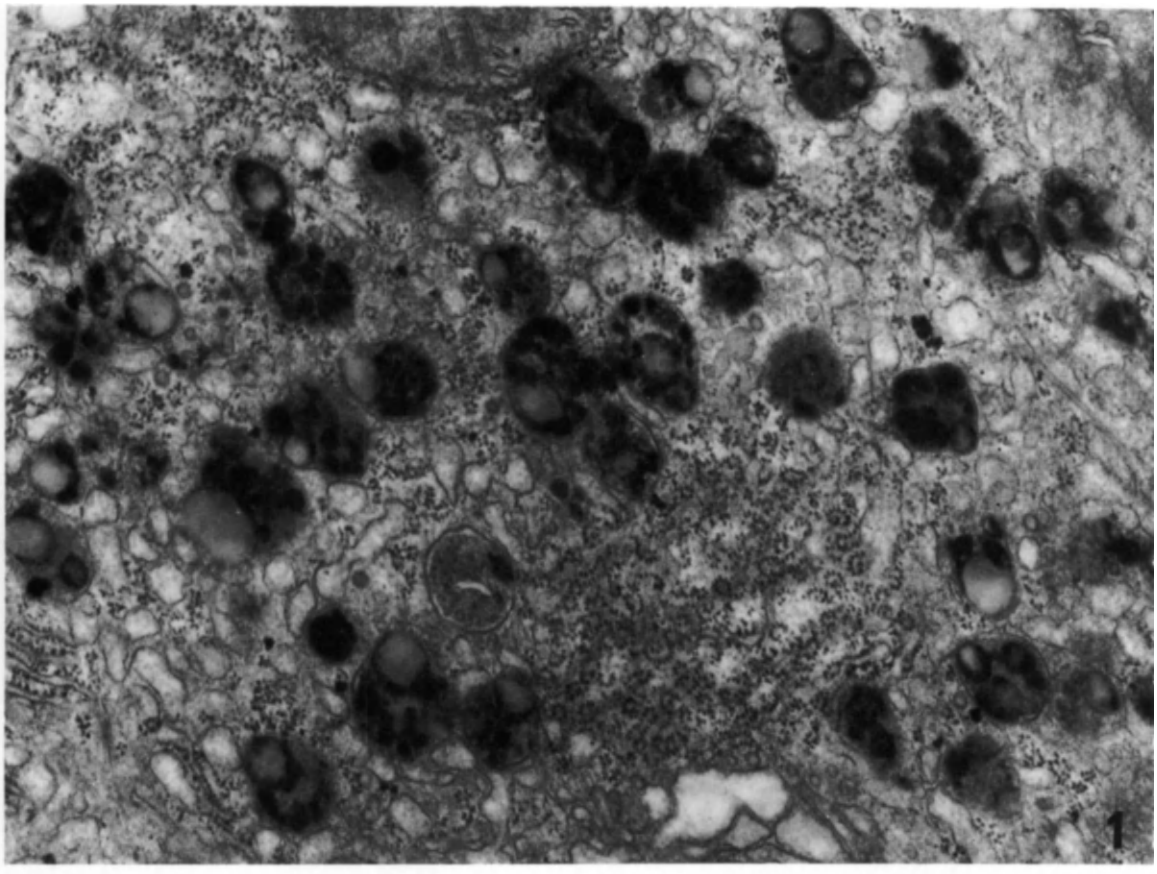
Despite these changes, light microscopic examination of routine histological preparations of the liver reveals either a normal-looking organ or one that shows only some cloudy swelling and occasional focal necrosis. However, ultrastructural studies have shown various changes in the liver of the tumour-bearing host. These include dilatation and vesiculation of the endoplasmic reticulum, mitochondrial swelling, reduction of liver glycogen, decrease in the number of microbodies (*see also* page 780) and as increase in hepatocellular lysosomes of the peribiliary dense body variety*. No overt increase in autolysosomes was seen by us in the liver except during the terminal stages of the life of the tumour-bearing host (Ghadially and Parry, 1965a; Parry and Ghadially, 1966). This increase in hepatocellular lysosomes can be quite marked; at times as many as 40-120 peribiliary dense bodies may be seen around a single bile canaliculus. This change has been quantified by Parry (1969) and shown to be statistically significant. He found that the mean number of lysosomes per bile canalicular section was 1.8 in the normal state; 4.4 in an animal bearing a 6 g tumour and 9.8 in an animal with a 293 g tumour. An increase in hepatocellular lysosomes or lysosomal enzymes has been seen in: (1) liver of rats bearing 7,12-dimethyl-α-anthracene-induced subcutaneous sarcoma (Ghadially and Parry, 1965a); (2) liver of rats bearing a variety of transplanted tumours such as squamous cell carcinoma of the skin, mammary carcinoma, hepatomas and sarcomas (Parry and Ghadially, 1967; Rogers *et al.*, 1967; Shamberger *et al.*, 1971; Blatteis *et al.*, 1974; Farbiszewski *et al.*, 1974; Ferguson *et al.*, 1979; Trew *et al.*, 1979); (3) mouse liver adjacent to 'solid' Landschutz sarcomas produced by inoculating tumour cells in the liver and also in the liver of mice bearing the ascites form of this tumour (Butterworth, 1970); (4) liver of mice bearing solid and ascites forms of Sarcoma 180 and Ehrlich's tumour (Ghadially *et al.*, 1980); (5) human liver adjacent to a well differentiated hepatoma (Ghadially and Parry, 1966a); (6) liver of a patient with Hodgkin's disease (*Plate 305*); (7) liver of patients with renal and gastric carcinomas (Schersten *et al.*, 1969; Stein *et al.*, 1971).

*Some of which resemble lipofuscin granules.

Plate 304

Fig. 1. Numerous lysosomes are seen in this hepatocyte of a rat bearing a carcinogen-induced subcutaneous sarcoma. × 30 000 (*From Parry, 1969*)

Fig. 2. Liver of rat bearing a carcinogen-induced sarcoma, showing a collection of lysosomes and other organelles. Tissue was fixed in glutaraldehyde and treated according to the method of Holt and Hicks (1961). The intensely electron-dense material deposited mainly on or close to the lysosomal membranes (arrows) is lead phosphate resulting from acid phosphatase activity in these organelles × 40 000 (*From Ghadially and Parry 1965a*)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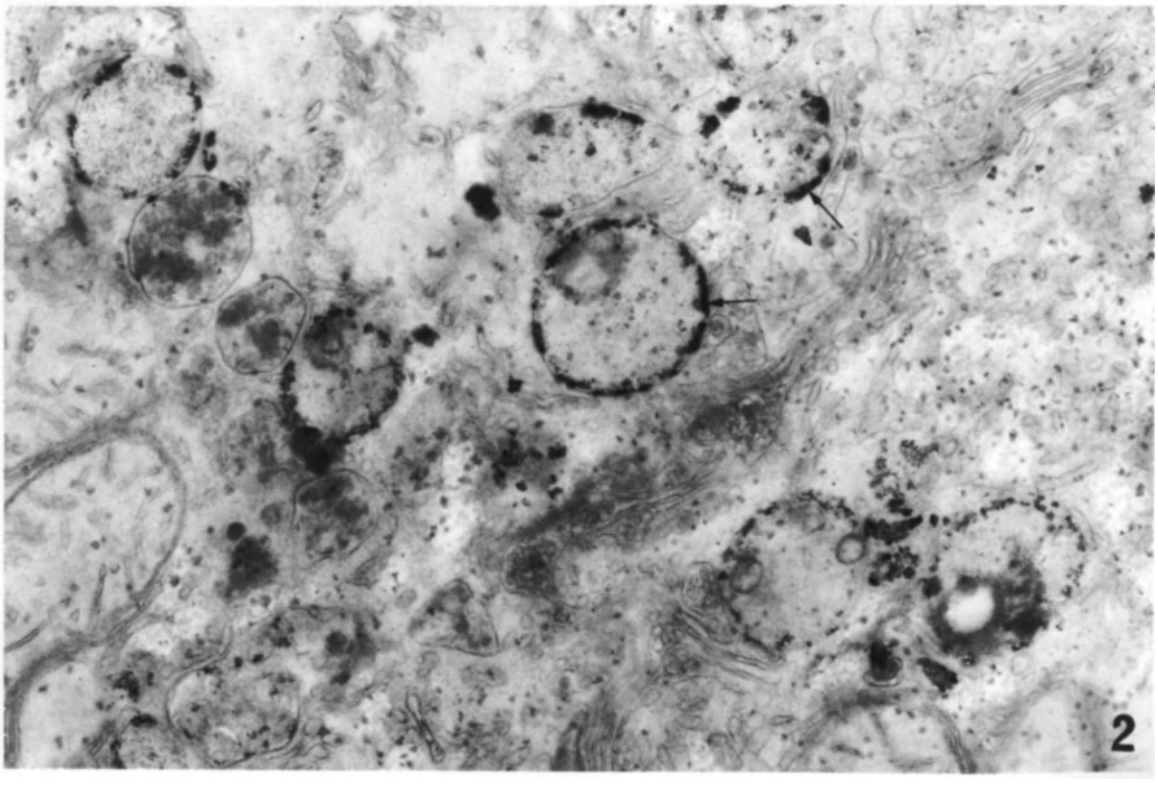
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Furthermore, we (Parry and Ghadially, 1969) have demonstrated that necrotic tumour tissue does not produce this effect, but injections of toxohormone, a much-studied polypeptide that can be extracted from a variety of tumours of experimental animals and man, does produce a statistically significant increase in the lysosome population (Parry and Ghadially, 1970).

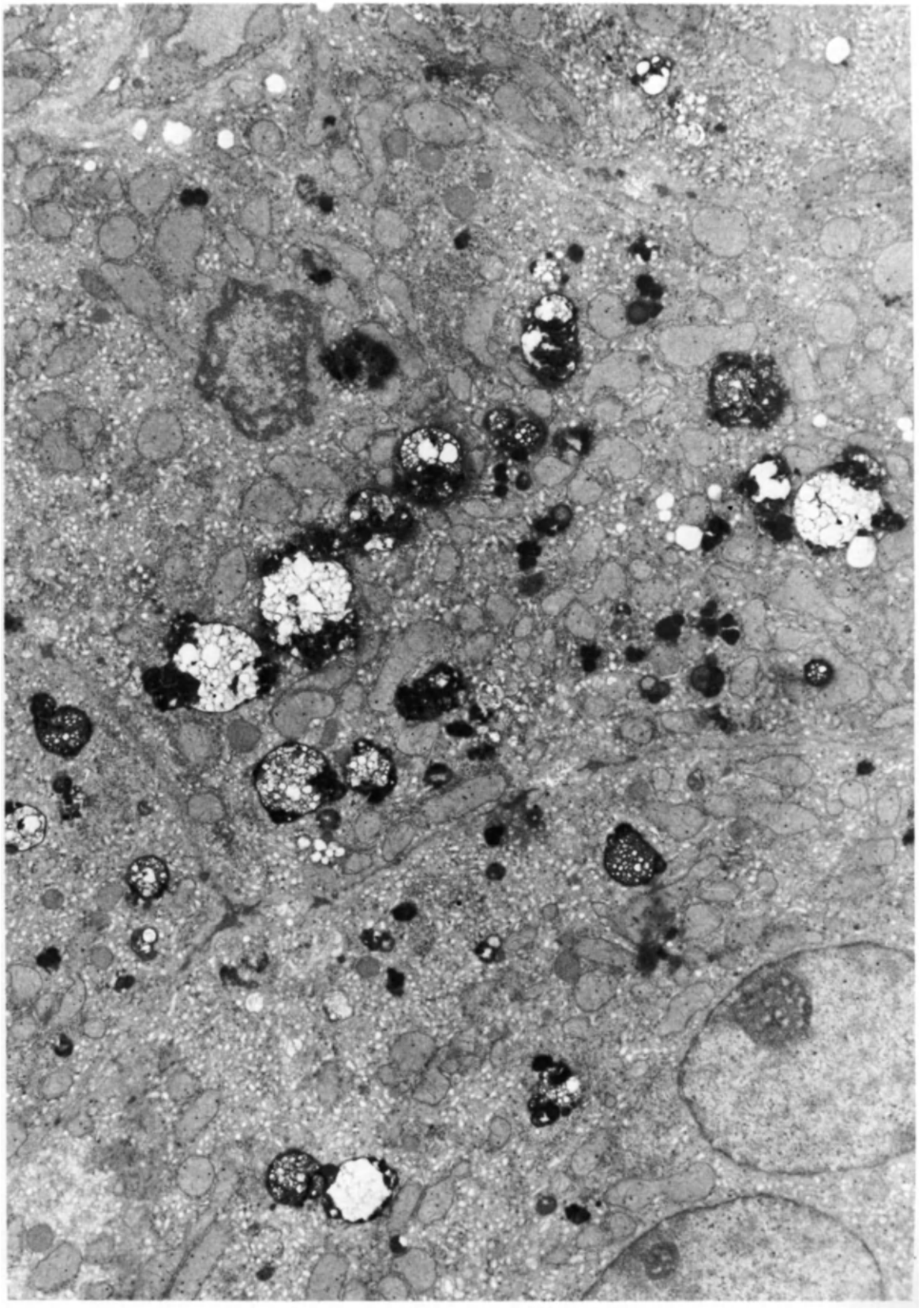
Contrary to the above mentioned findings are the reports by Khandekar *et al.* (1972) who did not find an increase in the hepatocellular lysosomes of rats bearing Walker carcinomas and Tsukada *et al.* (1966) and Lee and Aleyassine (1971) who could not detect an increase in hepatocellular lysosomes in mice bearing Ehrlich ascites tumour. However, biochemical studies show that in both the above mentioned tumour/host systems there is a statistically significant increase in the specific activity of several lysosomal enzymes (Trew *et al.*, 1979; Ghadially *et al.*, 1980). This difference between morphological and biochemical observations can be explained: (1) by the possibility that only gross increases in lysosome population are likely to be detected in the small samples examined with the electron microscope, but by biochemical methods whereby many grams of tissue are analysed, finer changes may be detected and/or (2) by the possibility that the enzyme content of individual lysosomes had increased. Since no increase in size was evident, the first hypothesis seems more attractive. In any case, one may conclude that there seems to be an increase in lysosomes and/or lysosomal enzymes in the liver of the tumour-bearing host in all tumour/host systems properly investigated to date and that at least at the moment there appears to be no proven exception.

The significance of the increase in the hepatocellular lysosomes noted in tumour-hearing rats is not too clear. A possible explanation would be that toxohormone (and perhaps also other toxic substances produced by viable tumour tissue) is taken into the hepatocytes by a process of endocytosis and is then sequestrated into the peribiliary dense bodies, as are metals and detergents. Thus, this phenomenon could be looked upon as a detoxicating mechanism of survival value to the tumour-bearing animal.

The precise cause of death of animals bearing tumours is not known. In the absence of haemorrhage or gross involvement of vital organs by tumour metastasis, it seems likely that death results from some biochemical disturbance. This idea is neither new nor novel and has been mooted by many workers (*see* review by Wiseman and Ghadially, 1958). Furthermore, it is known that some remarkable changes occur in the tumour-bearing rat just prior to its death. During this terminal stage in the life of the animal, when most of the normal tissues are markedly wasted, even the rate of growth of the tumour becomes retarded and the liver shows a sudden marked weight loss (McEwen and Haven, 1941). At this stage the light microscope shows degenerative changes and sizeable areas of hepatic necrosis. With the electron microscope the most salient feature is a swelling and rupture of many hepatocellular lysosomes, and also the presence of numerous autolysosomes (Parry and Ghadially, 1966). The appearances seen are in fact consistent with the 'suicide bag' hypothesis of de Duve whereby it is proposed that rupture of lysosomes or seepage of lysosomal enzymes could occur and the released acid hydrolases would then damage or kill the cell. Although in this instance one cannot be certain from static pictures alone whether cell death precedes or follows the rupture of lysosomes, there are now many situations in which tissue damage and cell death are thought to be mediated in this fashion (for example *see* pages 692 and 726).

Plate 305

Hepatocytes from a patient with Hodgkin's disease, showing a large number of lysosomes. × 8000 (*Ghadially, Ailsby and Larsen, unpublished electron micrograph*)



Angulate lysosomes

The term 'angulate lysosomes' is used to describe lysosomes which present an angular rather than the conventional rounded profile in sectioned material. On the basis of their contents angulate lysosomes may be divided into three groups. Those which contain: (1) 30–60 nm tubules (found in Gaucher's cells and in globoid cells of globoid leucodystrophy); (2) lamellar formations (usually paired lamellae mimicking longitudinally cut microtubules) and rarely also a few microtubules or tubules with a filament or microtubule in the centre (found in Gaucher-like cells in diverse different situations); (3) crystals of minerals and metals (found chiefly in pulmonary macrophages but also in other sites where macrophages have ingested metals and minerals).

In each of three above-mentioned instances, the angulate form of the lysosome stems from the presence of rigid linear or curved structures in its interior. If the rigid structures are aligned in a parallel fashion, a fusiform lysosome may develop (i.e. two sharp or not-so-sharp angles); if not so aligned a multiangulate lysosome may develop. In the text that follows, fusiform lysosomes and angulate lysosomes of other shapes are collectively referred to as angulate lysosomes.

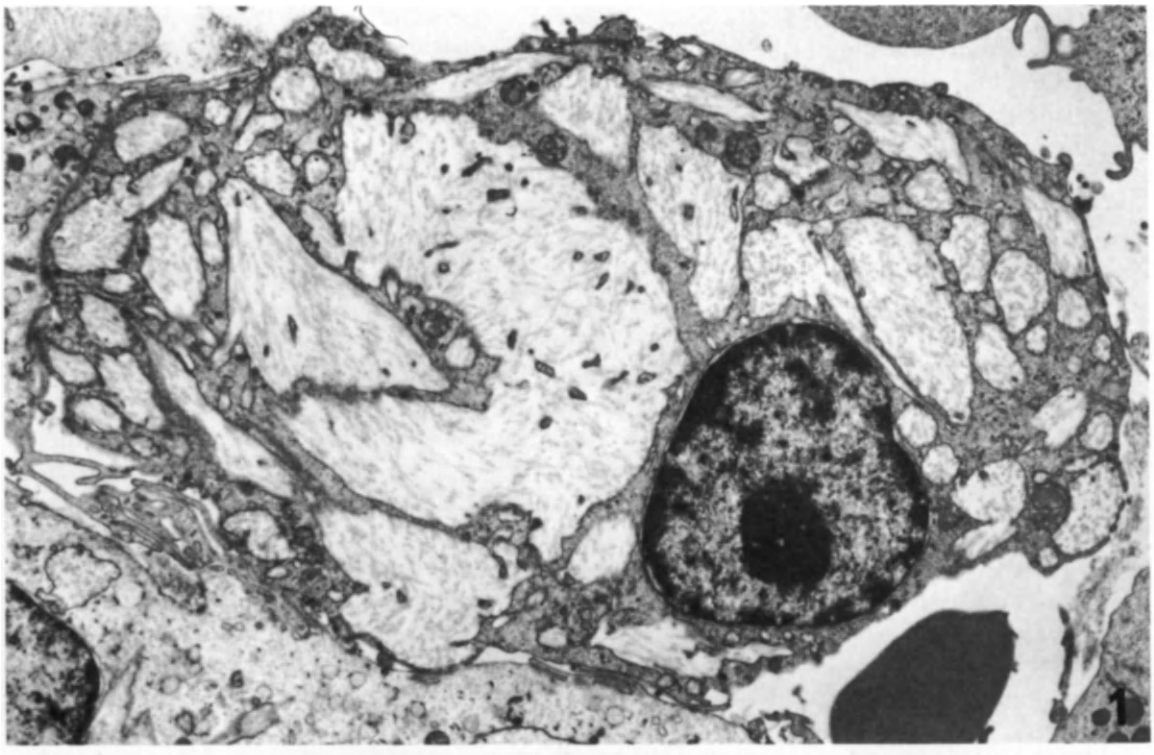
Angulate lysosomes occur almost exclusively in macrophages or histiocytes; only very rarely have they been seen in other cells (e.g. hepatocytes and glial cells). The conditions in which they have been seen include: (1) Gaucher's disease (Hers and Van Hoof, 1969; Beltrami *et al.*, 1973; (2) globoid leucodystrophy (Krabbe's disease) (Schochet *et al.*, 1969; Yunis and Lee, 1969; 1970); (3) generalized gangliosidosis type II (juvenile GM_1, gangliosidosis) (Suzuki *et al.*, 1968; Lowden *et al.*, 1974; Gilbert *et al.*, 1975; Hakozaki *et al.*, 1979); (4) leukaemias, particularly chronic myeloid leukaemia (Gelfand and Griboff, 1961; Sundberg *et al.*, 1964; Albrecht, 1966, 1967, 1969; Chang-lo *et al.*, 1967; Kattlove *et al.*, 1968, 1969; Smith *et al.*, 1968; Gerdes *et al.*, 1969; Witzleben *et al.*, 1970; Lee and Ellis, 1971; Dosik *et al.*, 1972; Keyserlingk *et al.*, 1972; Beltrami *et al.*, 1973; Ookuma, 1973; Hopfner *et al.*, 1974; Kirchen and Marshall, 1976; Takahashi *et al.*, 1977); (5) thalassemia (Zaino *et al.*, 1971; Hakozaki *et al.*, 1979); (6) congenital dyserythropoietic anaemia (Van Dorpe *et al.*, 1973); (7) schwannoma and so-called 'granular cell myoblastoma' which is now best regarded as a granular cell schwannoma (pages 684–689); (8) naevus of Ota (Dingemans *et al.*, 1983); (9) experimentally produced blue naevi in hamsters (Ghadially *et al.*, 1986); (10) normal-looking skin and skin with rash from cases of rheumatoid arthritis who received chrysotherapy (Ghadially *et al.*, 1978); (11) lichen nitidus (Dingemans *et al.*, 1983); (12) experimentally produced skin allergy (Dingemans *et al.*, 1983); (13) cerebrohepatocellular syndrome of Zellweger (Dingemans, 1983); (14) adenoleucodystrophy (Manz *et al.*, 1980; Jaffe *et al.*, 1982; Powers *et al.*, 1982); (15) infantile phytanic acid storage

Plate 306

From a block of tissue supplied by Dr M. Djaldetti.

Fig. 1. Gaucher's cell from human bone marrow. Numerous angulate single-membrane-bound bodies (Gaucher's bodies) are seen in the cytoplasm. × 7800

Fig. 2. Higher-power view of Gaucher's bodies showing tubules lying in an electron-lucent matrix. × 18 000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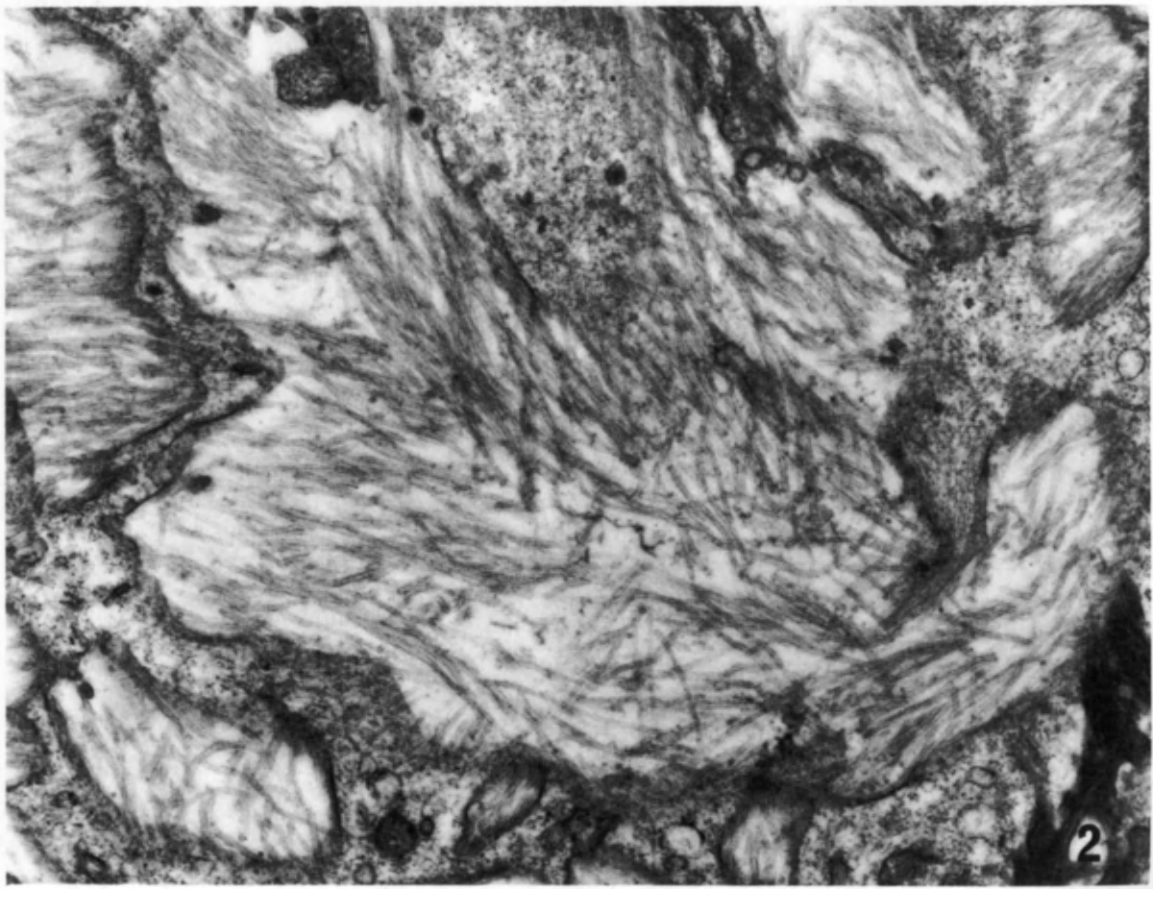
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disease (Scotto *et al.*, 1982); (16) Whipple's disease (Morningstar, 1975; Dingemans *et al.*, 1983); (17) necrotic area in pituitary adenoma (Dingemans *et al.*, 1983); (18) necrotic area in anaplastic epidermoid carcinoma (Sobel *et al.*, 1973); (19) chordoma (Soffer *et al.*, 1970); (20) salivary gland intraductal adenocarcinoma (Innes *et al.*, 1982); and (21) adenoma of breast (Ghadially, unpublished observation).

We have repeatedly noted in this chapter that there are four basic reasons why material may accumulate in lysosomes (angulate or otherwise). Accumulation may be engendered by: (1) a genetically induced 'primary' deficiency or absence of a specific enzyme so that the substrate on which the enzyme normally acts accumulates in the lysosomes; (2) incorporation of material or drugs (e.g. gold) in the lysosome which neutralizes or inactivates lysosomal enzymes so that material is not fully digested and hence accumulates in lysosomes; (3) a secondary deficiency of enzymes created by an overwhelming of the lysosomal apparatus with excessive amounts of substrate; and (4) ingestion of inherently indigestible materials (e.g. metals and minerals).

Let us now look at some of the conditions mentioned above (items 1–21) in greater detail. Gaucher's disease (item 1) is now regarded as a lysosomal storage disease. It comprises a group of hereditary disorders (infantile and adult forms) characterized by the presence of Gaucher's cells chiefly in the bone marrow (*Plates 306* and *307*), spleen and liver and to a lesser extent in various other organs (e.g. kidney (Chandler *et al.*, 1979)). In Gaucher's disease there is a deficiency of glucocerebrosidase* which is apparently one of the several α-glycosidases found in human tissues. This leads to an accumulation of glycocerebroside in the cells of the reticuloendothelial system (macrophages) in various organs. Such deposits occur in single-membrane-bound bodies (called Gaucher's bodies) acceptable as lysosomes, for acid phosphatase activity has been demonstrated in them (Takahashi *et al.*, 1978). There is also a large increase in acid phosphatase activity in the spleen and blood (Hers and Van Hoof, 1969; Beltrami *et al.*, 1973).

With the light microscope the Gaucher's cell is seen to be a large distinctive cell, with one or more eccentrically placed nuclei and a striated, grey or bluish cytoplasm, which has a wrinkled tissue-paper-like appearance. Since this appearance (striated cytoplasm) persists even after tissues have been treated with conventional lipid solvents it has long been suggested (Morrison and Hack, 1949; Uzman, 1951) that a non-lipid component is present in the deposits in Gaucher's cells. A correlated ultrastructural histochemical and chemical study (Elleder and Smid, 1977) has shown that in addition to cerebrosides there is also a component of protein, probably glycoprotein, in these deposits.

Electron microscopic examination of Gaucher's cells in various organs shows that the cytoplasm is filled with rounded, elongated or angulated sacs (i.e. angulate lysosomes) loosely

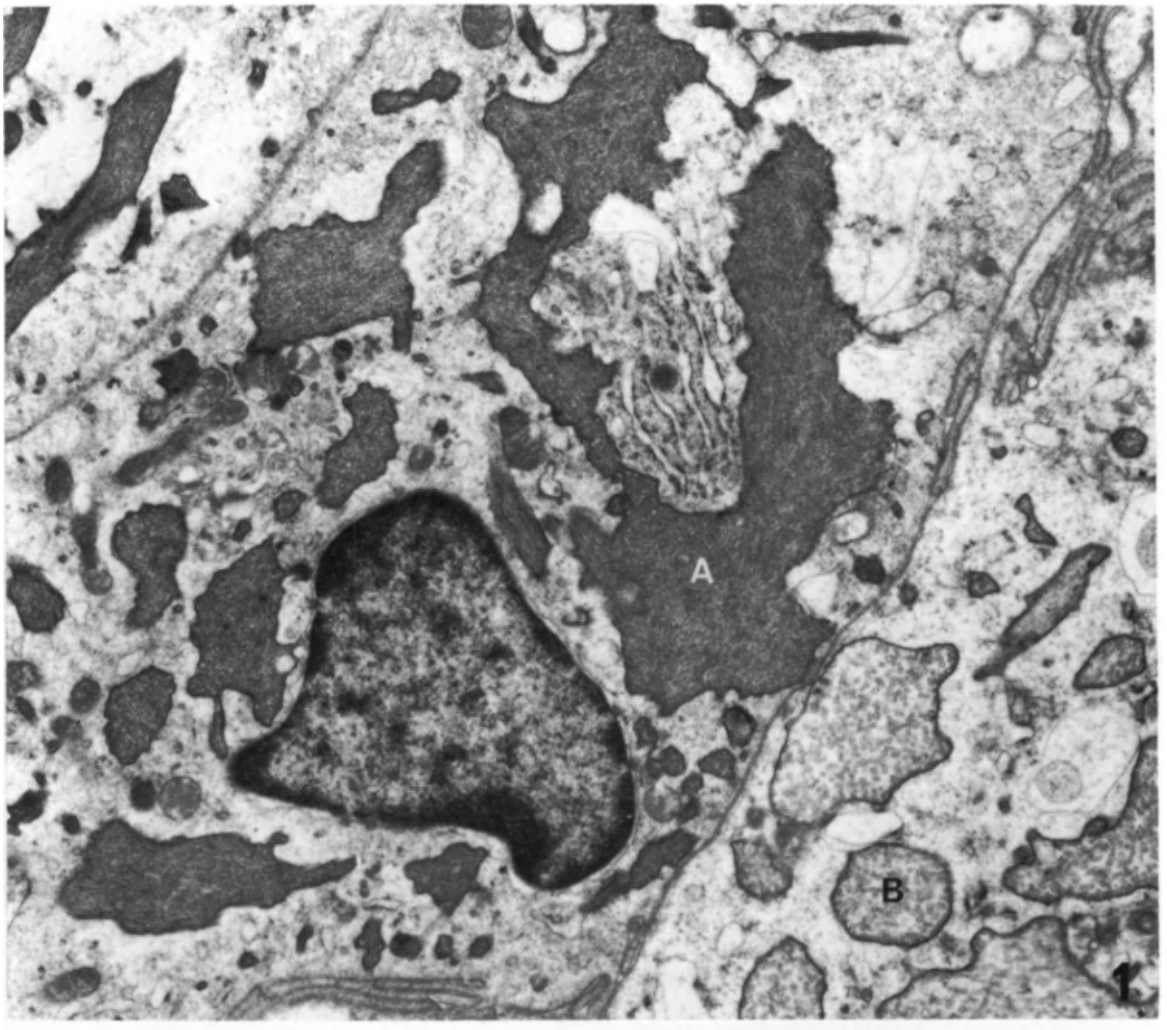
*A large number of glycosidases and related enzymes have been localized within lysosomes (Tappel, 1969) and according to Weinreb *et al.* (1968) the glucocerebrosidase of rat liver is localized in lysosomes.

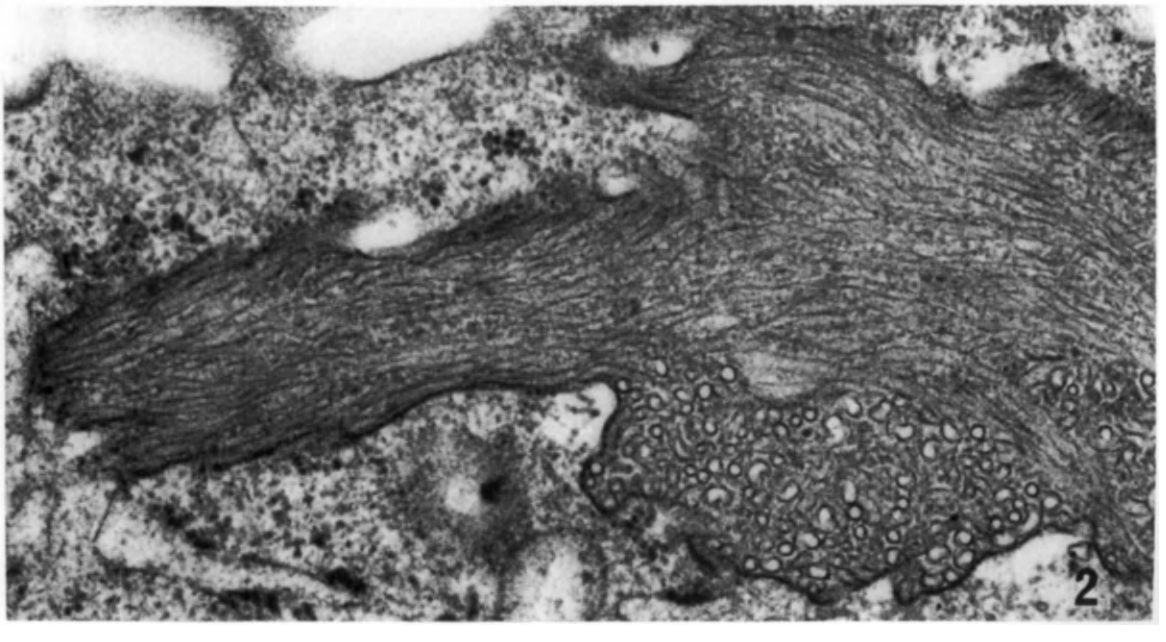
Plate 307

From a block of tissue supplied by Dr M. Djaldetti.

Fig. 1. Two types of Gaucher's bodies are seen in these Gaucher's cells from the bone marrow; one variety (A) appears electron-dense, the other (B) appears electron-lucent. Both are single-membrane-bound and contain tubules. × 13 000

Fig. 2. Part of a large dense Gaucher's body showing tubules cut in transverse and longitudinal planes. × 45 000


A
B
1


2

filled with a number of smooth walled tubules about 30–60 nm in diameter*. In longitudinal section, the tubules show a twisted or spiralled appearance; in cross section, they have a rounded or rectangular profile. Negative stained preparations and shadowed preparations of isolated tubules show the wall of the tubule is made up of 6–8 nm filaments (Lee and Ellis, 1971). The tubules are twisted in a right-handed helix (Yunis and Lee, 1970). In routine preparations (*Plates 306* and *307*), the tubules are seen to lie in a medium density or electron-lucent matrix within the lysosomal sac. Heterolysosomes containing ingested erythrocytes, leucocytes and platelets are also found in Gaucher's cells (Brady *et al.*, 1966; Bessler *et al.*, 1974; Djaldetti *et al.*, 1979) and it would appear that the undigested lipidic residues (glucocerebrosides) constitute, or contribute to the material in the angulate lysosomes.

The deposits in the lysosomes in Gaucher's cells are quite characteristic and almost diagnostic of this condition, but some caution is needed. For example, cells very similar to Gaucher's cells are seen in globoid leucodystrophy (Krabbe's disease) (Yunis and Lee, 1970).

In Krabbe's disease (item 2), extensive demyelination is associated with an accumulation of large numbers of globoid cells in the white matter of the brain. The globoid cells presumably stem from glial cells and macrophages that have ingested myelin, incomplete degradation of which produces the characteristic lipidic (cerebrosides) residues in angulate lysosomes. It is thought that there is a deficiency of cerebroside sulphotransferase or galactose cerebrosidase (O'Brien *et al.*, 1975; Yunis and Lee, 1970) leading to the storage of cerebroside in these cells. Ultrastructurally (routine preparations, negative stained preparations and shadowed preparations), the morphology of the tubules found in angulate lysosomes in this disease is virtually identical to the tubules in Gaucher's disease except perhaps that, in Krabbe's disease cross sections through the tubules show not only round and rectangular profiles but also occasional multiangular profiles (Schochet *et al.*, 1969; Yunis and Lee, 1969, 1970). However, not all workers agree on this point.

Gaucher-like cells are found in generalized gangliosidosis type II (juvenile GM_1 gangliosidosis) (item 3) where there is a profound deficiency of α-galactosidase. Deficiency of this lysosomal enzyme which normally acts on several substrates results in storage of keratan sulphate-like mucopolysaccharides and lipid (GM_1-ganglioside) in lysosomes. A variety of enlarged lysosomes occur; some quite electron-lucent ones (similar to those seen in genetic mucopolysaccharidoses) contain reticulogranular material (mucopolysaccharides), others contain whorled osmiophilic membranes and yet others (angulate lysosomes) contain filaments and/or 'microtubules' (more likely paired lamellae) about 16–22 nm in diameter (Suzuki *et al.*, 1968; Lowden *et al.*, 1974; Gilbert *et al.*, 1975; Hakozaki *et al.*, 1979). In this condition also the ultimate source of the filamentous and lamellar inclusions in the angulate lysosomes are blood cells ingested by the macrophage (i.e. the Gaucher-like cells).

*The term 'tubule' rather than 'microtubule' is more appropriate. Microtubules have a diameter of about 24 nm. When the diameter is 30 nm or more they should be called 'tubules' (*see* page 938 for more details).

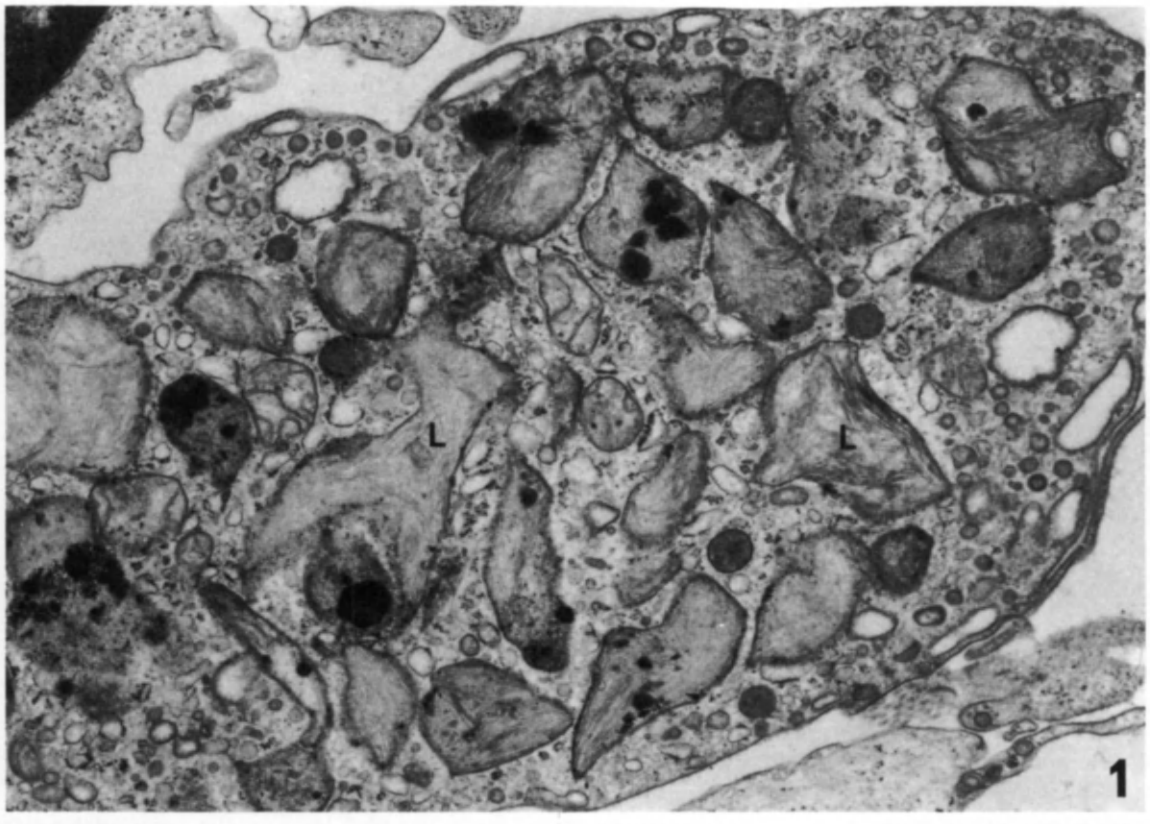
Plate 308

The lysosomes depicted on this plate are from the dermal macrophages of the skin of a patient who had received chrysotherapy for rheumatoid arthritis (*From Ghadially, DeCoteau, Huang and Thomas, 1978a*)

Fig. 1. A Gaucher-like cell containing numerous angulate lysosomes (L). They contain sheaves of filaments and lamellae mimicking microtubules but this is not clearly resolved at this low magnification. Some of the lysosomes also contain electron-dense material. × 21 000

Fig. 2. High-power view of an angulate lysosome. Paired lamellae or microtubules (arrowheads) appear to be present. The notion that these are microtubules is difficult to accept because of the absence of circular profiles acceptable as transverse sections through microtubules. × 50 000

Fig. 3. High-power view of lysosome containing tubules. A filament or microtubule (arrowhead) is seen in the centre of tubules cut transversely. × 100 000


L
L
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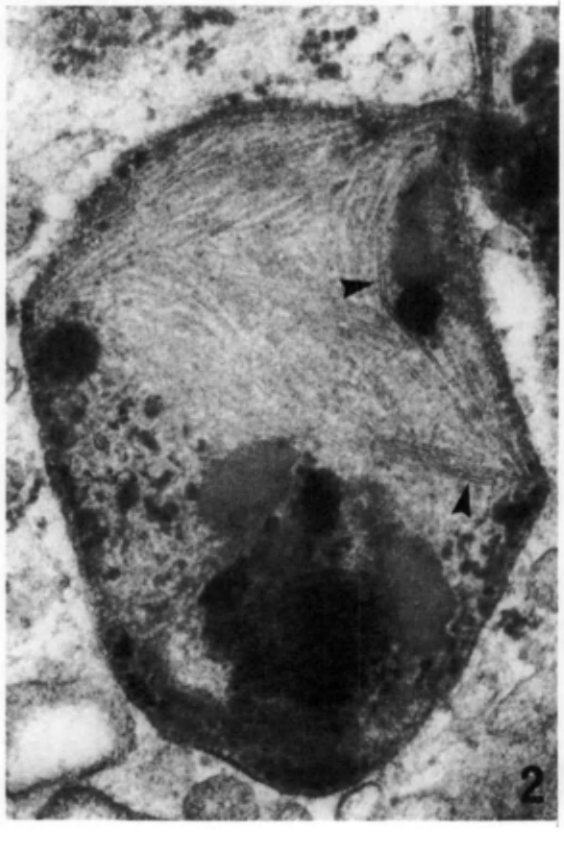
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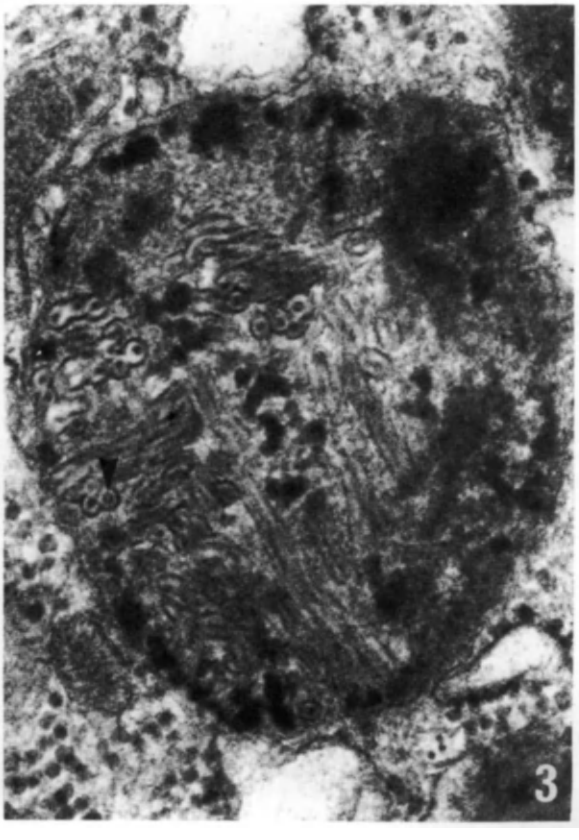

3

Gaucher-like cells are found in normal-looking skin* and in skin rashes of patients on chrysotherapy (item 10). Since gold deposits in lysosomes (*see* pages 730–735) and since gold is known to inactivate lysosomal enzymes, one may speculate that the enzymic defect engendered by it is responsible for the Gaucher-like bodies seen in dermal macrophages (*Plate 308*). The Gaucher-like bodies seen in this situation are quite pleomorphic. Most of them contain mainly paired lamellae mimicking longitudinally cut microtubules but a few contain tubules with a microtubule or filament in the centre (*Plate 308, Fig. 3*). Besides the above, these bodies may also contain gold deposits and melanosomes. Thus, the Gaucher-like bodies in the skin in this condition may be looked upon as modified melanophages.

Until now we have looked at situations where enzymic defect (genetic or drug-induced) in the lysosome produces Gaucher's cells and Gaucher-like cells containing angulate lysosomes. Let us now look at examples where an overwhelming of lysosomal apparatus (i.e. too much substrate and hence a relative deficiency of enzymes) is believed to be responsible for the formation of Gaucher-like cells containing angulate lysosomes.

Such examples include: (1) leukaemias† (item 4) where the Gaucher-like cells stem from macrophages (particularly in the spleen) which have endocytosed numerous leukaemic cells and other formed elements of the blood; (2) thalassaemia and dyserythropoietic anaemia (items 5 and 6) where the Gaucher-like cells stem from macrophages that have endocytosed erythrocytes and other formed elements of the blood; (3) Whipple's disease (item 16) where Gaucher-like cells stem from macrophages that have ingested numerous bacteria; (4) various naevi and tumours (items 7‡, 8, 9, 17, 18, 19, 20, 21) where the Gaucher-like cells presumably stem from macrophages that have ingested cell debris.

However, in several other situations we are not at all sure how or why Gaucher-like cells containing angulate lysosomes develop. In the case of adrenoleucodystrophy (item 14), infantile phytanic acid storage disease (item 15) and Zellweger's syndrome (item 13), Dingemans *et al.* (1983) speculate that it may be 'a primary metabolic defect that leads to the accumulation of mainly lipoid substances'. They also state that 'Such a hypothesis is in keeping with the fact that patients with Zellweger's syndrome seem to be completely devoid of peroxisomes, which normally play an important role in cellular lipid metabolism'. Adrenoleucodystrophy is a hereditary metabolic disorder characterized by the accumulation of esters of cholesterol with fatty acids of abnormally long chains. The presumed abnormal lipid composition of myelin causes its disintegration (for references *see* Manz *et al.*, 1980). One may speculate that endocytosis of this material by phagocytic cells produces Gaucher-like bodies (i.e. angulate lysosomes).

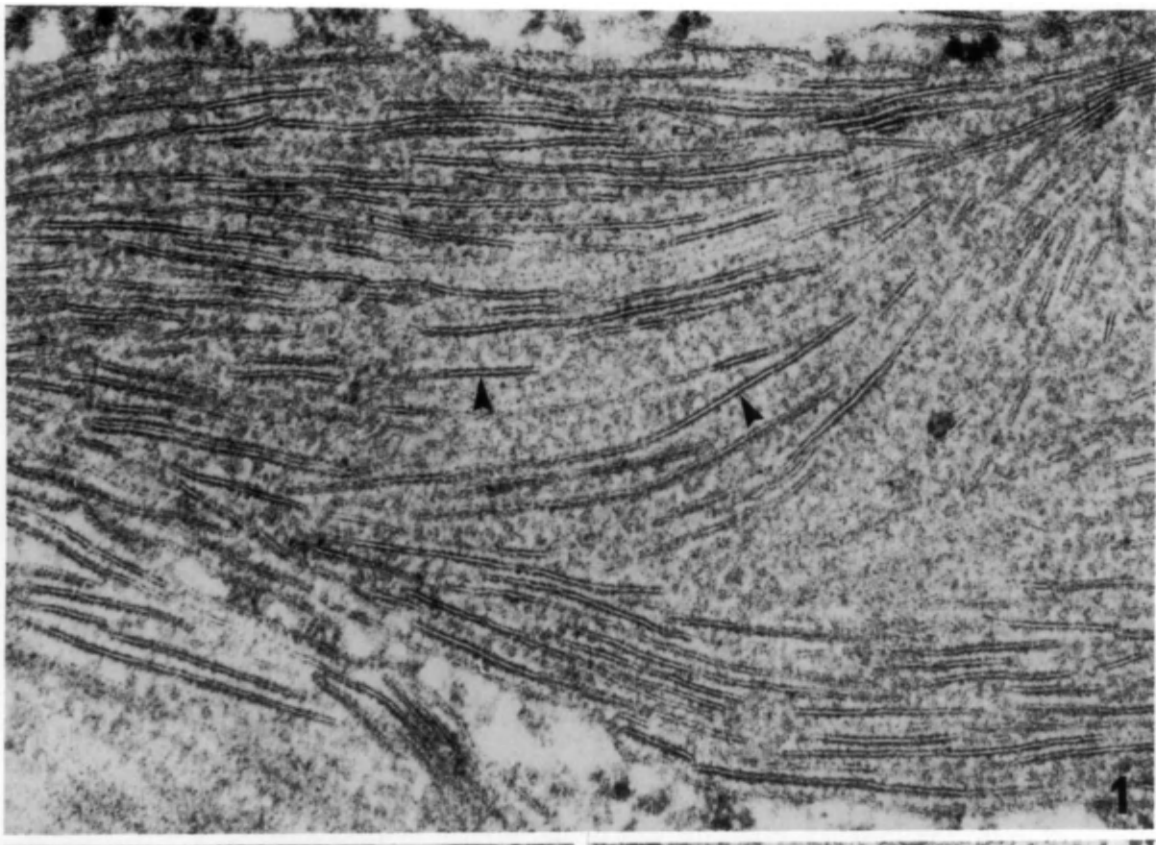
*This must not be construed to mean that Gaucher-like cells are at times seen in the normal skin of normal individuals.
†In certain cases of leukaemia, Gaucher-like cells and sea-blue histiocytes are seen and also cells with an intermediate morphology (Takahashi *et al.*, 1977)– that is to say macrophages containing lysosomes of the type seen in Gaucher-like cells and lysosomes of the type seen in sea-blue histiocytes. Furthermore, transitional forms suggesting that both these types of lysosomal bodies derive from ingested blood cells (mainly leucocytes) have been noted (Kattlove *et al.*, 1968, 1969, 1970; Dosik *et al.*, 1972; Keyserlingk *et al.*, 1972; Hopfner *et al.*, 1974).
‡I am in agreement with Dingemans *et al.* (1983) that in schwannomas and granular myoblastomas angulate lysosomes occur in histiocytes and not in the tumour cells themselves.

Plate 309

Fig. 1. From a Gaucher-like cell found in a needle biopsy of liver from a case of Zellweger's syndrome. A high-power view showing paired lamellae (arrowheads) mimicking longitudinally cut microtubules in angulate lysosomes. × 120 000 (*Electron micrograph supplied by Dr K. P. Dingemans*)

Fig. 2. From a macrophage (i.e. Gaucher-like cell) found in a neurofibroma which contained many granular cells. Seen here are obliquely cut Gaucher-like bodies. One of them has a spindle-shaped profile. Note the paired lamellae in their interior (arrowheads). × 61 000 (*From a block of tissue supplied by Drs G. Finkel and B. Lane*)

Fig. 3. A Gaucher-like cell found in an experimentally produced hamster blue naevus. Several elongated and angulated lysosomes are present. They contain giant melanosomes (G) and bundles of filaments (arrowheads). At higher magnifications circular profiles of transversely cut filaments were evident. The average diameter of the giant melanosome at the top of the picture is about 3 μm. × 8800 (*From Ghadially, Ghadially and Lalonde, 1986*)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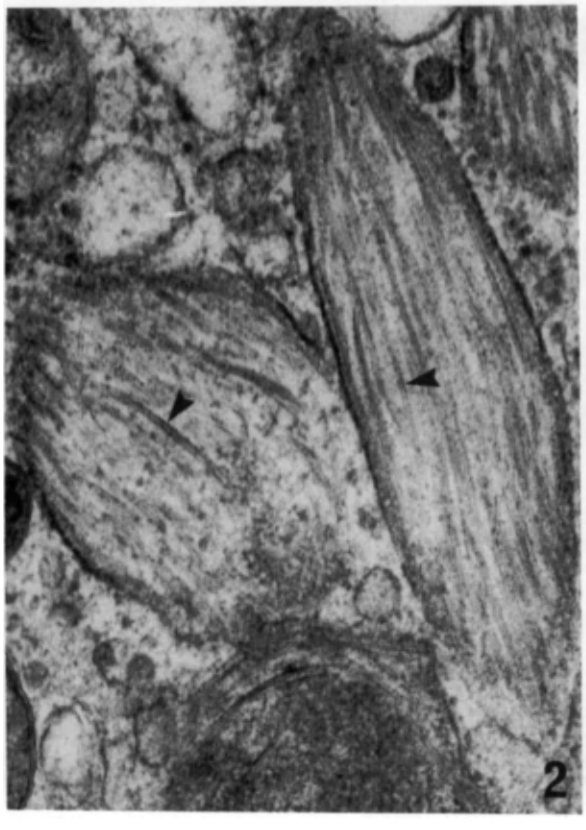
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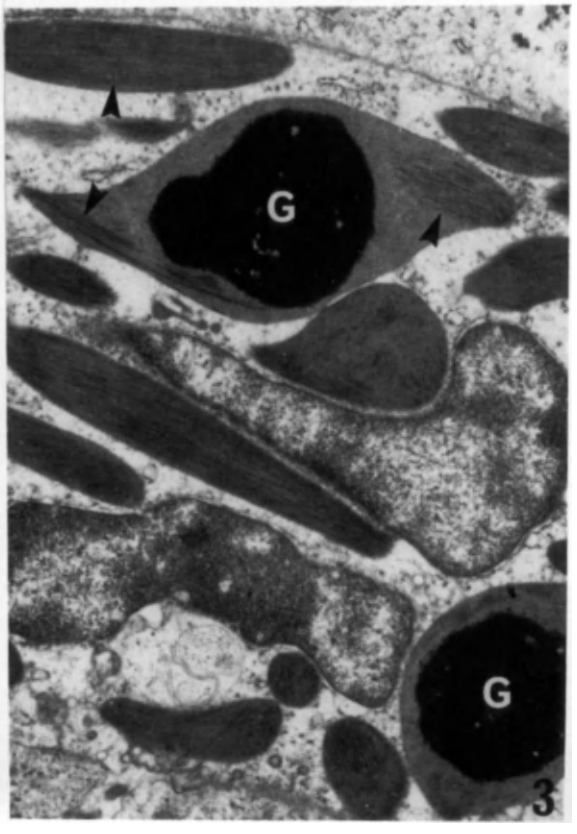

G
G
3

Both rounded and angulate lysosomes are produced by deposits of crystals of metals and minerals in lysosomes, but they are rarely distinguished in this manner or referred to as such in the literature. For example, lysosomes containing gold are called aurosomes (*Plate 323*), those containing uranium are called uraniosomes (*Plate 321*), those containing platinum are called platinosomes (*Plate 325*) simply because terms such as 'rounded lysosomes' and 'angulate lysosomes' though correct are not specific enough for adequately describing these structures. Many of the iron sulphide-containing lysosomes in melanosis duodeni are angulate (*Plate 296*) but no specific name has been coined to describe them.

The lungs are continuously exposed to inhaled dust which contains amorphous and crystalline minerals and metals in particulate form. Little wonder then that metal and mineral-containing lysosomes are found more frequently in alveolar and interstitial macrophages of the lung than in macrophages in other sites. It is said (Berry *et al.*, 1976) that in normal lungs the intralysosomal mineral deposits are amorphous* and only in pneumoconiotic lungs are crystalline deposits of minerals (and hence some angulate lysosomes) found.

Titanium is the most common metal and aluminium silicate the most common mineral found in the lysosomes of pulmonary macrophages of normal individuals. In the lungs of smokers, the angulate lysosomes of pulmonary macrophages contain hexagonal or irregular-shaped (i.e. broken) plates (i.e. crystals) of aluminium silicate. When cut normal to the surface such plates will present filamentous or rod-like profiles. Unaware of this simple fact, some workers mistakenly imagine that filamentous or rod-like crystals occur here. A quite characteristic inclusion is seen in lysosomes (usually angulate lysosomes) of smokers. This inclusion called 'smoker's inclusion' comprises electron-lucent filamentous or rod-like profiles lying in an electron-dense matrix (Brody and Craighead, 1975). What these profiles represent is not known but it is suggested that they may be 'casts' of aluminium silicate plates which have been lost or dispersed by lysosomal activity.

In surgically operated sites, one sometimes finds macrophages with angulate lysosomes containing plates of talc (magnesium silicate), which no doubt stem from the talc ('glove powder') used to lubricate surgical gloves (personal observations). Angulate lysosomes containing talc are seen in great abundance in pulmonary talcosis (*Plate 310*), a well known disease of drug addicts who intravenously self-administer crushed methadone tablets which contain talc as a filler material, or heroin or other drugs adulterated with talc (Ghadially *et al.*, 1984). As is well known, several narcotic drugs are relatively pure when they enter the illegal drug market, but as they pass through many venal hands before reaching the addict they are grossly adulterated with many materials such as: starch, lactose, baking soda and talcum powder. The macrophages and giant cells in talc granulomas which develop are loaded with angulate lysosomes containing plates of talc.

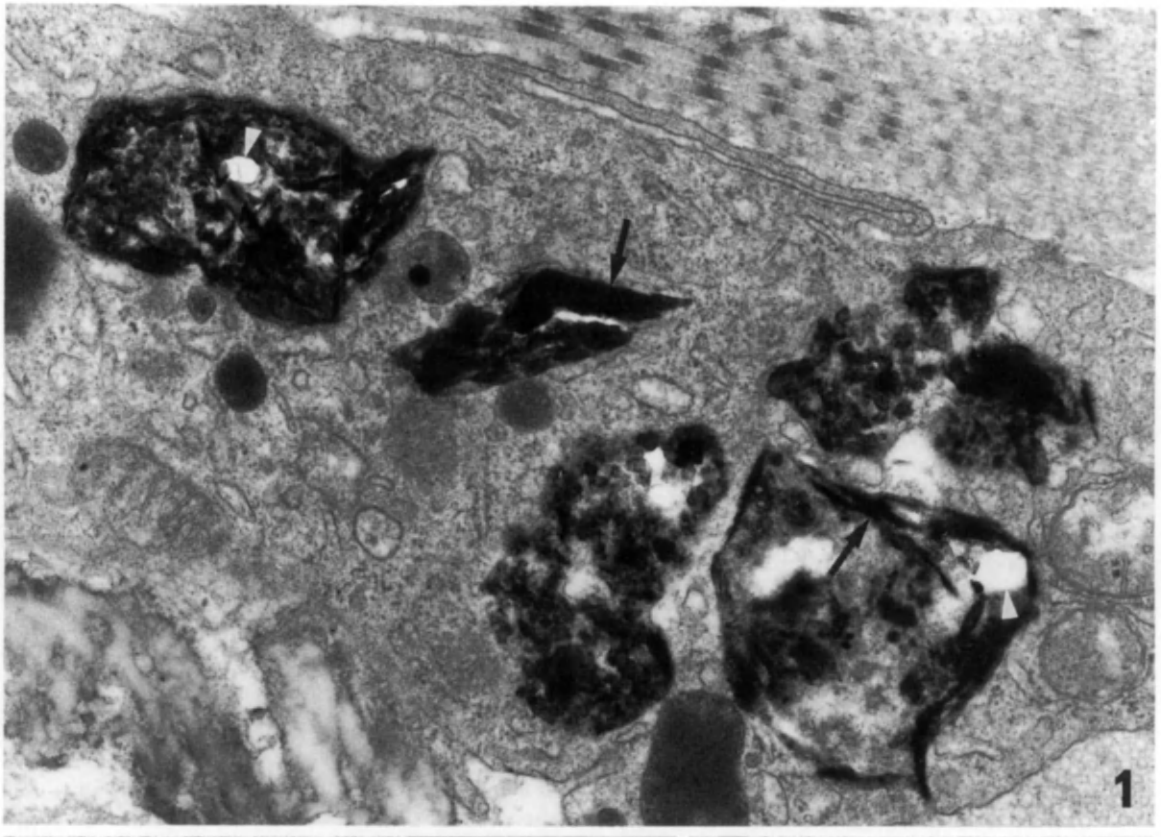
*This despite the fact that most of the inhaled mineral dust is crystalline. It is therefore postulated that the crystals are degraded to the amorphous state by the acid in the lysosome.

Plate 310

Fig. 1. From lung biopsy of a welder who had smoked half a packet of cigarettes a day for several years. Seen here is a septal (interstitial) macrophage containing angulate lysosomes. The electron-dense profiles seen in the lysosomes represent sections through plate-like crystals (arrows) and amorphous material. Some of the clear areas (white arrowheads) are artefactually produced holes in the section. × 29 000

Fig. 2. Same specimen as *Fig. 1.* An angulate lysosome containing crystalline and amorphous electron-dense material and another which contains smoker's inclusions (white arrowheads). × 37 000

Fig. 3. Lung biopsy from a drug addict suffering from talcosis. Plate-like electron-dense crystals of talc (arrows) are seen lying in a large angulate lysosome and a smaller elongated lysosome. Note also the artefactually produced holes (white arrowheads) in the section. Such holes and much larger holes are almost unavoidable in sections of crystals lying in tissues. × 46 000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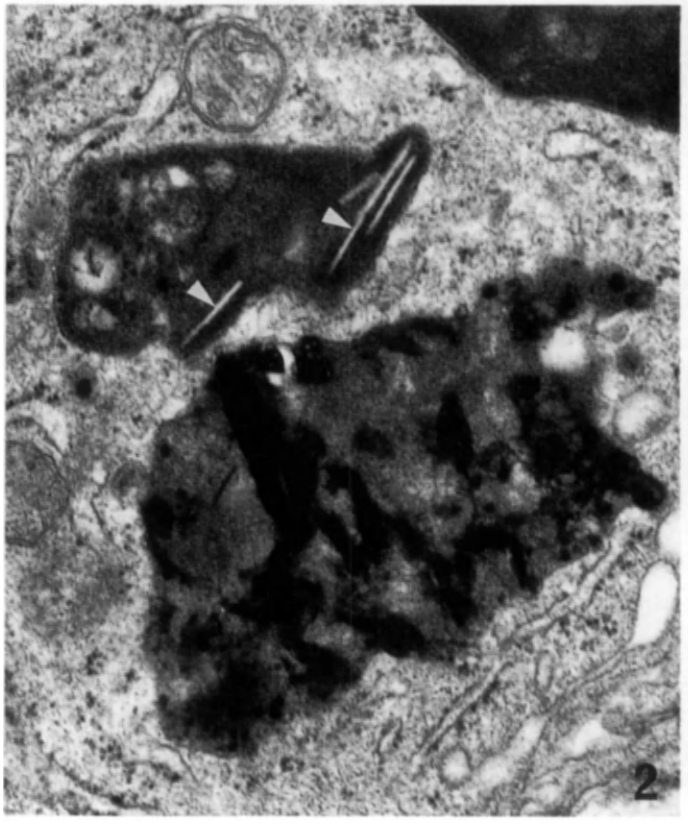
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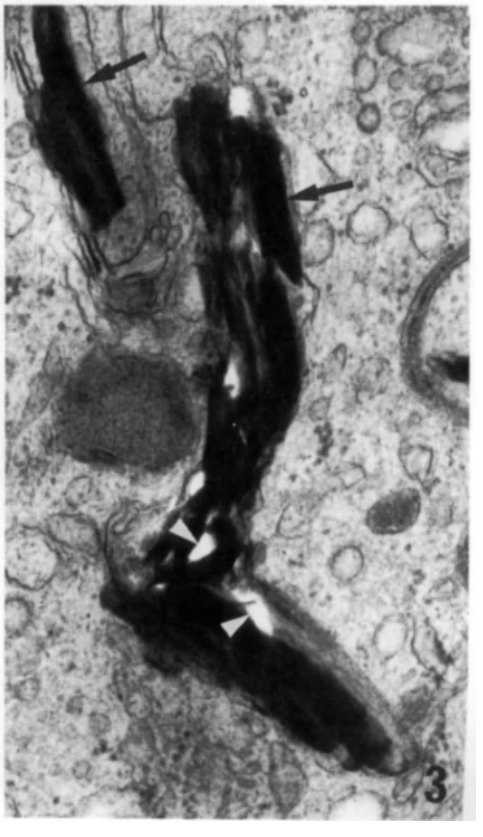
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Lysosomes in mucopolysaccharidoses

The mucopolysaccharidoses* comprise a group of inborn errors of metabolism characterized by intralysosomal storage and urinary excretion of partially degraded mucopolysaccharides (glycosaminoglycans). The best known and documented conditions are: (1) Hurler's disease where there is a deficiency of α-iduronidase. Heparan-sulphate and dermatan-sulphate are excreted in the urine; (2) Hunter's disease where there is a deficiency of sulphoiduronate sulphatase. Heparan-sulphate and dermatan-sulphate are excreted in the urine; and (3) Sanfilippo's disease, type A and B, where there is a deficiency of heparan-sulphatase and 2-acetylglucosaminidase respectively. Heparan-sulphate is excreted in the urine.

In the above mentioned diseases (*Plate 311*) the cells of various tissues come to contain single-membrane-bound bodies containing a sparse flocculent and particulate precipitate (referred to as 'reticulo-particulate material') lying in an electron-lucent matrix (Tondeur and Neufeld, 1973). The lysosomal nature of these bodies is attested by the fact that acid phosphatase can be demonstrated in them (Wallace *et al.*, 1966). The contents of these lysosomes give staining reactions which are consistent with the idea that the material in the lysosome comprises glycosaminoglycans (Dustin *et al.*, 1978). The lucent appearance no doubt reflects the ease with which much of the glycosaminoglycan is lost in routine fixative solutions.

Such lucent lysosomes with sparse flocculent precipitates have been seen in many sites such as: (1) hepatocytes (*Plate 311*) and Kupffer cells (Van Hoof and Hers, 1972); (2) kidney cells, glial cells and fibroblasts (Tondeur and Neufeld, 1973); (3) epithelial cells of conjunctiva† (Kenyon *et al.*, 1972); (4) sweat glands (Spicer *et al.*, 1974); (5) enterocytes (Daems *et al.*, 1973); (6) chondrocytes (Silberberg *et al.*, 1972); (7) muscle fibres (Van Hoof, 1973); and (8) leucocytes and cultured fibroblasts from cases of Hurler's disease (Conrad *et al.*, 1970).

On rare occasions in these modified lysosomes loaded with glycosaminoglycans one may also find some lipidic material in the form of myelinoid membranes or osmiophilic granules. These probably represent some stored glycolipids which have been detected by biochemical studies.

In the neurons, however, the enlarged lysosomes contain lipidic material (Zebra bodies, *see Plate 267*) not glycosaminoglycans. Zebra bodies may co-exist with the typical large lucent glycosaminoglycan-containing lysosomes in the glial cells and Kupffer cells (Haust *et al.*, 1965; Loeb *et al.*, 1968).

The clear or lucent lysosomes with reticulo-particulate content are of limited diagnostic value because lysosomes of the same or very similar morphology have been found not only in the commoner mucopolysaccharides described above but also in: (1) various other rarer mucopolysaccharidoses such as Scheie's disease (Kenyon and Sensenbrenner, 1971), Morquio's disease (Tondeur and Loeb, 1969; Maynard *et al.*, 1973) and Marotaux-Lamy disease (Van Hoof and Hers, 1972; Tondeur and Neufeld, 1973); (2) fucosidosis (Van Hoof, 1973); (3) Farber's disease (Van Hoof and Hers, 1973); (4) mannosidosis (Mónus *et al.*, 1977); (5) mucolipidoses Type I and II (Freitag *et al.*, 1971; Dustin *et al.*, 1978) and (6) GM_1-gangliosidosis (Landing's disease, also called 'pseudo-Hurler'), and GM_2-gangliosidosis (Tay–Sach's disease and Sandhoff's disease) (Dustin *et al.*, 1978).

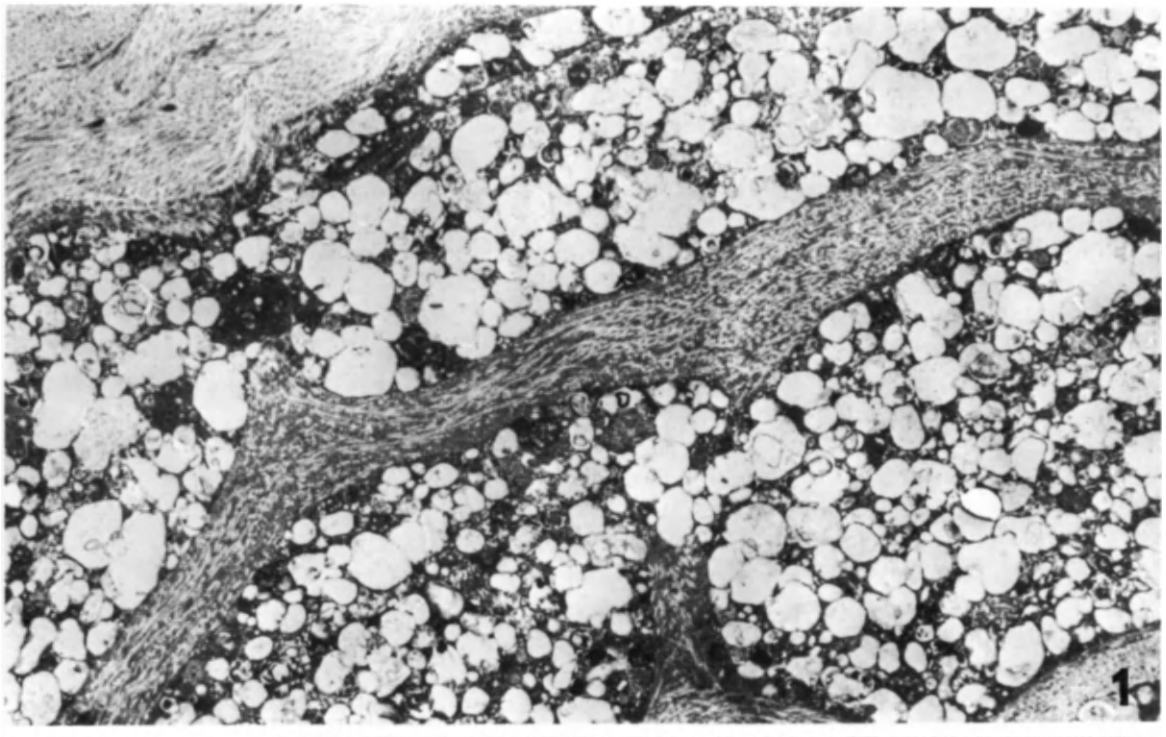
*Strictly speaking these should now be called 'glycosaminoglycanoses', but this might be confusing so in this section I retain the old terminology.

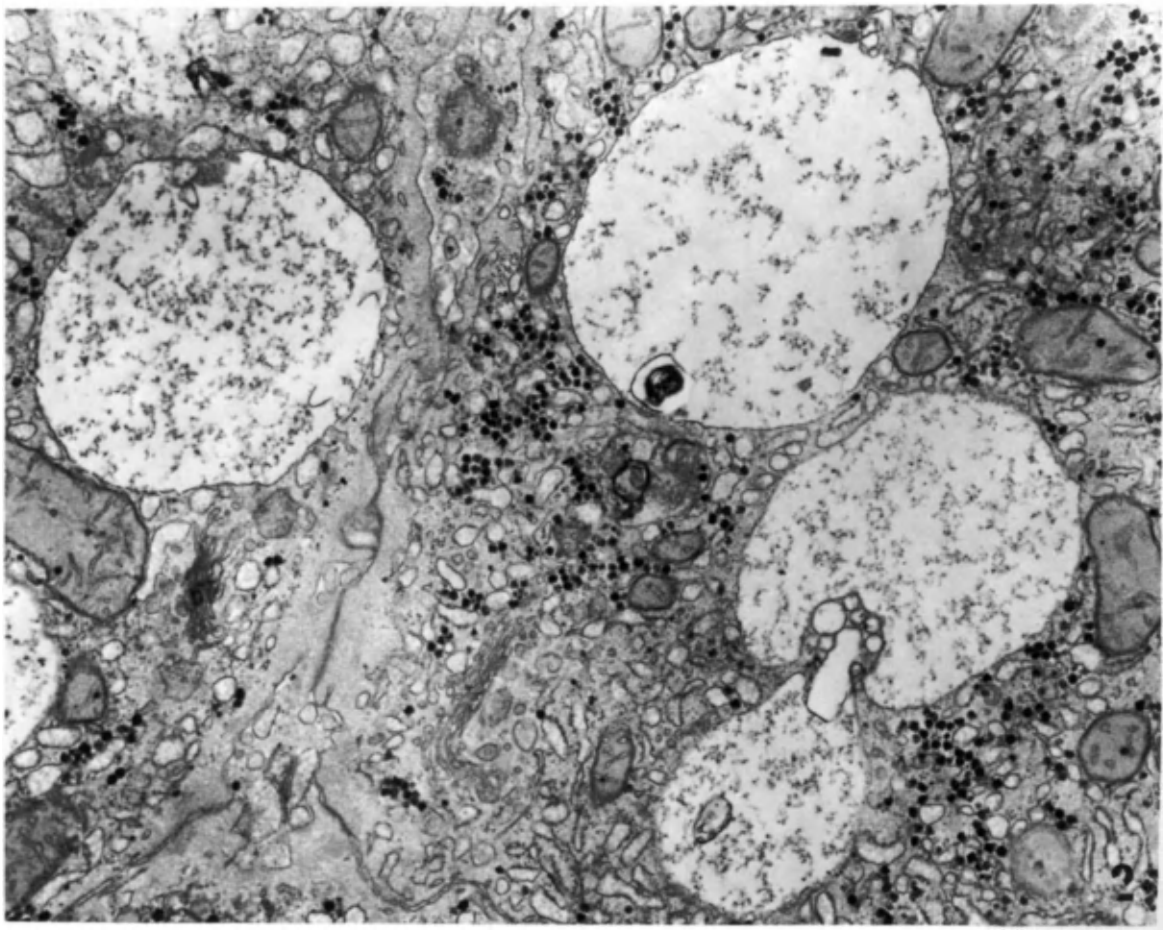
†Conjunctival biopsy is a simple procedure which permits early recognition and often accurate diagnosis of a large number of lysosomal storage diseases (Libert, 1980).

Plate 311

Fig. 1. Conjunctival biopsy from a case of Hurler's disease. The cells are loaded with innumerable lysosomes with lucent contents. × 7000 (*Dustin and Libert, unpublished electron micrograph*)

Fig. 2. Liver biopsy from a case of Hurler's disease. The large lucent lysosomes contain a reticulo-particulate material. × 18000 (*Dustin, unpublished electron micrograph*)





Lysosomes in metachromatic leucodystrophy (sulphatoidosis)

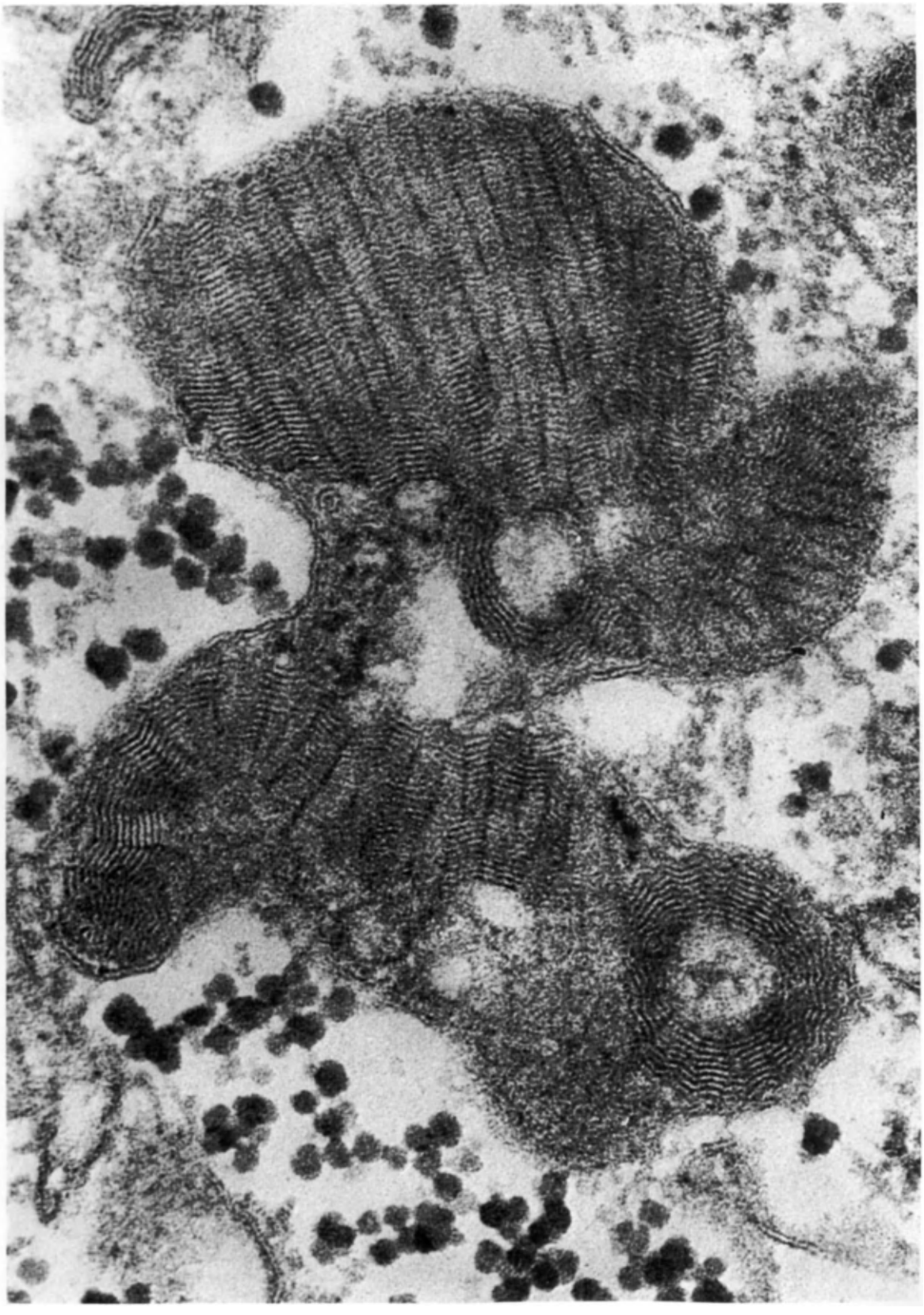
Metachromatic leucodystrophy (also called sulphatoidosis) is a lysosomal disease produced by the deficiency of the lysosomal enzyme arylsulphatase A. This leads to the accumulation of metachromatic lipids (ceramide galactosyl sulphate) in lysosomes (Austin *et al.*, 1965; Austin, 1973). These sulpholipids which accumulate in the brain, peripheral nerves, kidney, liver, spleen, gall bladder and various other organs are stained brownish red by toluidine blue. Several types or forms of the disease occur, death usually resulting from extensive destruction of myelin and resultant impairment of neuronal function.

The morphology of lysosome contents in sulphatoidosis is quite specific and different from that found in other lipoidoses. The sulpholipid deposits in lysosomes present as lamellar structures which show herringbone and prismatic patterns (*Plate 312*) (Grégoire, 1964; Grégoire *et al.*, 1966; Résibois, 1969, 1971; Résibois *et al.*, 1970; Hassoun *et al.*, 1971; Anzil *et al.*, 1973; Rutsaert *et al.*, 1973; Dustin *et al.*, 1978). Credit for first observing these inclusions goes to Grégoire (1964). The desquamation of renal cells containing characteristic sulphatide deposits is of diagnostic value (Pilz *et al.*, 1973).

The characteristic lysosomes with prismatic and herringbone patterned contents have been produced (Rutsaert *et al.*, 1973) by exposing cultured fibroblasts from cases of sulphatoidosis (and from a heterozygous carrier) to sulphatides extracted from normal human brain. On rare occasions such deposits are also found in normal fibroblasts after treatment with much larger doses of sulphatides. Anzil *et al.* (1973) have succeeded in producing the lysosomes with characteristic contents by intracerebral injections of sulphatides in the rabbit.

Plate 312

Cultured fibroblast from a case of sulphatoidosis exposed to 20 μg/ml of sulphatides showing a large lysosome with herringbone formations in its interior. × 200 000 (*From Rutsaert, Menu and Résibois, 1973*)



Curvilinear bodies in lysosomes

Curvilinear membranous formations (C-shaped or worm-like profiles) have been seen lying free in the cytoplasmic matrix in several conditions (page 512). These structures derived from the endoplasmic reticulum are a variety of membrane complex. Quite distinct and different are the much smaller structures usually called 'curvilinear bodies' (to be more accurate, profiles) which are found constantly in lysosomes in certain lysosomal diseases and rarely also in other conditions (*see below*). Two types of curvilinear bodies can be identified in published electron micrographs. The first variety (which one may refer to as Type A curvilinear bodies) presents as small electron-dense, C-shaped or worm-like profiles about 15 to 20 nm thick. The average width of the curved profiles is about 0.1 μm. In some published electron micrographs, alternating dense and light 'lines' have been resolved in these crescentic elements. The periodicity of these is said to be 4–4.5 nm (e.g. Gonatas *et al.*, 1968). The second type (which one may refer to as Type B curvilinear bodies) also present as C-shaped or worm-like profiles. These bodies at first sight appear to be longitudinal sections through tubules. However, convincing circular profiles representing transverse sections of tubules are rarely if ever encountered, hence, one has to concede that these formations are not tubules but perhaps paired lamellae with a lucent space between them (overall width as calculated from published electron micrographs appears to be about 30–40 nm).

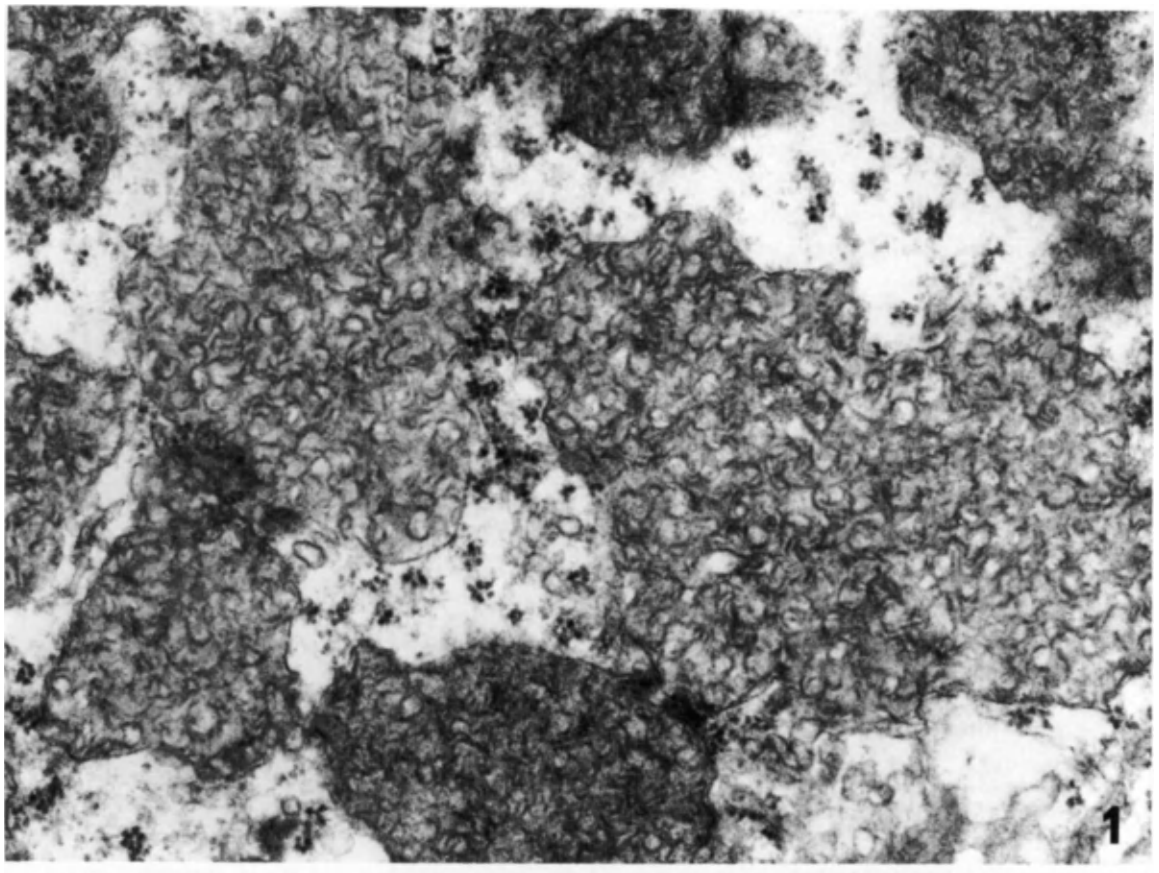
Lysosomes containing Type A curvilinear bodies (*Plate 313*) have been found in certain types of ceroid-lipofuscinoses (Zeman and Siakotos, 1973; Zeman, 1974). The ceroid-lipofuscinosis may be classified as follows (Dustin *et al.*, 1978): (1) infantile form (onset first year); (2) late infantile form (Jansky-Bielchowsky disease onset one to four years; death before five years); (3) juvenile form (Batten's disease or Spielmyer-Sjogern disease; onset five to eight years); and (4) adult form (Kuf's disease; onset 20 years or more).

Lysosomes containing Type A curvilinear bodies have been seen in ceroid-lipofuscinosis with onset between two to four years (Zeman and Donahue, 1963; Gonatas *et al.*, 1968; Carpenter *et al.*, 1972; Williams *et al.*, 1977). In cases with later onset one usually finds lysosomes containing whorled or stacked myelin figures, but cases also occur (intermediate types) where all three varieties (containing whorled, stacked and curvilinear profiles) of lysosomes are found (Zeman and Donahue, 1963). In addition to the central nervous system, lysosomes containing curvilinear bodies have been found in virtually every organ in the body. This suggests a

Plate 313

Fig. 1. An irregular shaped lysosome containing Type A curvilinear bodies in the cerebral neuron of a case of Batten's disease. × 49 000 (*From a block of tissue supplied by Dr A. H. Cameron*)

Fig. 2. Lysosomes containing Type B curvilinear bodies found in lung tissue from a case of Farber's disease × 36 000 (*Dustin, unpublished electron micrograph*)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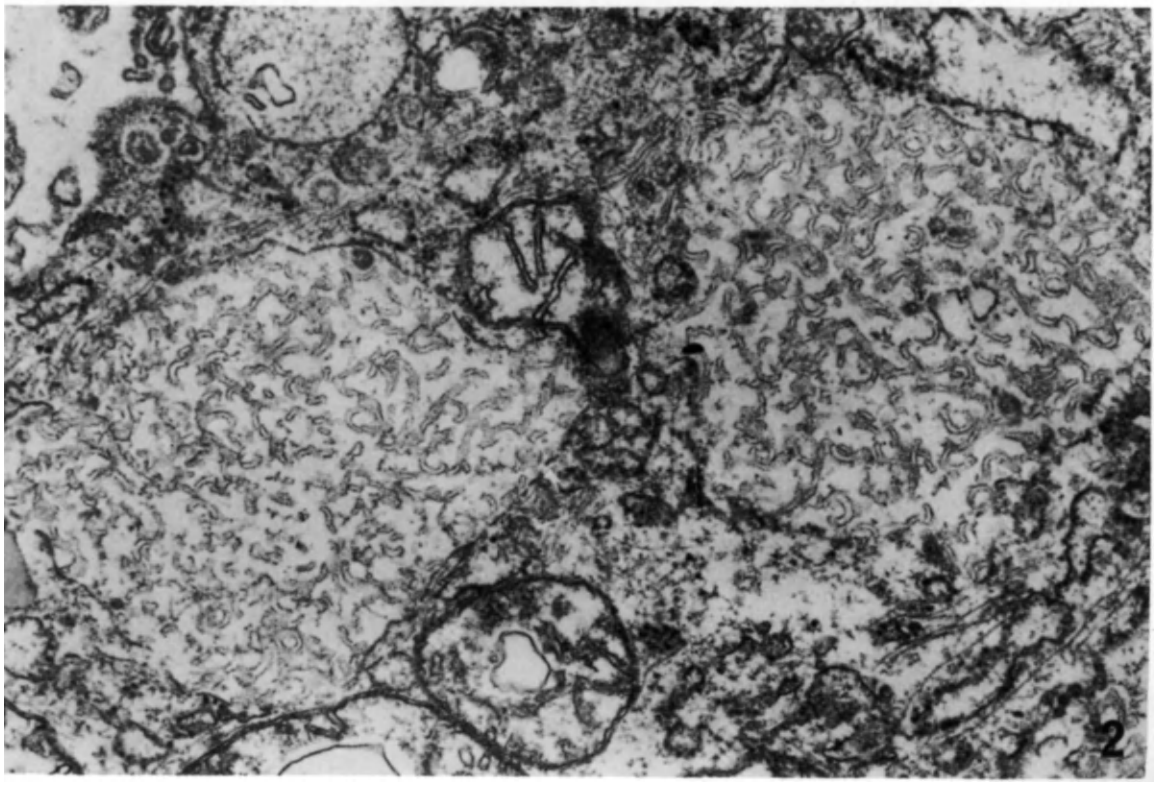
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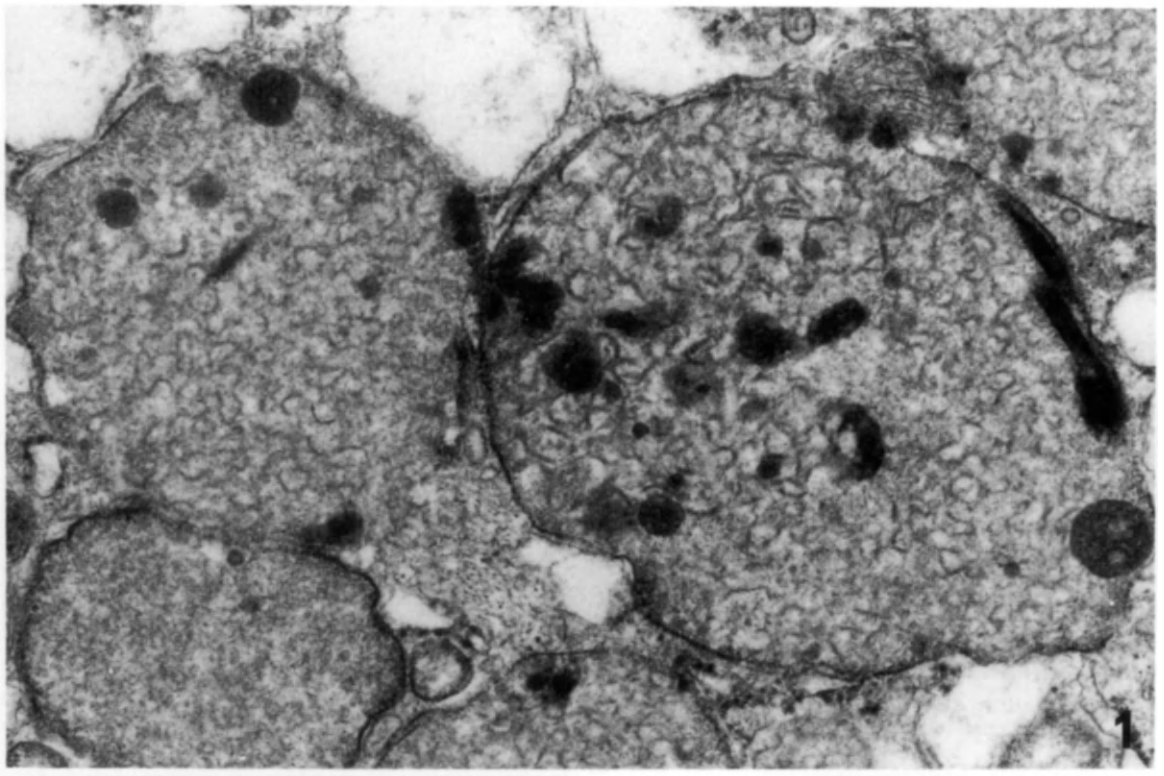
generalized metabolic disorder but little is known about the biochemical defect underlying this group of diseases. Ultrastructurally the lysosomes containing these curvilinear bodies do not resemble lipofuscin granules, but like lipofuscin they have a golden yellow colour and are autofluorescent. Lysosomes containing Type B curvilinear bodies have been seen in ceramidosis (Farber's disease, also called 'disseminated lipogranulomatosis') (Van Hoof and Hers, 1973; Rutsaert *et al.*, 1977; Schmoeckel and Hohlfed, 1979; Tanaka *et al.*, 1979; Burck *et al.*, 1985; Takahashi and Naito, 1985). In this rare disease nodular accumulations of histiocytes containing ceramide occur usually in tissues around the joints. This accumulation of ceramide in lysosomes is due to the deficiency of the lysosomal enzyme ceramidase. Rutsaert *et al.* (1977) found that when cultured fibroblasts from a patient with Farber's disease were exposed to ceramides (non-hydroxylated fatty acids), typical lysosomes containing Type B curvilinear bodies developed. Normal fibroblasts overloaded with ceramides showed identical though less numerous inclusions.

What has been said above may have created the impression that lysosomes containing Type B curvilinear bodies are diagnostic of Farber's disease, while the Type A variety are diagnostic of ceroid-lipofuscinosis. This may or may not be correct. The number of published illustrations available for study is exceedingly small, hence dogmatic assertions are unwarranted. Further, as pointed out at the beginning of this section of the text, a few curvilinear profiles in lysosomes or a few lysosomes containing numerous curvilinear profiles are at times seen even when no genetic lysosomal disease is present. The best example of this (*Plate 314*) that I have seen was in a lymph node biopsy from a case of T-cell lymphoma. Lysosomes containing curvilinear bodies were found in cells acceptable as stromal fibroblasts rather than macrophages or neoplastic cells because they were often fusiform and contained much vesiculated rough endoplasmic reticulum.

Plate 314

From a biopsy of a lymph node from a case of T-cell lymphoma.
Figs. 1–3. Lysosomes containing curvilinear bodies. × 43 000; × 35 000; × 67 000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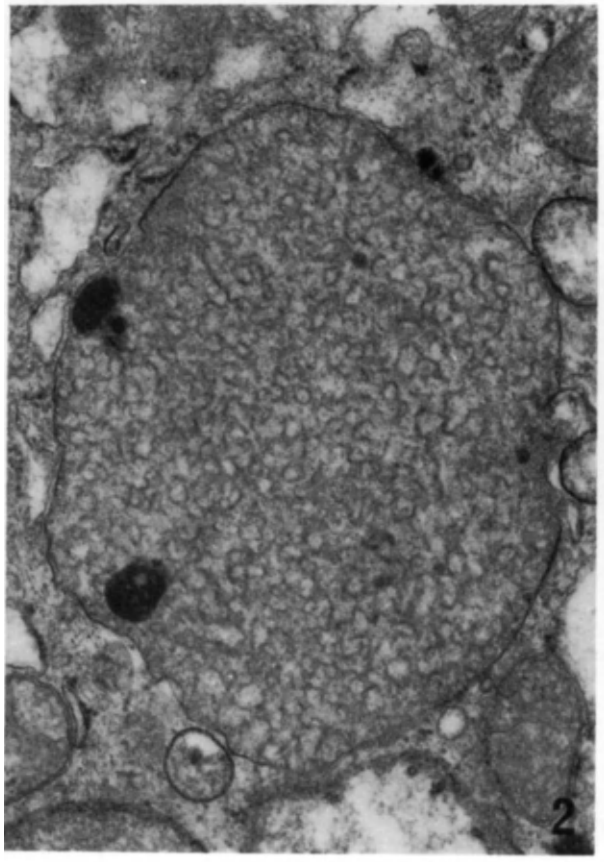
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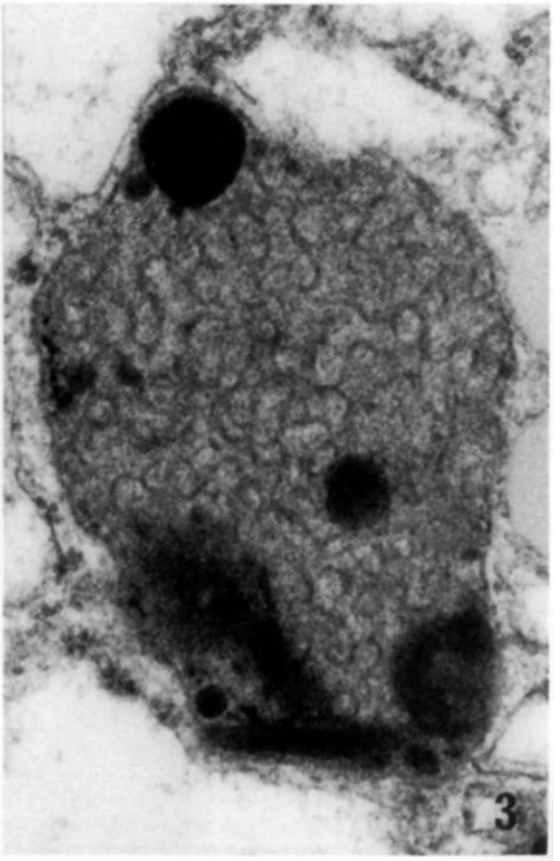

3

Collagen in lysosomes

It is now generally accepted that while the structural units of collagen are synthesized intracellularly, aggregation into fibrils occurs outside the cell. However the manner in which collagen fibrils are resorbed or removed is less well established. In most tissues collagen appears to be quite stable and provides little opportunity for studying its turnover. This phenomenon may be studied more readily in situations where substantial amounts of collagen are degraded and removed over a short period of time. Such situations include the resorption and remodelling of tissues which occurs during: (1) morphogenesis and metamorphosis (Weber, 1963; Gross, 1964; Usuku and Gross, 1965; Woessner, 1968); (2) wound healing (Grillo and Gross, 1967; Ross and Odland, 1968; Williams, 1970); and (3) post-partum involution of the uterus (Deno, 1936; Harkness and Moralee, 1956; Maibenco, 1960; Lobel and Deane, 1962; Woessner, 1962, 1965; Luse and Hutton, 1964; Schwarz and Guldner, 1967; Parakkal, 1968).

Many biochemical studies have shown a marked increase in acid hydrolases in tissues undergoing resorption and remodelling (Harkness and Moralee, 1956; Lobel and Deane, 1962; Weber, 1963; Goodall, 1965; Woessner, 1965, 1968) but early attempts to demonstrate collagenase in such systems were unsuccessful. However, Gross and his colleagues (Gross *et al.*, 1963; Gross, 1964; Grillo and Gross, 1967) have now succeeded in demonstrating a collagenase in regressing tissue which at physiological temperatures and neutral pH (hence non-lysosomal) is capable of lysing a reconstituted collagen gel.

Animal collagenase, unlike bacterial collagenase, appears to be incapable of completely degrading collagen, and Woessner (1968) has proposed that the collagen fibrils are first fragmented by the collagenase (non-lysosomal) and then picked up by phagocytic cells where they suffer further degradation within lysosomes.

Various electron microscopic studies (e.g. on involuting rat and mouse uterus) support such a concept for cells (macrophages and fibroblasts) where numerous single-membrane-bound vacuoles containing collagen are found (*Plates 315* and *316*). These have been regarded as heterosomes and heterolysosomes since disintegrating collagen fibrils and acid phosphatase activity has been demonstrated in these structures and the dense bodies that evolve from them (Parakkal, 1968).

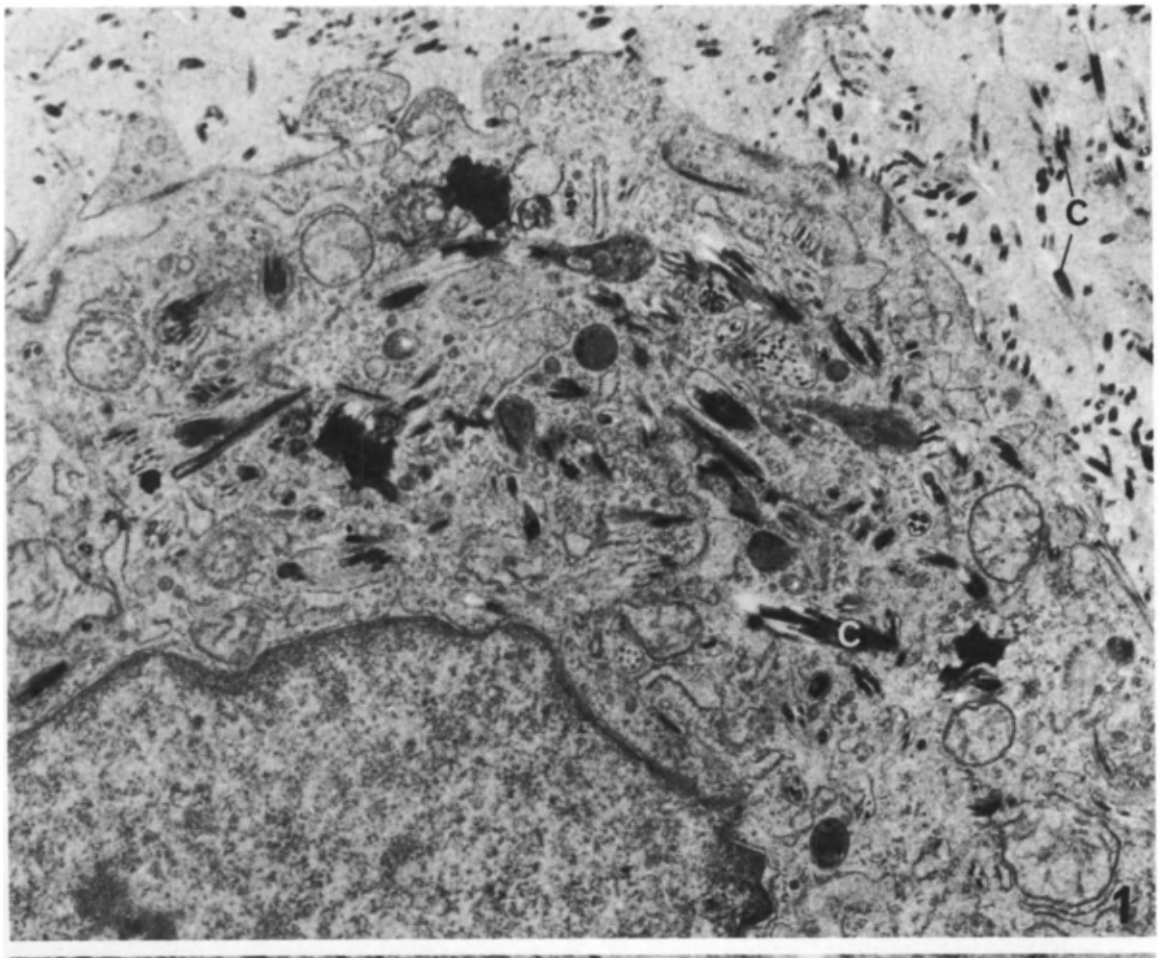
However, the sighting of collagen fibrils in membrane-bound vacuolar or tubular spaces (referred to as 'intracellular collagen') does not always imply phagocytosis of collagen, for there are instances where such images are produced by sections through collagen fibrils lying in bays or invagination of the cell membrane and there are also instances where such images have been interpreted as collagen fibril synthesis occurring in an intracellular location. These matters are discussed later*. Here we review only those studies where intracellular collagen has been regarded as phagocytosed collagen.

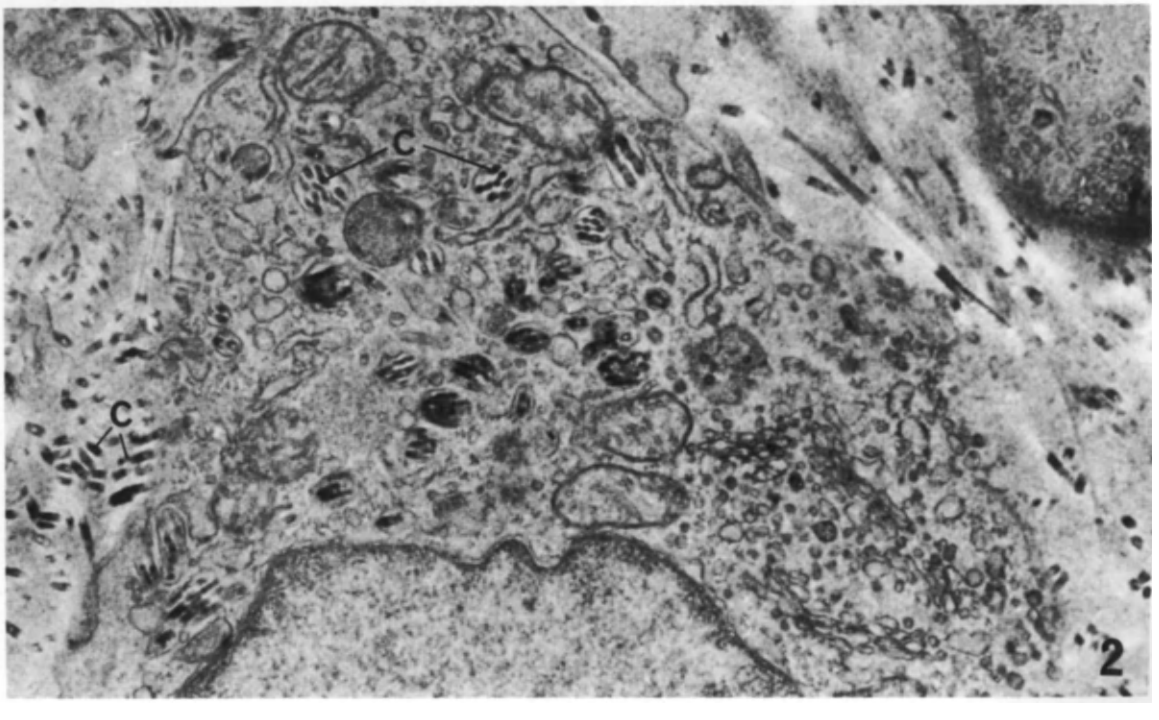
Intracellular collagen fibrils which have been interpreted as phagocytosed collagen fibrils have been seen in: (1) macrophages, fibroblasts and smooth muscle cells during postpartum involution of the rat and mouse uterus (Luse and Hutton, 1964; Schwartz and Guldner, 1967; Parakkal, 1968; Brandes and Anton, 1969); (2) macrophages in the skin of mouse during catagen phase of hair follicle cycle (Parakkal, 1969); (3) macrophages in human skin from cases

*This essay should be read in conjunction with the essay on intracellular and intracytoplasmic collagen fibrils (pages 996–1001) where references and discussion about interpretation of images referred to as 'intracellular collagen' and 'intracytoplasmic collagen' are presented.

Plate 315

Figs. 1. and 2. Macrophages from involuting rat uterus three days after delivery, showing vacuoles containing collagen fibrils cut in various planes. In this preparation the fibrils (C) in the matrix and vacuoles are intensely stained by phosphotungstic acid. × 20000, 23000


C
C
1


C
C
2

of epidermolysis bullosa dystrophica (Pearson, 1962); (4) macrophages and fibroblasts in carrageenan granulomas produced in the subcutis of the guinea-pig and rabbit (Perez-Tamayo, 1970); (5) macrophages and fibroblasts in inflamed (silver nitrate 'burn') rabbit cornea (Graf *et al.*, 1970; Faure *et al.*, 1970a, b); (6) fibroblasts in granulation tissue from joints of rats with an induced adjuvant polyarthritis (Cullen, 1972); (7) macrophages from the tail of frog tadpoles during metamorphosis (Fox, 1972a, b); (8) macrophages from the tail of frog tadpoles treated with thyroxin (Gona, 1969; Usuku and Gross, 1965); (9) cultured fibroblasts from the periodontal ligament (Svoboda *et al.*, 1979); (10) fibroblasts and osteoblasts from cultured bone of chick embryo (Glauert *et al.*, 1969); (11) normal and rheumatoid synovial cells in cultured synovial membrane (Krey *et al.*, 1971); and (12) cells at the pannus–cartilage junction in rheumatoid arthritis (Harris *et al.*, 1977).

From the list presented above it will be apparent that degradation of collagen in lysosomes seems to occur only in certain circumstances characterized by a rapid involution of connective tissue. It will also be apparent that the main cell involved in the resorption of collagen is the macrophage, but the fibroblast also appears to be capable of performing this function. There is, however, some doubt and controversy about this in the literature.

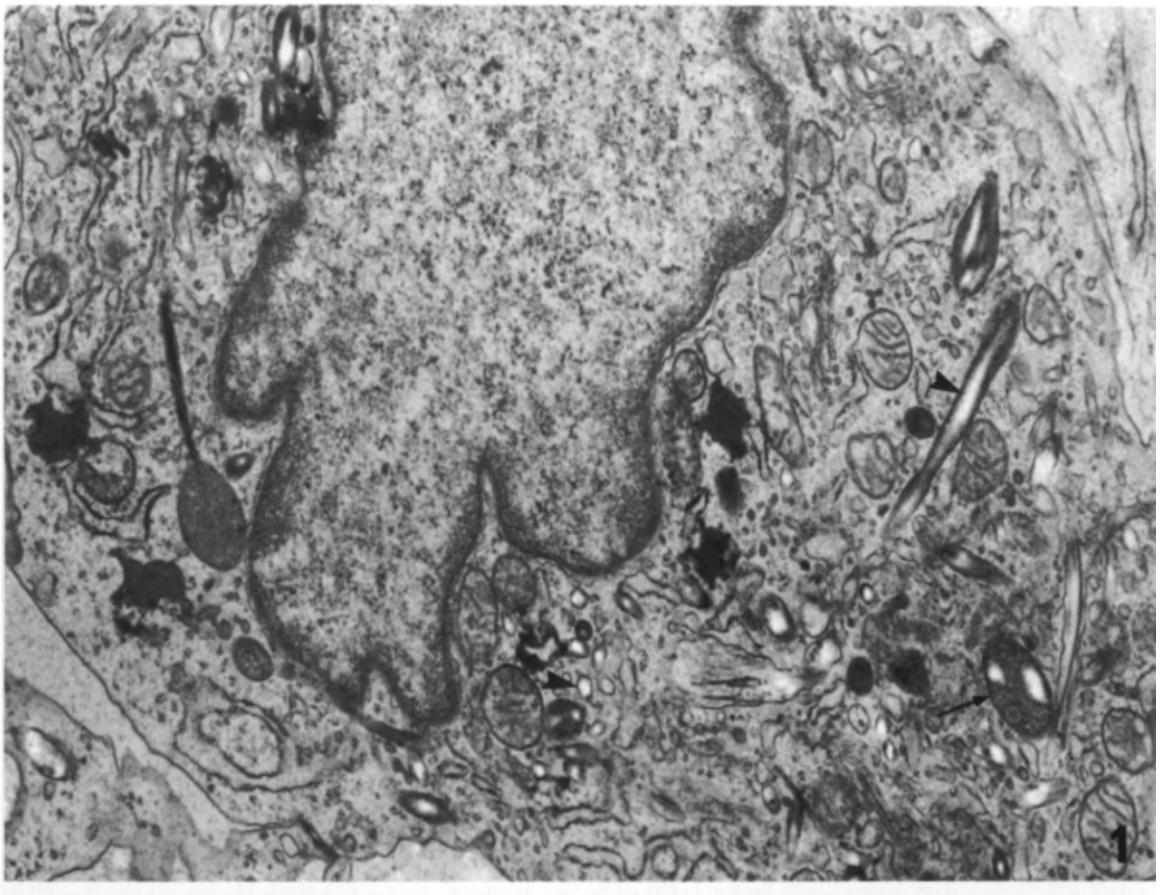
For example, in the case of the involuting uterus, Luse and Hutton (1964) concluded that it was the fibroblast which was the phagocytic cell responsible for the removal of collagen, while Schwartz and Guldner (1967), and Parakkal (1968) consider the phagocytic cells to be macrophages. A major distinction between the fibroblast and macrophage is that in the fibroblast the rough endoplasmic reticulum is abundant but there is usually a paucity of rough endoplasmic reticulum in the macrophage*. However, it is the resting or not too active macrophage which shows a paucity of rough endoplasmic reticulum. Stimulated macrophages (i.e. ones actively engaged in phagocytosis) are somewhat better endowed with rough endoplasmic reticulum, presumably necessary for the synthesis of hydrolytic enzymes and primary lysosomes (for references *see* Parakkal, 1968). Thus there could be occasions when it is difficult to be certain whether one is looking at an activated macrophage or a rather quiescent fibroblast. A further point of distinction which might be helpful is that the rough endoplasmic reticulum of the macrophage appears to be sparsely populated by ribosomes; this is because the polyribosome chains are quite short (four or five ribosomes) as compared to those found in fibroblasts (20 to 30 ribosomes) (Leibovich and Ross, 1975).

However, there is little doubt that fibroblasts are also capable of phagocytosis although usually to a much more limited extent than macrophages. Thus after a haemorrhage many siderosomes develop in macrophages but a few are also seen in cells that are unmistakably fibroblasts (personal observations). Similarly, it would appear that fibroblasts are involved not only in laying down of collagen fibrils but also in their removal.

*In a monograph on the macrophage (Carr, 1973) and in Carr *et al.* (1977) macrophages are repeatedly described as possessing 'a prominent granular endoplasmic reticulum'. However, the illustrations presented to support this statement effectively belie such a contention. One of these (*Fig. 1.6* in Carr *et al.*, 1977) shows a macrophage with so few and such minute wisps of rough endoplasmic reticulum that it could more aptly be labelled as 'poorly endowed with rough endoplasmic reticulum'. The other macrophage (*Fig. 1.5* in Carr *et al.*, 1977) contains some heterolysosomes and shows evidence of some activation. In keeping with this it has slightly more rough endoplasmic reticulum. If such cells are described as having 'prominent rough endoplasmic reticulum' one wonders what term one would use to describe the rough endoplasmic reticulum in plasma cells and fibroblasts.

Plate 316

Figs. 1 and 2. From involuting rat uterus three days after delivery. In this preparation (prepared in the usual way) most of the collagen fibrils (arrowheads) have failed to stain and hence appear electron-lucent. This is popularly referred to as 'negatively stained' collagen (For more details about this phenomenon, *see* page 1216). This is a fortunate situation which permits clear visualization of collagen in lysosomes (arrow). × 19 000, × 19 000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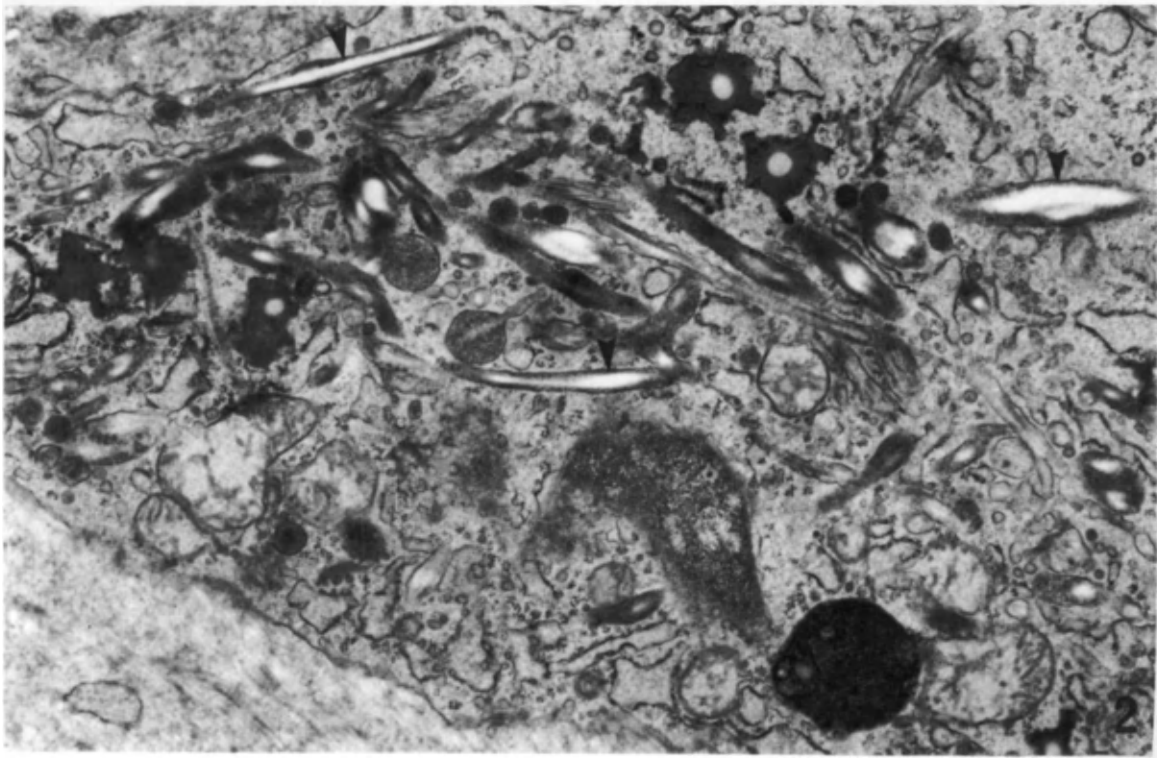
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Glycogen in lysosomes (glycogenosomes)

Accumulation of glycogen in lysosomes has been noted in various conditions but the most marked example of this is seen in Pompe's disease which is also called 'glycogenosis type II'. The diseases we call 'glycogenoses', or 'glycogen storage diseases' are all inherited disorders of glycogen metabolism. Eight types of glycogenoses are known to occur in humans, each characterized by: (1) a deficiency of a particular enzyme involved in glycogen metabolism; (2) the nature of the polyglycan storage (glycogen or amylopectin); and (3) the location of the excess glycogen (for references *see* Dustin *et al.*, 1978). In this chapter devoted to the lysosome, we deal only with the type II glycogenosis which is characterized by the presence of large amounts of glycogen in lysosomes.

In Pompe's disease (glycogenosis type II) there is a deficiency of lysosomal acid α-glucosidase (acid maltase) and large amounts of glycogen accumulate in single-membrane-bound bodies (Hers, 1963; Baudhuin *et al.*, 1964; Cardiff, 1966; Hug and Schubert, 1967; Hers and Van Hoof, 1969), which are regarded as lysosomes because acid phosphatase activity has been demonstrated in them (Garancis, 1968). These glycogen-containing bodies called 'glycogenosomes' have been found in various sites such as striated muscle (heart and skeletal), liver, kidney, pancreas, skin, brain and eye (Witzleben, 1969; Pokorny *et al.*, 1982). However, in striated muscle much of the glycogen lies outside the lysosome (*Plate 317*). It is thought that this might be due to lysosomal rupture by mechanical pressure from muscle contraction. The concomitant release of acid hydrolases would explain the degenerate and necrotic muscle fibres, cell debris and myelin figures seen with the electron microscope and the well known signs and symptoms related to the destruction of myocardium and skeletal muscle seen in patients with Pompe's disease (Hers and Van Hoof, 1969). Not all, however, agree with the idea that extralysosomal glycogen derives from ruptured lysosomes, some hold that it might stem from an additional metabolic defect which involves cytoplasmic glycogen metabolism (for references *see* Walvoort *et al.*, 1985). Most workers, however, do not accept this idea.

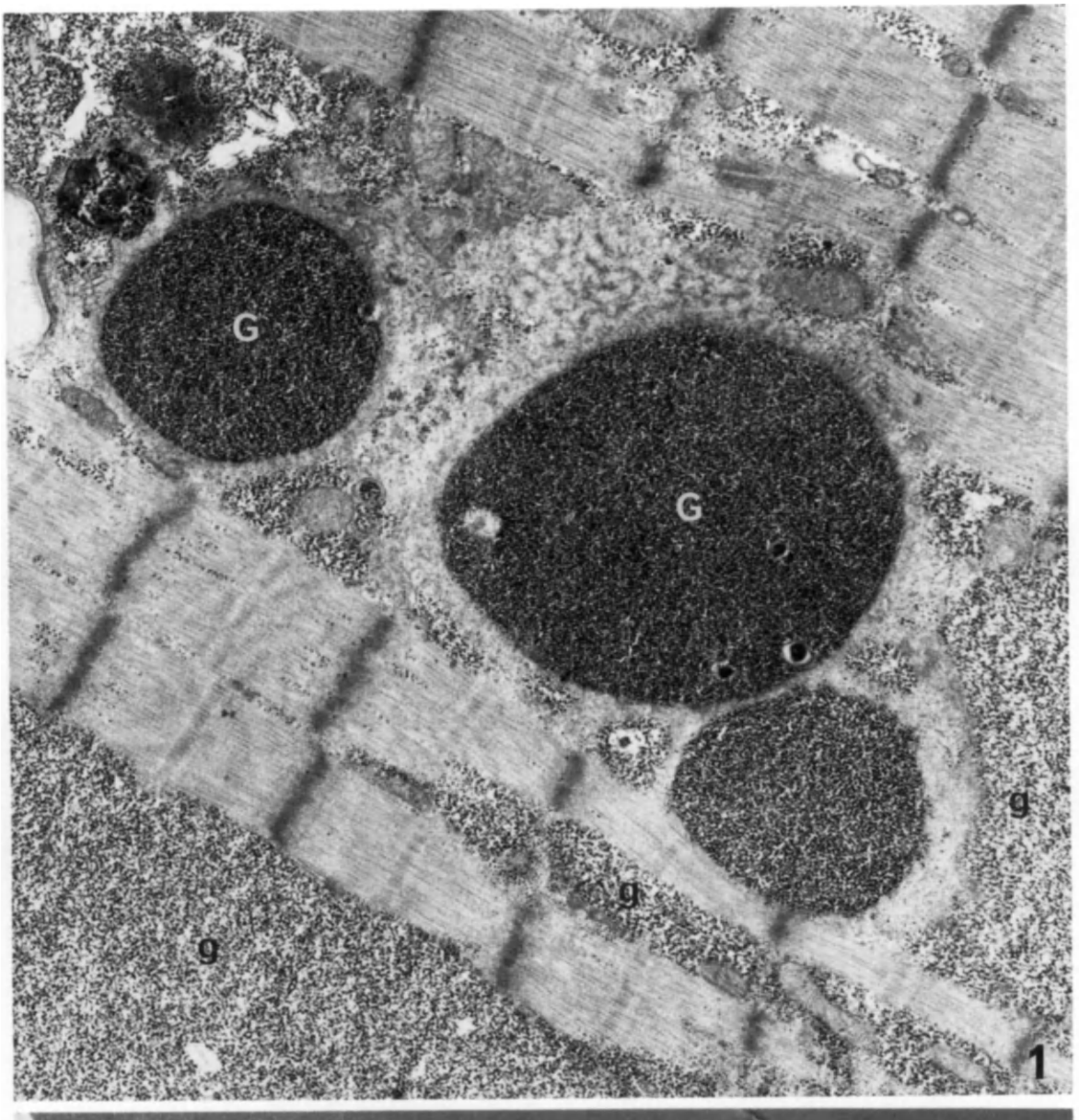
Pompe's disease has also been reported to occur in: (1) cats (glycogenosomes were present in the central nervous system) (Sandstrom *et al.*, 1969); (2) dogs (Mostafa, 1970; Walvoort *et al.*, 1981); (3) cattle (Richards *et al.*, 1977; O'Sullivan *et al.*, 1981); and (4) Japanese quail (Murakami *et al.*, 1980).

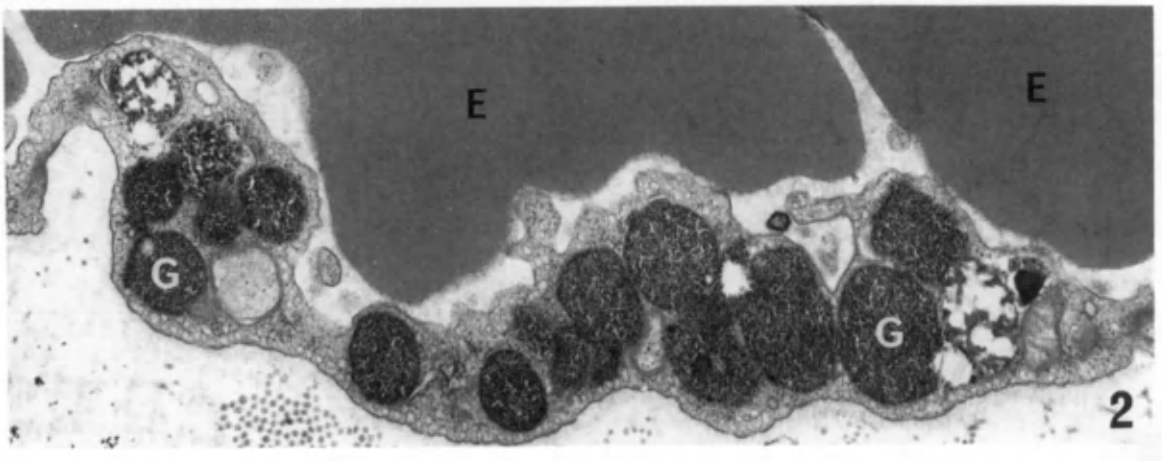
Plate 317

Muscle biopsy from a case of Pompe's disease. (*From a block of tissue supplied by Dr Y. S. Lee*)

Fig. 1. Glycogenosomes (G) and large accumulations of intracytoplasmic glycogen (g) are seen between the myofibriles. × 23 000

Fig. 2. A capillary with erythrocytes (E) in its lumen. The endothelial cells contain several glycogenosomes (G). × 19 000


G
G
g
g
g
1


E
E
G
G
2

It is worth noting that single-membrane-bound structures containing glycogen have been seen in muscle tissues on several occasions, and not only in Pompe's disease. We have noted occasional membrane-bound structures containing glycogen in biopsies of human myocardium and skeletal muscle (*Plate 318, Figs. 1 and 2*). Engel (1969) states that 'occasional glycogen sequestration in simple membrane-bound sacs can be seen in any myopathy while the presence of glycogen in ordinary autophagic vacuoles with heterogeneous contents occurs in chloroquine myopathy, polymyositis and other chronic myopathies'. His studies show that acid phosphatase activity can be demonstrated in some but not all single-membrane-bound structures containing glycogen.

Autophagic vacuoles (autolysosomes) containing glycogen have been found in many cell types. Even so, it must be conceded that this is rather a rare phenomenon. We have found autolysosomes containing glycogen in: (1) erythrocytic cells from cases of erythroleukaemia (*Plate 318, Fig. 3*); (2) a carcinogen-induced subcutaneous sarcoma of the rat (*Plate 318, Fig. 4*); (3) a normal cow hepatocyte (*Plate 318, Fig. 5*); (4) cells in the mucosa and submucosa in melanosis coli (*Plate 319, Figs. 1 and 2*); and (5) in the liver of rats treated with toxohormone (Parry and Ghadially, 1970). In most of these examples, the amount of glycogen found in the lysosome is quite small and one might contend that such inclusions of glycogen in autolysosomes are fortuitous.

On rare occasions a few glycogen particles have been seen in some lysosomes of apparently normal adult rat liver, but lysosomes containing much glycogen are not hard to find in the livers of newborn mice and rats (Jézéquel *et al.*, 1965; Phillips *et al.*, 1967).

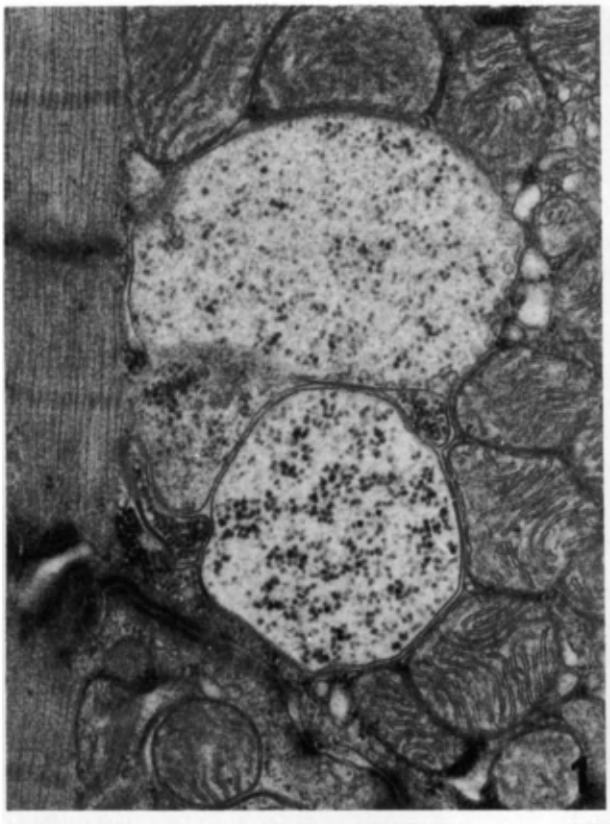
Plate 318

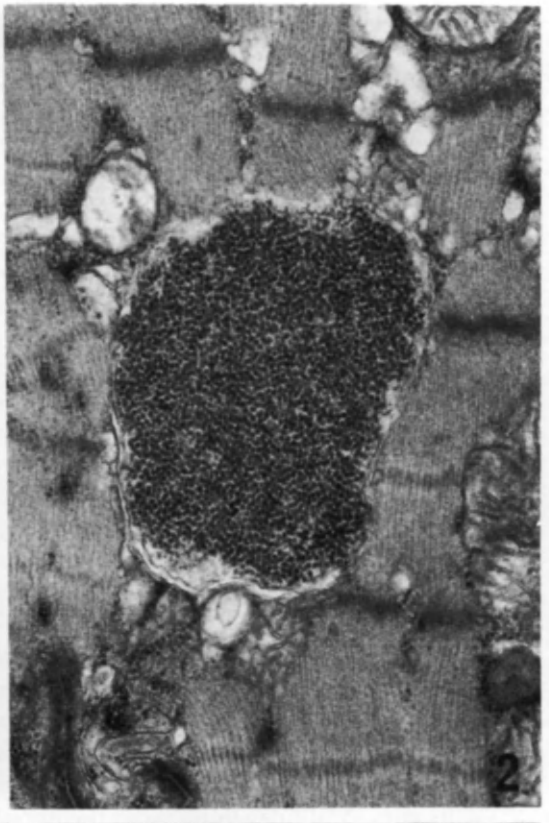
Figs. 1 and 2. Membrane-bound collections of glycogen found in human myocardium. Whether these structures are lysosomes or not is debatable. × 30 000; × 30 000

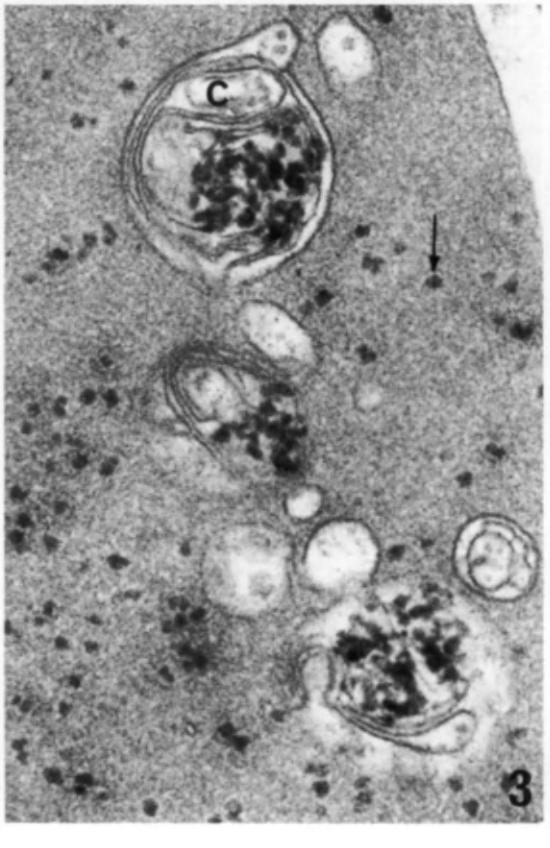
Fig. 3. Erythroleukaemia. Erythrocyte with monoparticulate glycogen in cytoplasm (arrow) and several autolysosomes (C), some containing glycogen deposits. × 63 000 (*From Skinnider and Ghadially, 1973*)

Fig. 4. Glycogen-containing autolysosomes found in a tumour cell. From a carcinogen-induced subcutaneous sarcoma of the rat. × 28 000 (*Parry and Ghadially, unpublished electron micrograph*)

Fig. 5. Autolysosome containing glycogen rosettes found in a normal cow hepatocyte. × 72 000 (*Ghadially and Ailsby, unpublished electron micrograph*)



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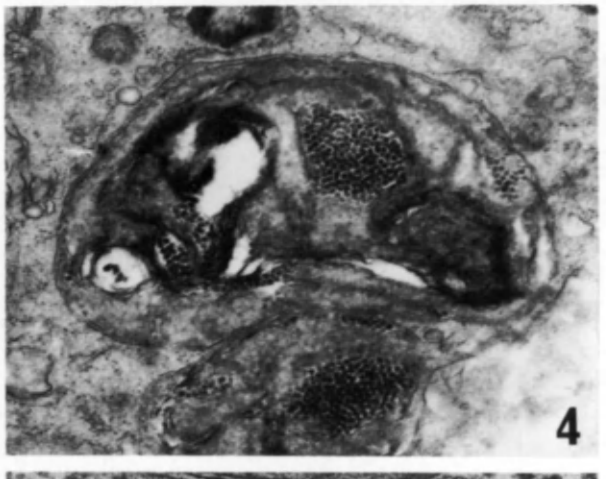
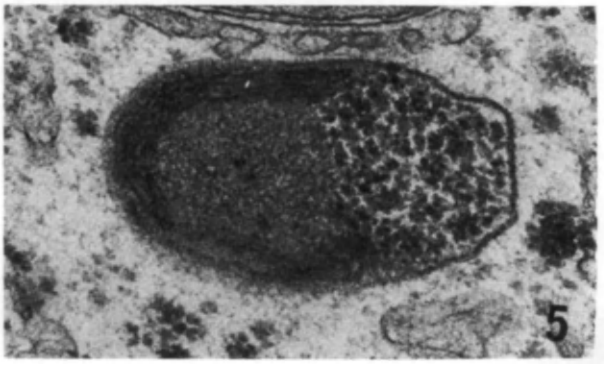




Glycogen-laden lysosomes have not been reported to occur in experimental animals after the administration of barbiturates, and our (Ghadially and Bhatnagar, unpublished observations) experience is generally in keeping with this. However, in one hamster that had been so treated numerous massively enlarged glycogen-containing lysosomes were noted (*Plate 319*). Some of the lysosomes seemed to contain only glycogen but others contained small amounts of membranous and osmiophilic material also. The appearances seen here are in every way similar to those found in Pompe's disease but the significance of this finding is hard to assess on the limited data available. Glycogen-containing lysosomes have also been found in the tubular cells of the kidney of diabetic spiny mice (*Acomys cahirinus*) and in rats made hyperglycaemic by the administration of streptozotocin (Junod *et al.*, 1969; Orci *et al.*, 1970; Orci and Stauffacher, 1971). Here also some 'pure' glycogen-containing lysosomes were seen but others contained lipid and membranous structures as well as glycogen.

The functional basis of the accumulation of glycogen in lysosomes is not established unequivocally except in the case of Pompe's disease. Here it is clear that a genetic defect leading to the absence of a single lysosomal enzyme (acid maltase), necessary for the breakdown of glycogen to glucose, produces accumulations of glycogen in lysosomes. Although the lysosomal pathway for glycogen breakdown is said to be a minor one compared to phosphorylytic degradation, and the latter system is normal in patients with Pompe's disease, it is not difficult to visualize how, over a period of time, massive lysosomes containing glycogen develop.

Regarding the occurrence of glycogen-containing lysosomes in newborn animals, it is thought that there might be a transient enzyme deficiency in the lysosome during the neonatal period or that a relative deficiency of enzyme develops due to a rapid increase of glycogen metabolism at this stage of life (Jézéquel *et al.*, 1965; Phillips *et al.*, 1967). In diabetic animals there is an excess of cytoplasmic glycogen in the kidney tubules; therefore a relative enzyme deficiency could develop due to the excessive amounts of substrate taken in by the lysosomes. On the other hand, one could speculate that there might be a primary diabetes-induced enzyme defect produced in these animals (Orci and Stauffacher, 1971). In this connection it may be noted that hyperglycaemia occurs in these animals and it has been found that the presence of glucose is known to diminish the activity α-1,4-glucosidase.

An occasional lysosome containing glycogen may be found in several types of tumours (e.g. Ewing's tumour and rhabdomyosarcoma), particularly when abundant intracytoplasmic glycogen is present. However, the sugar tumour of the lung is in a class by itself because the cells are loaded with innumerable single-membrane-bound bodies containing glycogen* (for references *see* Ghadially, 1985 and Andrion *et al.*, 1985). Massive amounts of monoparticulate and rosette forms of glycogen are found also in the cytoplasm of the tumour cells.

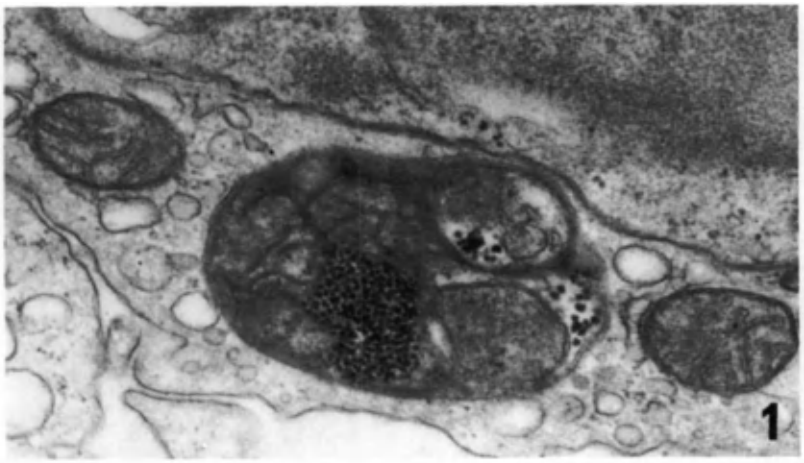
The significance of the occurrence of glycogen in presumably lysosomal bodies in the sugar tumour is obscure. One might, however, speculate that the somatic mutation which presumably leads to the production of the sugar tumour also encompasses another defect — namely an inability to produce acid maltase, and hence, glycogen accumulates in the lysosomes of this tumour (Ghadially, 1985).

*Glycogenosomes have been reported to occur in all sugar tumours studied with the electron microscope, except in a case described by Zolliker *et al.* (1979) where only abundant intracytoplasmic glycogen was present. I too have seen a tumour which was clinically and histologically acceptable as a sugar tumour where I could not find glycogenosomes but abundant intracytoplasmic glycogen was present.

Plate 319

Figs. 1 and 2. Autolysosomes containing glycogen found in an unidentified cell in the submucosa of a case of melanosis coli. *See also Plate 293, Fig. 2.* × 34 000; × 34 000 (*Ghadially and Parry, unpublished electron micrographs*)

Fig. 3. Lysosomes containing glycogen found in the hepatocyte of a hamster that had received phenobarbitone. × 36 000 (*Ghadially and Bhatnagar, unpublished electron micrograph*)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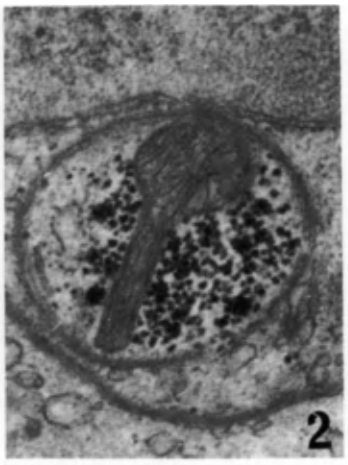
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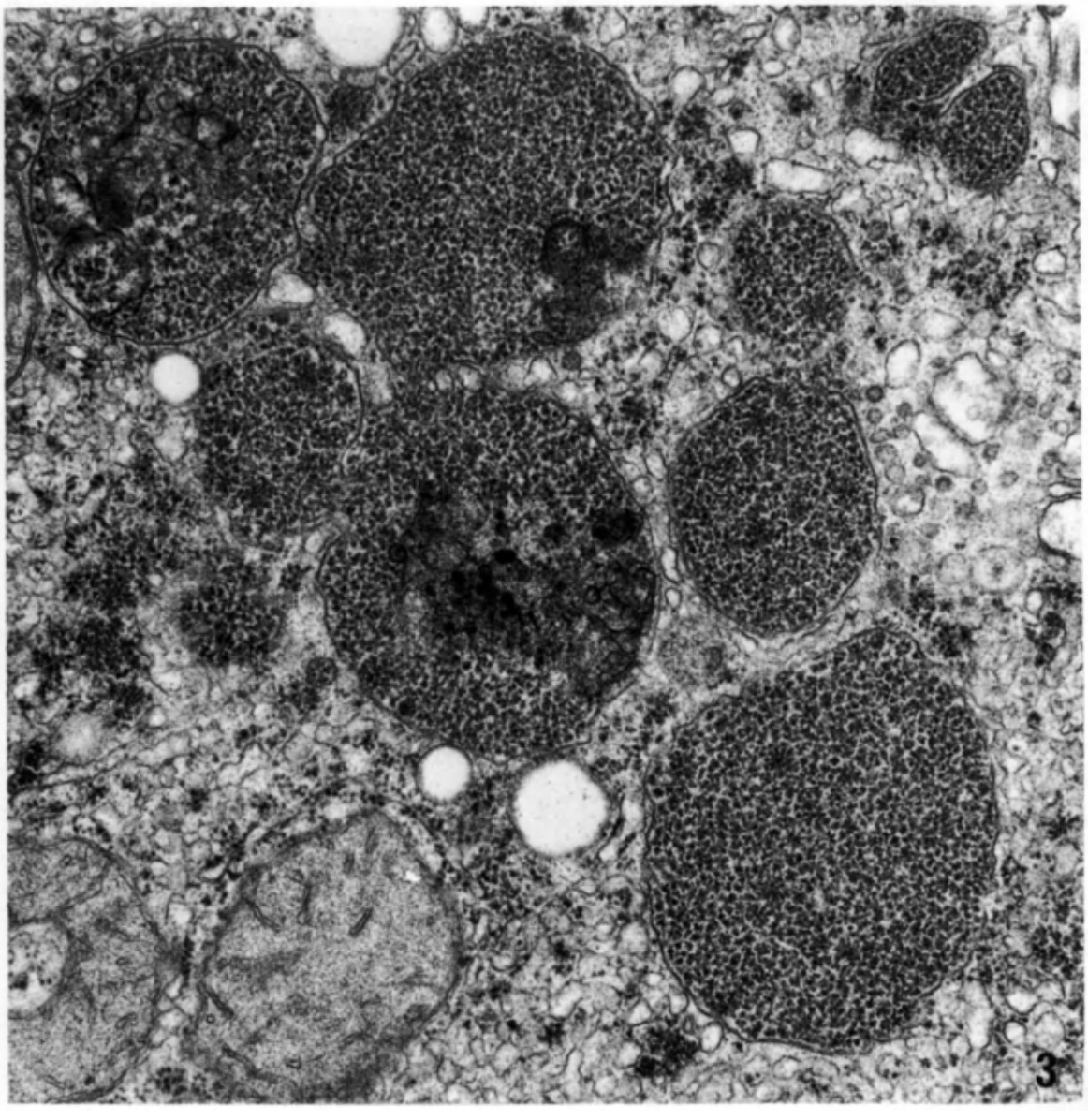
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Metals in lysosomes

Numerous studies now attest to the fact that lysosomes in various tissues come to contain metals, such as iron (*Plate 320*), uranium (*Plate 321*), gold (*Plates 322–324*), platinum (*Plate 325*),copper, silver, lead, mercury and thorium when compounds of these metals are administered to experimental animals (Kent *et al.*, 1963a, b, 1965; Koenig, 1963; Taylor, 1965; Ganote *et al.*, 1966; Ericsson, 1969; Wessel *et al.*, 1969; Southwick and Bensch, 1971; Oryschak and Ghadially, 1974; Gooneratne *et al.*, 1980)*. Needless to say this is not the only site where metals can accumulate, but in various tissues lysosomes are the most common site of accumulation of a variety of metals and there is evidence that this may be of pathological import.

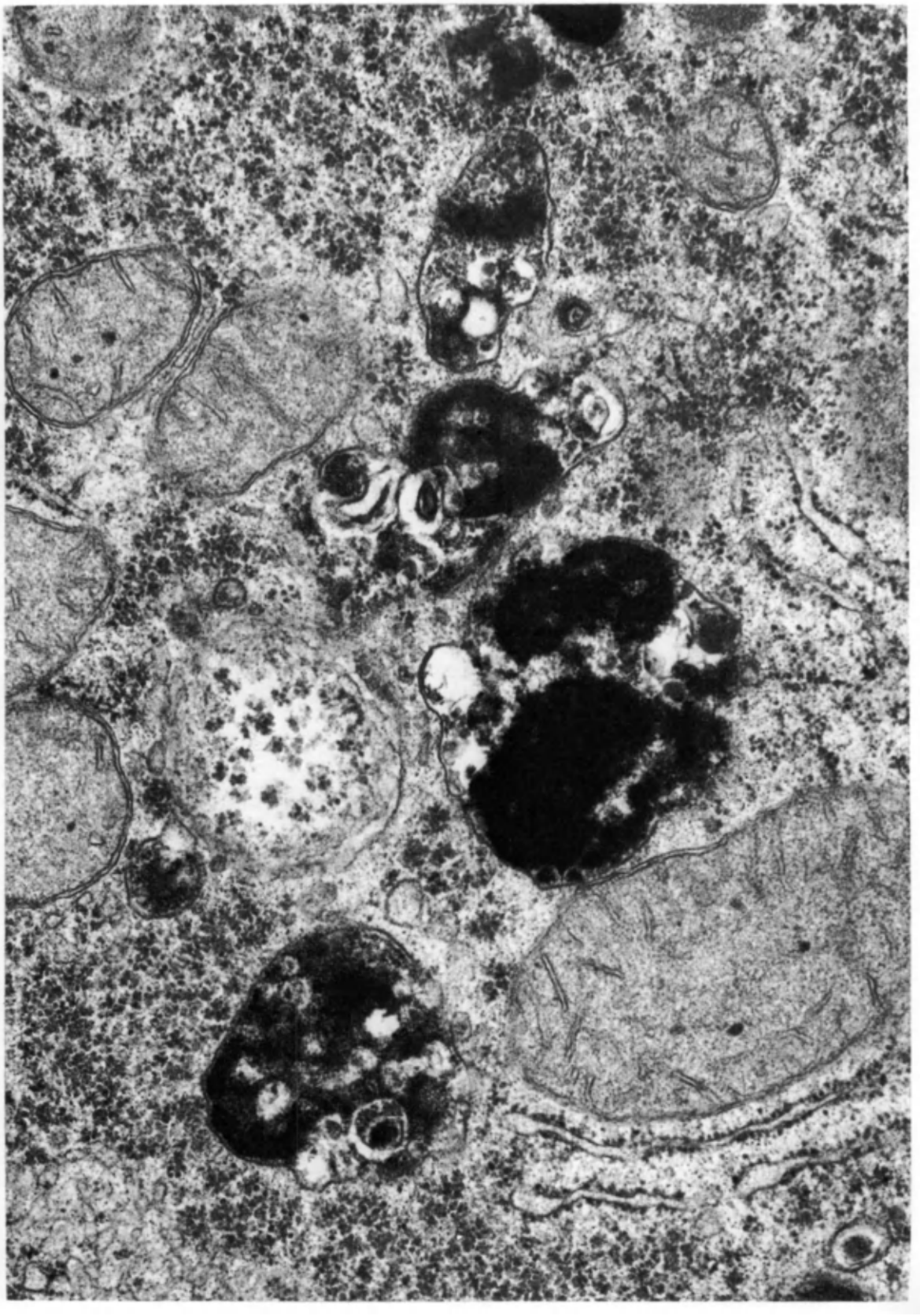
The accumulation of metals in lysosomes is also seen in some human diseases. For example, in Wilson's disease where there is an inherited disorder of copper metabolism, copper accumulates in the hepatocellular lysosomes (Goldfischer and Moskal, 1966; Hanaichi *et al.*, 1984), while in haemachromatosis iron deposits occur in hepatocellular lysosomes (Novikoff and Essner, 1960; Kent *et al.*, 1963a, b). Similarly, in human and experimentally produced chronic haemarthrosis, synovial membrane and articular cartilage come to contain numerous iron-loaded lysosomes (siderosomes). (For a review and references, *see* Ghadially, 1985.) In all the above-mentioned instances, varying degrees of cell necrosis and/or fibrosis are seen, and it has long been debated to what extent, if any, the metallic deposits in the tissue are responsible for such pathological changes. Against such an idea it has been pointed out that: (1) the normal fetal and newborn liver can tolerate concentrations of copper comparable to those seen in Wilson's disease and that large amounts of copper may be present with normal liver function; (2) in haemachromatosis the cirrhosis can antedate the iron deposits, and there is evidence that nutritional deficiencies are also operative which may be responsible for the cirrhosis; and (3) the studies of Kent *et al.* (1963a, b) have shown that substantial quantities of iron are not taken up by hepatocytes unless these cells are first damaged by hepatotoxic agents.

Nevertheless, there is now a growing body of evidence which suggests that metallic deposits in lysosomes are not innocuous and that they can contribute to the pathological changes that occur. It is thought that if lysosomes are overloaded with indigestible materials, or substances which inhibit enzyme activity so that their normal function is impaired, rupture of lysosomes or seepage of enzymes may occur, leading to cell damage and necrosis.

*Intralysosomal crystalline deposits of metals and minerals may produce lysosomes with angulate profiles. Such lysosomes called 'angulate lysosomes' are described on page 698–707. Not all metallic deposits produce angulate lysosomes, and in fact the term angulate lysosomes is rarely used to describe lysosomes containing metals because it is much more informative to name the lysosome by the metal it contains.

Plate 320

Iron-loaded electron-dense lysosomes found in the liver of a rabbit that had received injections of iron dextran. × 48 000

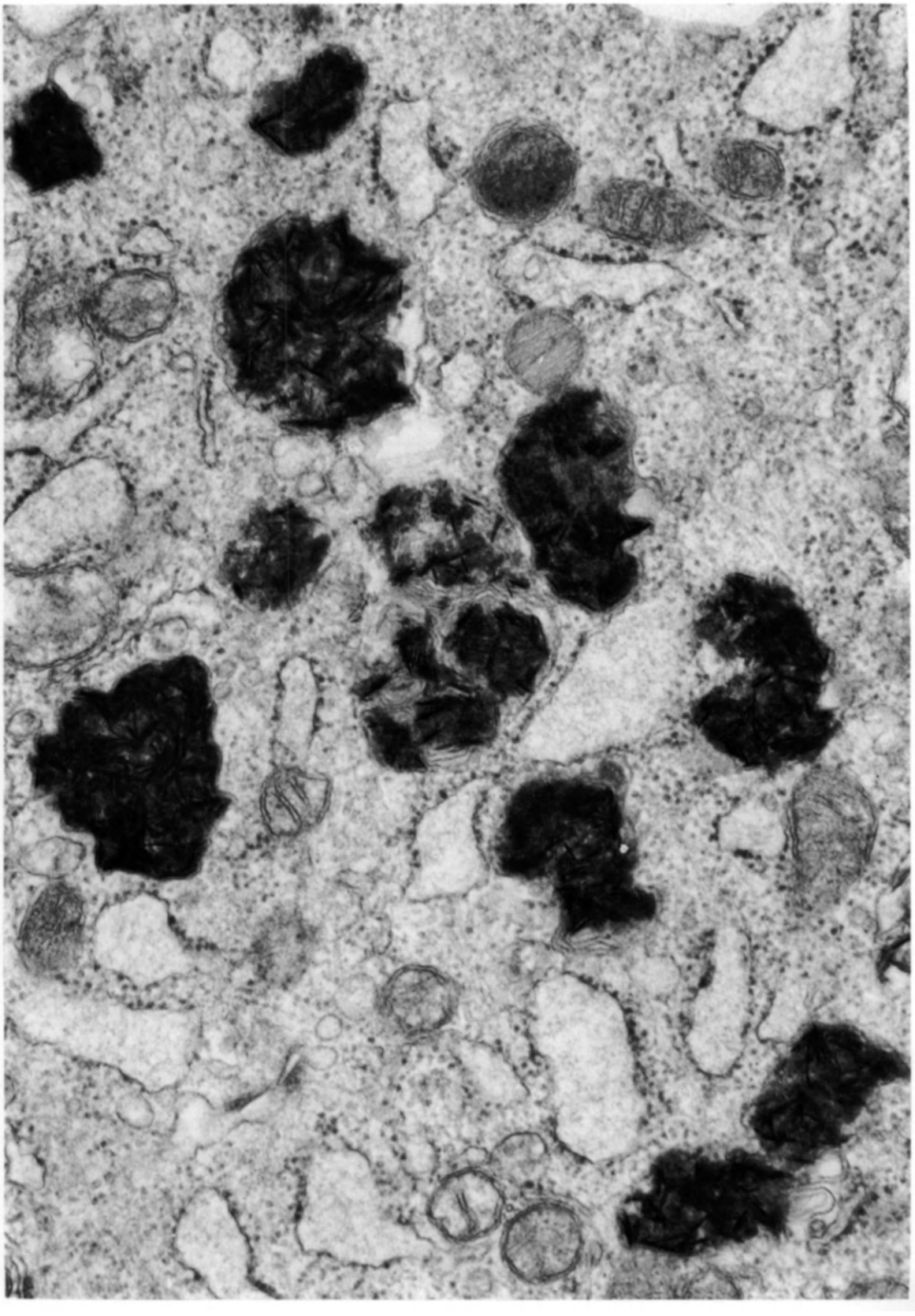


Thus, for example, *in vitro* studies on isolated lysosomes show that the activity of acid phosphatase and β-glucuronidase is inhibited by copper and that a high concentration of iron and copper can produce a marked leakage of enzymes from lysosomes (Schaffner *et al.*, 1962; Koenig and Jebril, 1962). It has also been shown (Peters and Seymour, 1976; Peters *et al.*, 1977; Seymour and Peters, 1978) that in haemachromatosis there is a markedly enhanced fragility of hepatocellular lysosomes and that removal of excess iron by venesection is followed by return of lysosomal fragility to normal. Similarly, Allison *et al.* (1966) have shown that when lung macrophages take up silica or asbestos, the lysosomal membrane becomes unstable and hydrolytic enzymes are released into the cell with resultant injury or cell death. Such an event releases the silica particles which are taken up once more by another cell, and it seems feasible that such a self-perpetuating mechanism could lead to necrosis and fibrosis as seen in silicosis.

Finally, it is worth pointing out that certain non-metallic (but also indigestible) materials can also lead to a release of hydrolytic enzymes. For example, when the detergent Triton WR-1339 is injected into rats, the hepatocellular lysosomes become loaded with this material and there is a marked increase in the acid phosphatase content of the bile, no doubt released from the lysosomes.

Plate 321

A cell showing single-membrane-bound presumably lysosomal bodies (called 'uraniosomes'), containing uranium crystals, which present electron-dense needle-like profiles. From a mouse spleen culture exposed to 25 μg/ml of uranium acetate for 71.5 hours. × 53 000 (*Ghadially and Yang-Steppuhn, unpublished electron micrograph*)



Aurosomes

The term 'aurosome' was coined to describe lysosomal bodies containing gold which develop in man and experimental animals after the administration of gold (Oryschak and Ghadially, 1974). Interest in these bodies stems from the fact that soluble gold salts, and to a lesser extent colloidal gold also, have been used for the treatment of rheumatoid arthritis for over 60 years. The inertness of this noble metal makes histochemical demonstration of gold deposits (aurosomes) in cells difficult and doubtful, while the small size of many such deposits puts them beyond the reach of the light microscope. It is for such reasons that our knowledge of gold deposits in cells stem largely from recent ultrastructural and x-ray analytical studies.

Virtually all such studies show that gold is deposited in lysosomal bodies but there is one study (Stuve and Galle, 1970) where it is claimed that it is deposited in mitochondria. In 1966 Ganote *et al.* reported the occurrence of single-membrane-bound lysosomal bodies containing electron-dense filamentous and granular material in the kidney of rats after administration of sodium aurothiosulphate (but not after administration of sodium thiosulphate) and they quite correctly surmised that these electron-dense deposits indicated the presence of gold. However, the situation became confused when in 1968, Norton, Lewis and Ziff reported that they had found bodies of a similar morphology not only in the synovial intimal cells of rabbits that had received intra-articular injections of sodium aurothiomalate, but also in rabbits that had received only thiomalic acid. This claim was later repeated about similar deposits in rat kidney (Strunk and Ziff, 1970). They stated that sodium thiomalate produced deposits 'similar though not identical' to those produced by sodium aurothiomalate and they therefore concluded that 'the formation of these deposits appeared to be dependent on the sulphur-containing portion of the gold thiomalate molecule'. Our studies (Oryschak and Ghadially, 1974, 1976a; Ghadially *et al.*, 1977; Lalonde and Ghadially, 1981) do not support such contentions, for (1) neither sodium thiomalate nor sodium thioglucose produced such lysosomal bodies but sodium aurothiomalate and aurothioglucose did; and (2) sodium chloroaurate (which does not contain sulphur) can also produce aurosomes which are indistinguishable from those produced by sulphur-containing gold salts.

The morphology of the aurosome produced by colloidal gold is quite different from that produced by soluble gold salts (*Plates 322–324*). The aurosome produced by colloidal gold contains spherical electron-dense granules while the mature aurosome produced by soluble gold salts has quite characteristic crystalline contents which present as electron-dense lamellar, rod-shaped and filamentous profiles studded with electron-dense particles and granules. Such details of structure are better appreciated in unstained sections of unosmicated tissues (*Plate 324*).

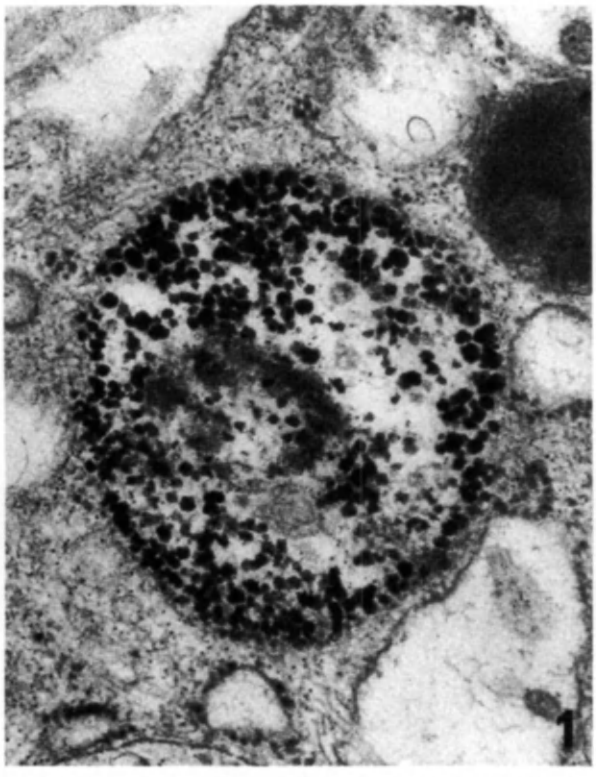
Plate 322

Fig. 1. Aurosome found in a rabbit synovial intimal cell four hours after intra-articular injection of sodium aurothiomalate. At this early stage of development the aurosome produced by this soluble gold salt has a granular content; but two or three days later the characteristic crystalline structures develop. × 64 000

Fig. 2. Aurosomes found in a rabbit syovial intimal cell eight days after intra-articular injection of sodium aurothiomalate. Electron-dense filamentous and rod-like profiles set in a lucent matrix are evident. × 32 000 (*From Oryschak and Ghadially, 1976a*)

Fig. 3. Aurosomes found in a rabbit synovial intimal cell one month after intra-articular injection of aurothioglucose. Note the characteristic electron-dense contents, basically similar to those shown in *Fig. 2.* × 44 000 (*From Ghadially, Thomas and Lalonde, 1977*)

Fig. 4. Aurosome found in a synovial intimal cell ten days after intra-articular injection of colloidal gold. This aurosome contains spherical electron-dense granules. The morphology is quite different from that seen in aurosomes produced by soluble gold salts. × 63 000 (*From Ghadially, Thomas and Lalonde, 1977*)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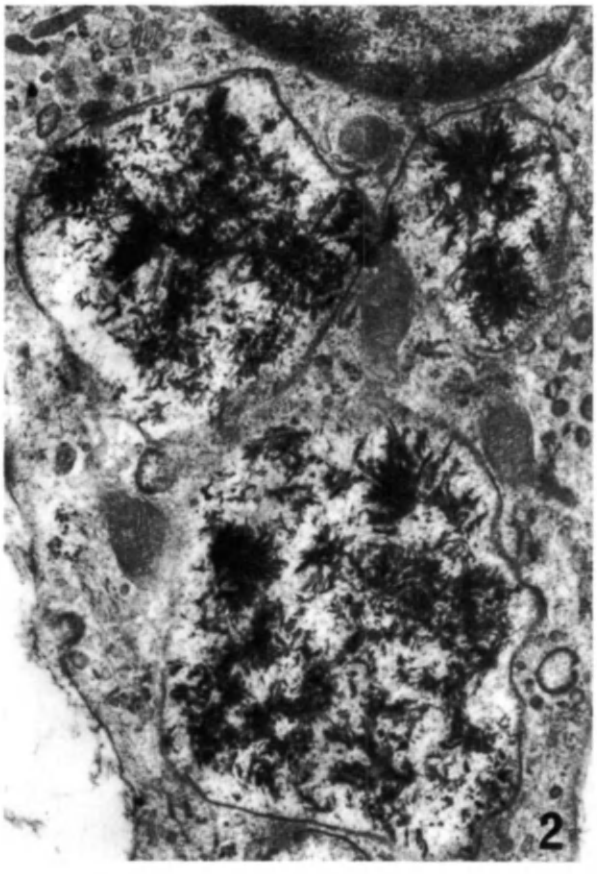
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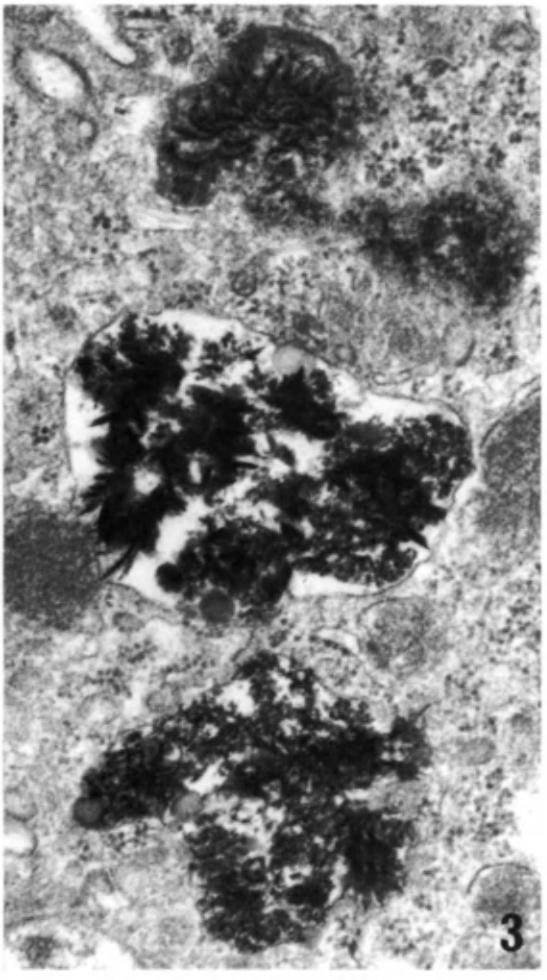
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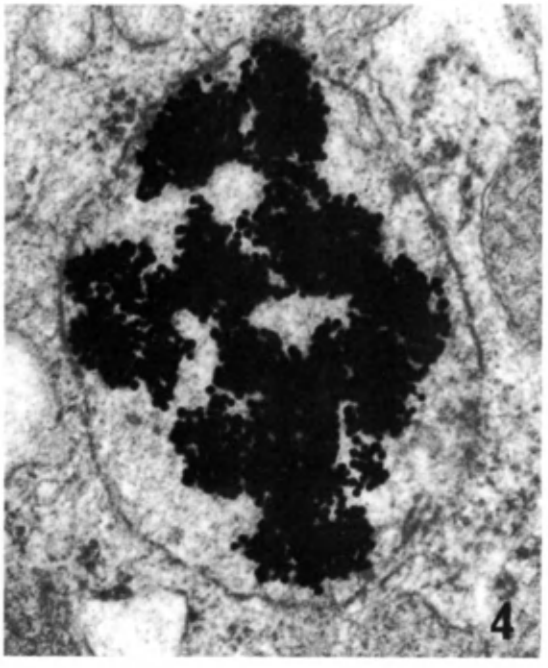
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However, it must be noted that the aurosomes found in the rabbit synovial membrane one day after intra-articular injection of the soluble gold salt sodium aurothiomalate have a granular content and only with the passage of time do the characteristic lamellar, rod-like and filamentous profiles studded with particles and granules develop*. A comparison of aurosomes seen three days and 18 months after injection of sodium aurothiomalate into the joint clearly shows that the lamellar and rod-like profiles are more prominent in the latter instance (Ghadially *et al.*, 1978b).

Aurosomes produced by soluble gold salts have now been found in cells of many organs and tissues (after intra-articular or intramuscular injection) such as; synovial membrane, articular cartilage, kidney, liver, spleen, lymph nodes, skin, lung, jejunum and colon (Ganote *et al.*, 1966; Norton *et al.*, 1968; Silverberg *et al.*, 1970; Strunk and Ziff, 1970; Katz and Little, 1973; Oryschak and Ghadially, 1974, 1976a, b; Yarom *et al.*, 1975, 1976; Ghadially *et al.*, 1976d, 1977, 1978a, b; Nakamura and Igarashi, 1977; Viol *et al.*, 1977; Ghadially and Lalonde, 1978; Ghadially, 1979c; Pääkkö *et al.*, 1984; and Ghadially, unpublished observations). Aurosomes have also been produced *in vitro* by exposing cultured human circulating monocytes to sodium aurothiomalate (Ugai *et al.*, 1979; Ghadially *et al.*, 1982). Fewer studies have been published on the aurosomes produced by colloidal gold (after intra-articular injection) in synovial intimal cells and subsynovial macrophages or by fibroblasts in culture (Gordon *et al.*, 1965; Southwick and Bensch, 1971; Yarom *et al.*, 1973, 1975; Ghadially *et al.*, 1977).

A review of this literature and my published and unpublished observations leads me to conclude that the morphology of the aurosome produced by soluble gold salts is quite distinctive and that this characteristic appearance of its contents is not materially affected by which gold salt is used (i.e. sodium aurothiomalate, aurothioglucose, sodium aurothiosulphate, and sodium chloroaurate), by which particular route it is administrated (i.e. intra-articular or intramuscular) or by the site (i.e synovial membrane, articular cartilage, liver, kidney, spleen, bone marrow or skin) in which the aurosome develops, or by species (i.e. rat, rabbit and man). Hence one can legitimately speak about the aurosome produced by soluble gold salts but it must be noted that oral administration of chlorotriethylphosphine gold (SK & F 36914) produces aurosomes of a similar morphology in the synovial intimal cells and subsynovial macrophages (Thomas and Ghadially, 1977).

Electron microscopic studies have now unequivocally demonstrated a remarkable increase in the lysosome population in the rheumatoid synovial membrane (page 692) and there is also ample evidence that perpetuation of the inflammatory process and the ensuing destruction of articular tissues that occurs is due to release of hydrolytic enzymes either by seepage or rupture of lysosomes. There is no evidence that gold stabilizes the lysosomal membrane as some drugs do but it has been shown that gold inactivates lysosomal enzymes (Persellin and Ziff, 1966).

In the normal synovial membrane of experimental animals treated with gold salts fresh lysosomes ('*de novo*' lysosomes) containing gold develop. These usually have an electron-lucent matrix. If this were to happen to any extent in the rheumatoid synovial membrane it would only add to the burden of lysosomes present in this tissue. However, in the post-chrysotherapy

*Since we are looking at sectioned material one may argue that the filamentous and rod-like formations are sections (profiles) through lamellar structures. This is no doubt true in many cases, but this does not preclude the possibility that besides the lamellar formations some rod-like and filamentous structures also occur.

Plate 323

Fig. 1. Aurosomes found in rabbit synovial intimal cells 18 months after intra-articular injection of sodium aurothiomalate. Lamellar and rod-like profiles are prominent here while filamentous formations are more prominent in the eight-day aurosome shown in *Plate 322, Fig. 2.* × 82 000 (*From Ghadially, 1979c*)

Fig. 2. Lysosomes found in the synovial intimal cells of a case of rheumatoid arthritis before chrysotherapy. For comparison with *Fig. 3.* × 48 000 (*From Ghadially, Oryschak and Mitchell, 1976d*)

Fig. 3. Post-chrysotherapy rheumatoid synovial membrane. The lysosomes contain characteristic gold deposits (arrows). × 48 000 (*From Ghadially, 1979c*)


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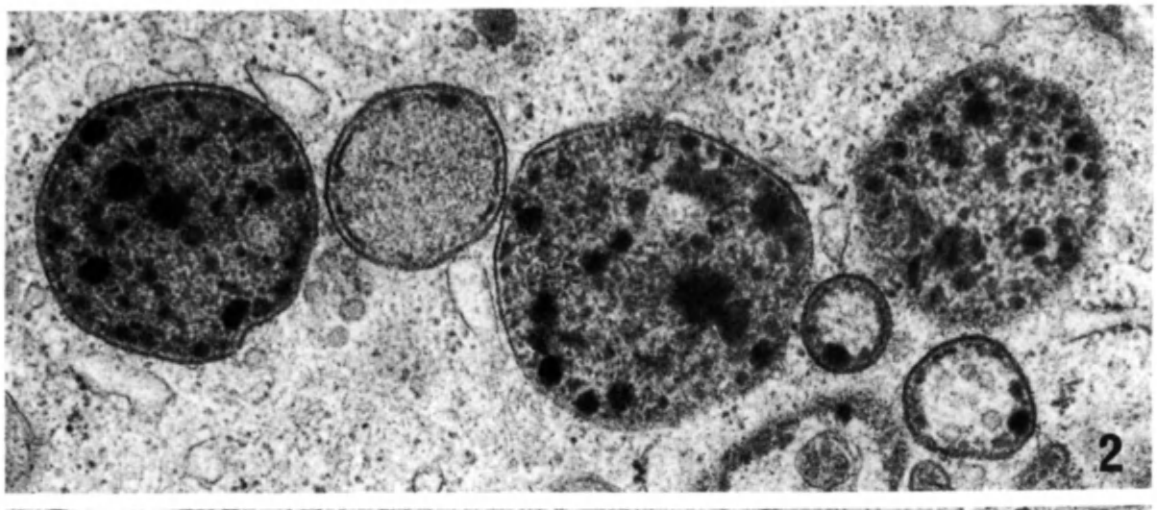
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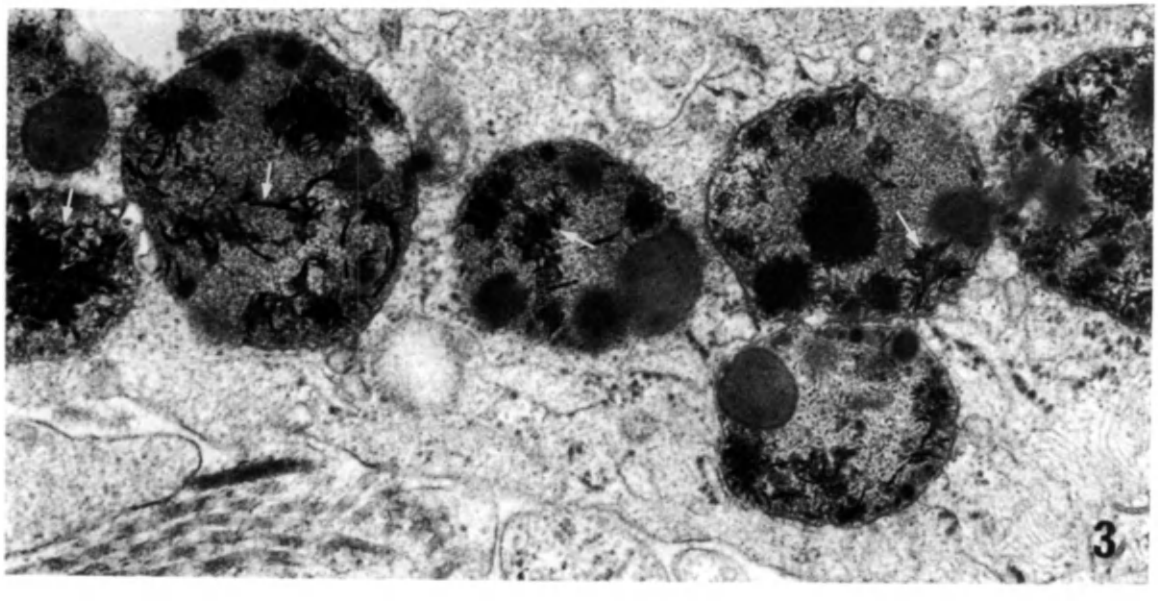
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rheumatoid synovial membrane *'de novo'* gold-containing lysosomes with a lucent matrix are only very rarely seen, most of the gold is found in bodies similar to the lysosomes found in the rheumatoid synovial membrane. Hence, it would appear that gold enters what may be interpreted as 'pre-existing' lysosomes where presumably it neutralizes and inactivates the enzymes which would, if released, perpetuate the inflammatory process and destroy articular tissues.

One of the toxic manifestations of gold therapy is the development of a cutaneous rash. Ultrastructural studies (Ghadially *et al.*, 1978a) show that the characteristic electron-dense deposits indicating the presence of gold occur within compound melanosomes and other secondary lysosomes in dermal macrophages. However, there are no qualitative or quantitative differences discernible in this regard in affected and non-affected skin. The production of a rash seems to be dependent on the discharge of mast cell granules in the affected skin.

Electron-probe x-ray analysis has been employed to show gold and other elements in the aurosome. The first study of this kind demonstrating gold in the aurosome produced by colloidal gold was by Yarom *et al.* (1973). However, we have now shown that a dual population of coarse granules containing gold and fine particles containing iron occur in the aurosome produced in synovial cells after intra-articular injection of colloidal gold (Ghadially and Lalonde, 1980).

The first study demonstrating gold in aurosomes produced by soluble gold salts was by Oryschak and Ghadially (1974). In this and later studies (Ghadially *et al.*, 1976d; Oryschak and Ghadially, 1976a) we showed that besides gold one could detect phosphorus and in some instances also calcium in the aurosome.

With more sophisticated equipment, computer programs and improvements in our techniques of analysis, it became possible to show that gold, phosphorus and sulphur are of constant occurrence in aurosomes produced by sodium aurothiomalate and in aurosomes produced by sodium chloroaurate (Ghadially *et al.*, 1978b; Ghadially, 1979c; Lalonde and Ghadially, 1981). Hence it was concluded that the 'thio' component in the drug is not essential for production of the characteristic morphology of aurosome contents, and that sulphur in the aurosome can be derived from the biological milieu. Considerations of the atomic ratios of the elements led us to conclude that there is more than enough sulphur present for all gold to occur as a sulphide but there is not enough gold here to account for all the sulphur plus phosphorus present and therefore, this might probably reflect the presence of some inorganic compound(s) containing phosphorus and/or sulphur.

Other workers have also attempted to analyse the aurosomes produced by soluble gold salts. Yarom *et al.* (1975) report the occurrence of gold but make no mention of other elements, while Nakamura and Igarashi (1977) found both gold and sulphur but they do not comment about phosphorus.

Plate 324

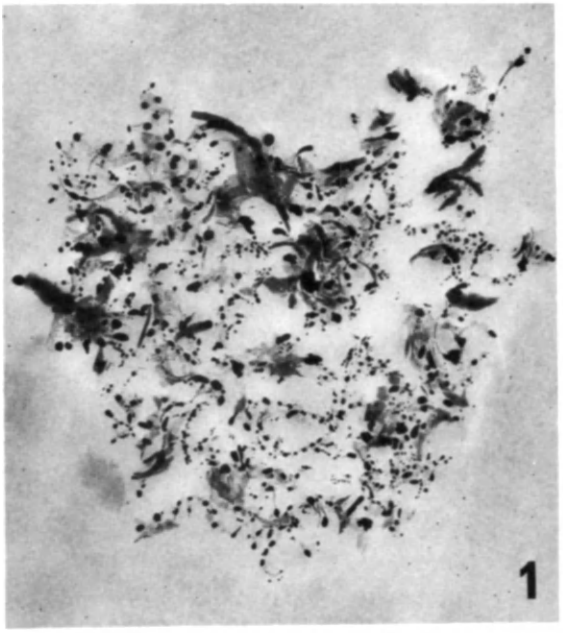
Aurosome contents as seen in unosmicated unstained synovial cells at various time intervals after intra-articular injection of various gold preparations in the rabbit knee joint. As is to be expected cytomembranes and cellular details are not visualized, but the inherently electron-dense gold deposits are easily seen.

Fig. 1. Three days after sodium aurothiomalate. Particles and granules are prominent but lamellar and rod-like profiles are sparse. × 60 000 (*From Ghadially, Lalonde, Thomas and Massey, 1978*)

Fig. 2. Eighteen months after sodium aurothiomalate. Prominent lamellar, rod-like and filamentous profiles studded with particles and granules are evident. The very fine particles (arrowheads) probably contain iron, but this has not been established by electron-probe x-ray analysis. × 60 000 (*From Ghadially, Lalonde, Thomas and Massey, 1978*)

Fig. 3. Three days after aurothioglucose. Lamellar, rod-like and filamentous profiles studded with particles and granules are evident. × 80 000 (*From Ghadially, Thomas and Lalonde, 1977*)

Fig. 4. Ten days after colloidal gold. Spherical electron-dense granules containing gold (arrowheads) are evident in the aurosome and also very fine particles which contain iron (arrows). × 100 000 (*From Ghadially, Thomas and Lalonde, 1977*)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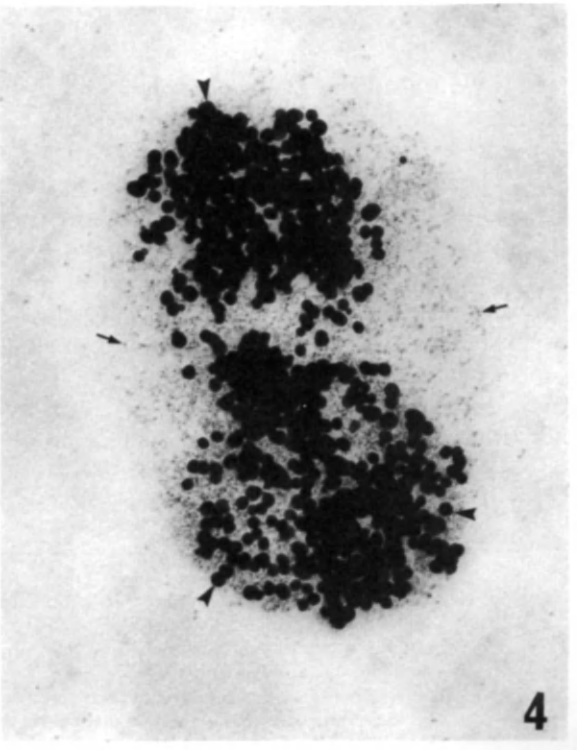
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Platinosomes

Many platinum compounds have been shown to have antitumour activity and some are being used for the treatment of neoplastic diseases in humans (Rosenberg, 1973). The best known and most widely used compound is *cis*-dichlorodiamine platinum (II) (*cis*-Pt) but later studies have shown that platinum-pyrimidine blues are less toxic, more potent antitumour agents (Davidson *et al.*, 1975).

Little information is available on tissue distribution of platinum (Litterst *et al.*, 1976), its site of intracellular localization and the mechanism(s) by which it produces its therapeutic effect. On the basis of *in vitro* studies it is speculated that *cis*-Pt binds to DNA and that it inhibits DNA, RNA and protein synthesis (Harder and Rosenberg, 1970; Kara *et al.*, 1971; Roberts and Pasco, 1972), but there is no real proof that this in fact occurs *in vivo*.

The term 'platinosome' is used to describe single-membrane-bound presumably lysosomal bodies containing platinum (Ghadially *et al.*, 1981b, c) (*Plate 325*). Platinosomes are basically single-membrane-bound bodies with an electron-lucent or medium density matrix in which are set electron-dense granules which at times have a tendency to form linear or reticular patterns. Platinosomes have been produced in: (1) cultured macrophages after incubation with *cis*-Pt bound to DNA (Heinen, 1978a, b); (2) HeLa cells and RPMI 6410 cells (a human lymphoblastoid cell line) incubated with platinum uracil blue (Ghadially *et al.*, 1981b); and (3) synovial intimal cells, subsynovial macrophages and subsynovial lipocytes after intra-articular injection of platinum uracil blue (Ghadially *et al.*, 1981c).

Strangely enough, platinosomes have not been found in animals injected with *cis*-Pt or in cultured cells incubated with this compound (Heinen, 1978a, b; Ghadially *et al.*, 1981b, c). Perhaps this is because the compound is so toxic* that doses adequate for ultrastructural visualization or electron-probe x-ray analytical detection cannot be attained.

In contrast to this is the report by Khan and Sadler (1978) who claim to have found platinum with the aid of electron-probe x-ray analysis in the nucleus (heterochromatin aggregates adjacent to the nuclear envelope) and nucleolus of HeLa cells exposed for four hours to quite high concentrations of *cis*-Pt in dimethylsulphoxide (final concentration 200 μm Pt, 5% $SOMe_2$).

We (Ghadially, Yang-Stepphun and Lalonde, unpublished observations) have repeated the experiment of Khan and Sadler (1978) and are unable to confirm their results in that we could not detect platinum in the nucleus, nucleolus or any other cell compartment.

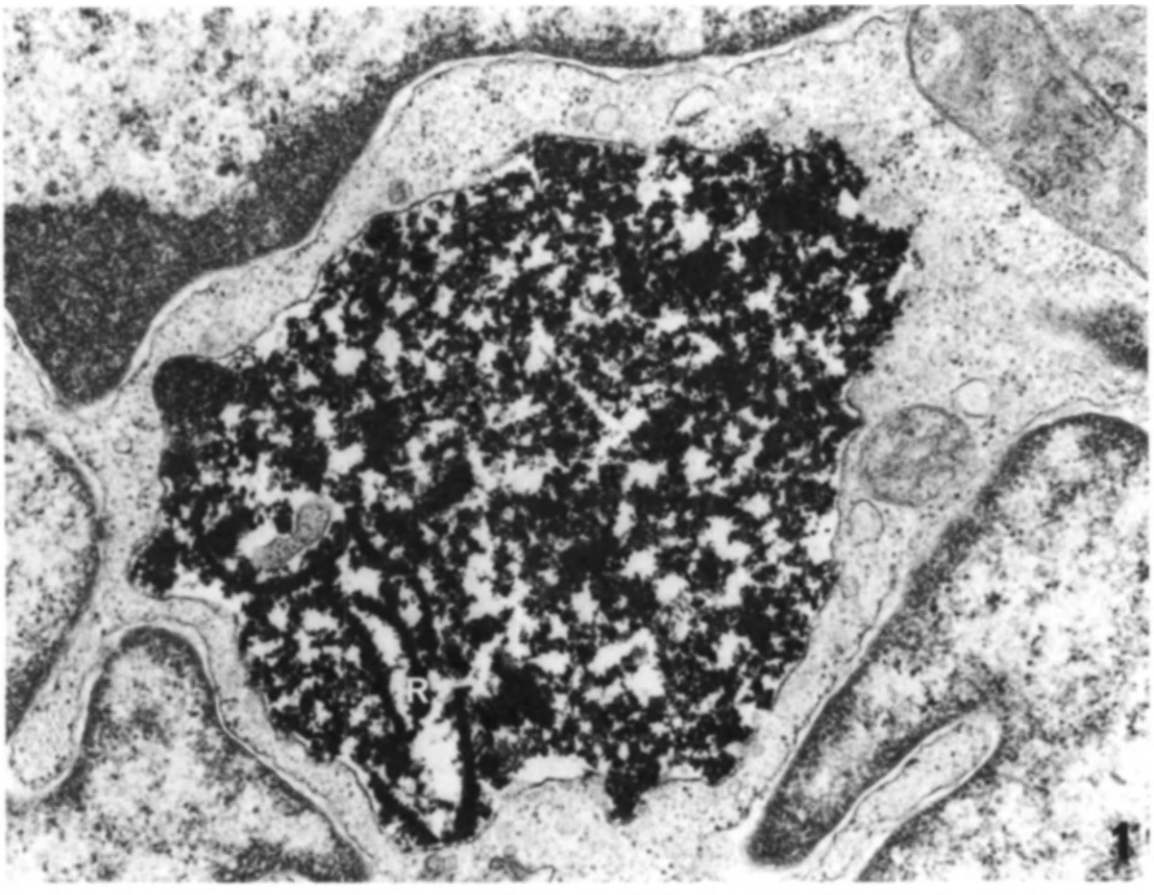
In any case, the cells analysed in such an experiment are dead cells (a point that is also evident from *Figs. 1* and *2* in Khan and Sadler (1978) which show necrotic cells) and it is well known that platinum, like uranium, can bind to DNA and RNA and indeed platinum compounds have been used as stains for electron microscopy (Aggarwal *et al.*, 1975). Thus even if platinum was demonstrated by Khan and Sadler (1978) one could contend that what was being witnessed was a staining of dead cells with platinum similar to the well known electron microscopic technique of block staining tissues with uranium. Thus it would appear that there are as yet no compelling reasons for believing that *cis*-Pt produces its antitumour effect by combining with DNA, even though this is an attractive possibility.

*5 μg/ml of *cis*-Pt kills cultured cells within 24 hours.

Plate 325

Fig. 1. A large platinosome produced in a RPMI cell after exposure to 100 μg/ml of platinum uracil blue for 70 hours. This cell had a markedly irregular nucleus beset by invaginations. Besides electron-dense granules this single-membrance-bound platinosome also contains rope-like formations (R). × 33000 (*From Ghadially, Lock, Yang-Steppuhn and Lalonde, 1981*)

Fig. 2. Platinosomes found in a subsynovial macrophage two weeks after injection of 10 mg of platinum uracil-blue into the rabbit knee joint. The granular electron-dense contents are forming a coarse reticular pattern. × 33000 (*From Ghadially, Lock, Lalonde and Ghadially, 1981*)



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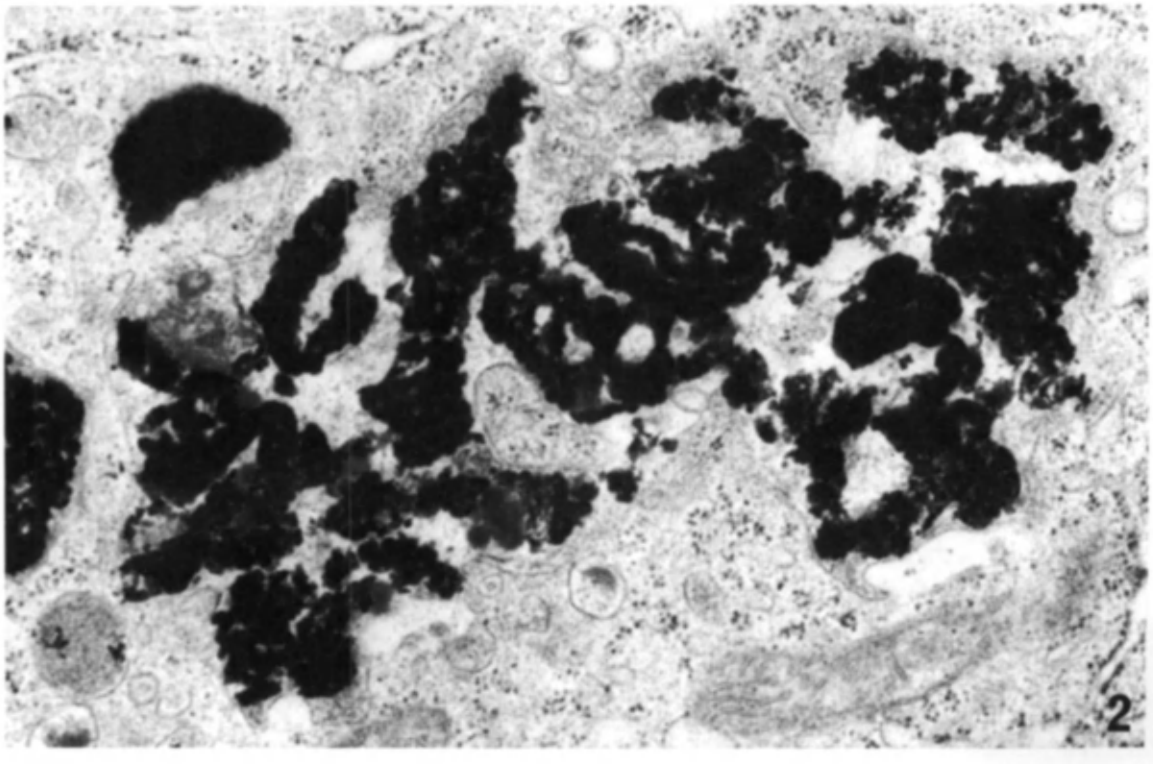


2

Interlysosomal crystalline plates (zipper-like structures)

The term 'zipper-like structures'* is frequently used to describe structures which are at times seen uniting two or more lysosomes. Since these flat or plate-like structures have a highly ordered substructure they could more appropriately be called 'interlysosomal crystalline plates'. When the crystalline plate is cut at right angles (i.e. normal) to its surface a linear or curved rod-like profile is seen. The 'rod' is composed of flattened parallel adjoining lysosomal membranes (which show the characteristic trilaminar structure) connected by zig-zag or parallel subunits or 'lines'. In markedly oblique or tangential sections a crystalline lattice composed of hexagonal subunits is evident (*Plate 326*).

Interlysosomal crystalline plates have been seen in: (1) neoplastic cells of clear cell adenocarcinoma of the endometrium (Ferenczy and Richart, 1974); (2) hepatocytes from livers containing metastatic malignant melanoma, adenocarcinoma of colon and adenocarcinoma of stomach (Soares, 1982); (3) gall bladder epithelial cells, from a gall bladder with deposits of malignant melanoma (Sobel and Marquet, 1983); (4) malignant cells of: (a) primary adenocarcinoma of lung and duodenum; (b) central nervous system metastasis from a large cell carcinoma of the lung; and from an 'atypical endocrine' tumour (primary probably in the oesophagus) (Orenstein, 1983); and (5) reactive mesothelial cells in a benign pleural effusion from a woman with adenocarcinoma of ovary (Orenstein, 1983).

It will be evident from the list presented above that interlysosomal crystalline plates have no diagnostic value, because they have been seen in several types of tumour cells and also in some normal cells. Regarding the significance of these structures, two ideas have been put forward: (1) that they may represent a mechanism by which lysosomes fuse (Mackay, 1982; Orenstein, 1983); and (2) that they are a tissue preparative artefact (Rafel, 1983). I am inclined to favour the latter idea because: (1) Nistal *et al.* (1978) have shown that interlysosomal crystalline plates (which the authors consider to be similar to septate junctions) can develop between lysosomes when formaldehyde is used as the primary fixative; (2) Hausmann (1977) has shown that tissue damage produced by experimental or other means can induce artefactual fusion of membranes; and (3) intramitochondrial rod-like crystals (*Plate 138*) are seen in autopsy tissue (but not in promptly fixed biopsy tissue) and their number increases with the passage of time after death.

Regarding the mechanism of formation of interlysosomal crystalline plates, one may speculate that they develop when lipids and proteins leach out of cell membranes and then crystallize. Tissues where such leaching is likely to occur include: (1) necrotic tissue; (2) tissue obtained at autopsy; and (3) tissue altered by improper or inadequate fixation. This, however, cannot be the complete answer because it leaves unexplained why crystalline structures form in certain specific sites (e.g. between the membranes of a mitochondrial crista and between membranes of adjacent lysosomes) and not in others. Clearly some local differences in the composition of these membranes must also be a factor responsible for the production of these crystalline structures.

*This is a poor term because the structure bears little resemblance to a zipper; zig-zag would be more appropriate.

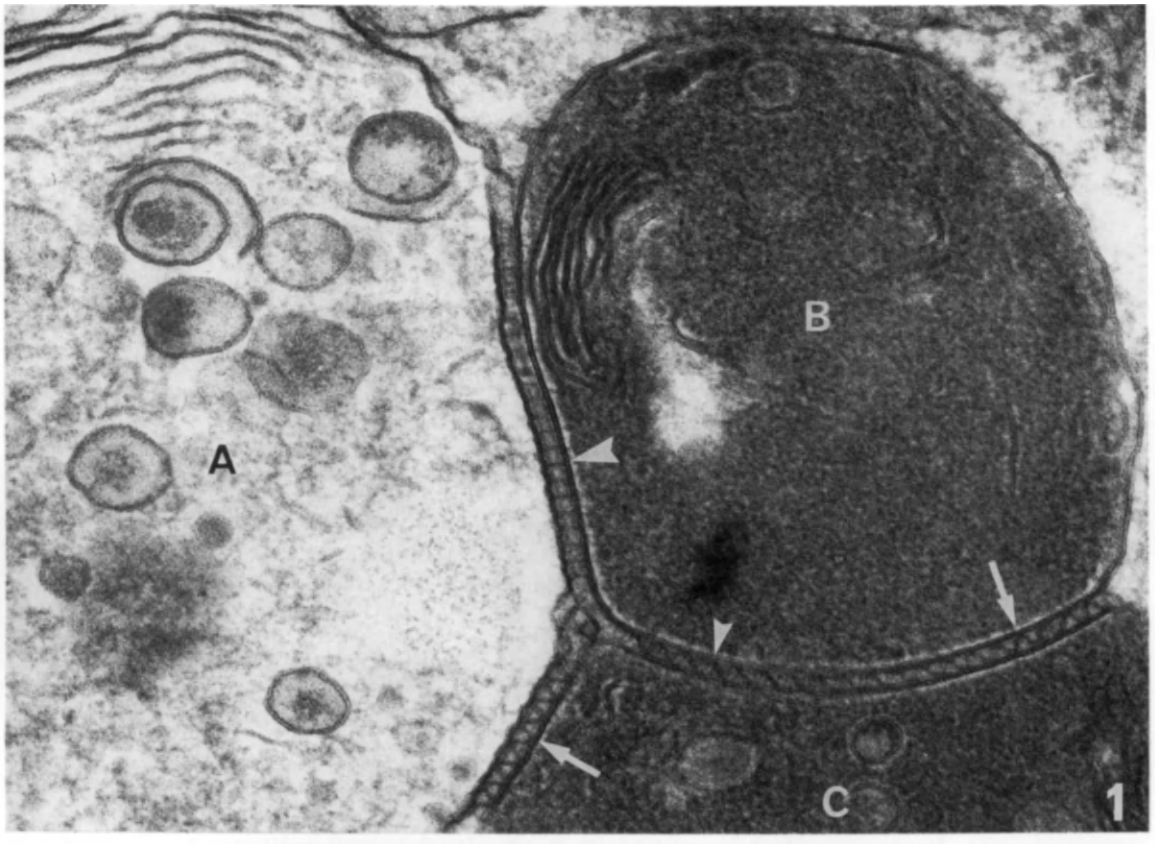
Plate 326

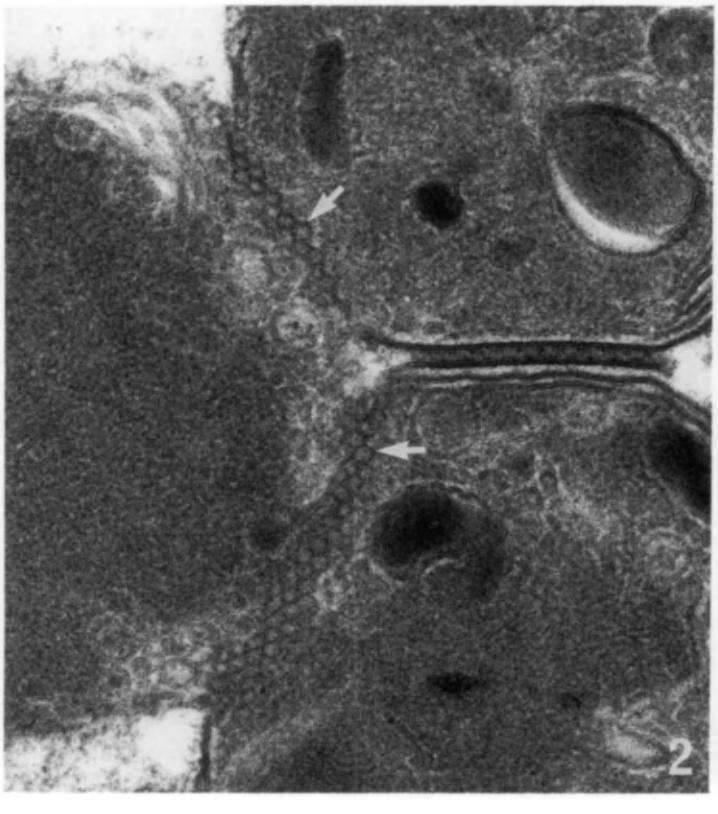
Tumour cells from a pulmonary adenocarcinoma (*From grids supplied by Dr Delia Beju*).

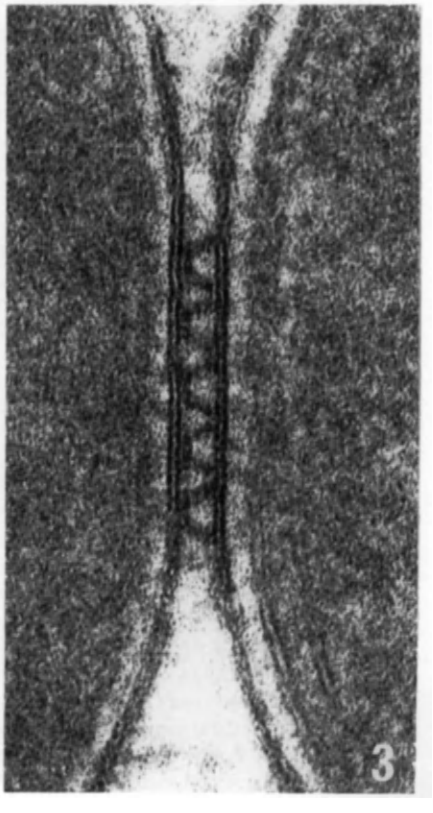
Fig. 1. Normal section through interlysosomal plates between three lysosomes (A, B and C). The interconnecting 'lines' or subunits in the crystalline plates show a zig-zag pattern (arrows) or a pattern of parallel lines deployed at right angles to (large arrowhead), or oblique to (small arrowhead), the adjoining lysosomal membranes. × 109 000

Fig. 2. A markedly oblique or tangential cut through two interlysosomal plates reveals a crystalline lattice which appears to be composed of hexagonal subunits (arrows). × 88 000

Fig. 3. High-power view of a normal section through a plate showing the zig-zag pattern. × 244 000


A
B
C
1


2


3

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8

Microbodies (peroxisomes, microperoxisomes and catalosomes)

Introduction

The term 'microbody' was coined by Rhodin (1954, 1956) to describe certain single-membrane-bound organelles (containing a fine granular matrix) found in the proximal tubular cells of the mouse kidney. Morphologically similar bodies but with a dense crystalloid set in the granular matrix were subsequently found in rat hepatocytes (Gansler and Rouiller, 1956; Rouiller and Bernhard, 1956). Later biochemical studies (de Duve, 1965; de Duve and Baudhuin, 1966; de Duve, 1969a, b) on isolated microbodies showed that they contain several oxidases, such as urate oxidase, D-amino acid oxidase, L-α-hydroxy acid oxidase and isocitrate dehydrogenase, which generate hydrogen peroxide, and also catalase which destroys hydrogen peroxide. Because of their involvement in hydrogen peroxide metabolism, the term 'peroxisome' was coined by de Duve (1965) to describe them. Later studies have demonstrated the activities of other enzymes such as NAD^+: α-glycerol phosphate dehydrogenase, NADH-cytochrome C reductase and carnitine acetyltransferase in isolated peroxisome fractions (Donaldson *et al.*, 1972; Markwell *et al.*, 1973; Gee *et al.*, 1974).

The presence of urate oxidase (uricase) within the crystalloid of the hepatic microbodies of some species led Afzelius (1965) to suggest that such microbodies be called 'uricosomes'. This term has not gained much popularity. The discovery in a variety of cells of small (often 0.3 μm or less in diameter) peroxisomes (*Plate 327*) or peroxisome-like organelles which stain positively for catalase led Novikoff and Novikoff (1972) and Novikoff *et al.* (1972) to introduce the term 'microperoxisomes' to describe them. Other terms used to describe these structures include: (1) 'catalase-positive particles' (Graham and Karnovsky, 1966; Novikoff and Goldfischer, 1969); (2) 'DAB-positive particles' (DAB is the abbreviation for 3,3′ diaminobenzidine) (Shio *et al.*, 1974); and (3) 'catalosomes' (Reddy and Krishnakantha, 1975).

Microbodies found in the liver and kidney are usually round or oval organelles about 0.5 μm in diameter. However, there are many interesting morphological differences discernible in microbodies from various sites and species (page 770). Initially, it appeared that microbodies were confined to a few plant cells and protozoa and to the hepatic and renal tubular epithelial cells of vertebrates. On the basis of this observation and their enzyme content it was speculated that microbodies were perhaps vestigial organelles representing a phylogenetically primitive oxidative pathway. However, the development of a cytochemical method for staining microbodies at the ultrastructural level (Fahimi, 1968; Hirai, 1968; Novikoff and Goldfischer,

1968) and also morphological and biochemical studies have now shown that small catalase-containing organelles acceptable as microbodies are found in many other vertebrate cells (*Plate 327*). Such studies have also shown that all microbodies contain catalase (in rat hepatic microbodies up to 40 per cent of the microbody protein is catalase) but the hydrogen-peroxide-producing oxidases differ in number and specificity in microbodies found in various sites (de Duve, 1969a, b). Thus, catalase has become the marker for the identification of microbodies as has acid phosphatase for lysosomes.

However, strictly speaking a peroxisome or microperoxisome should contain oxidases and catalase. A catalosome is an organelle revealed by catalase staining; it may or may not contain oxidases. For example, the small catalase-positive organelles in the myocardium and epithelial cells of the gut contain oxidases (Connock *et al.*, 1974; Herzog and Fahimi, 1974), but Böck *et al.* (1975) could find no evidence of the presence of oxidases in the catalase-positive organelles of the rat Harderian gland.

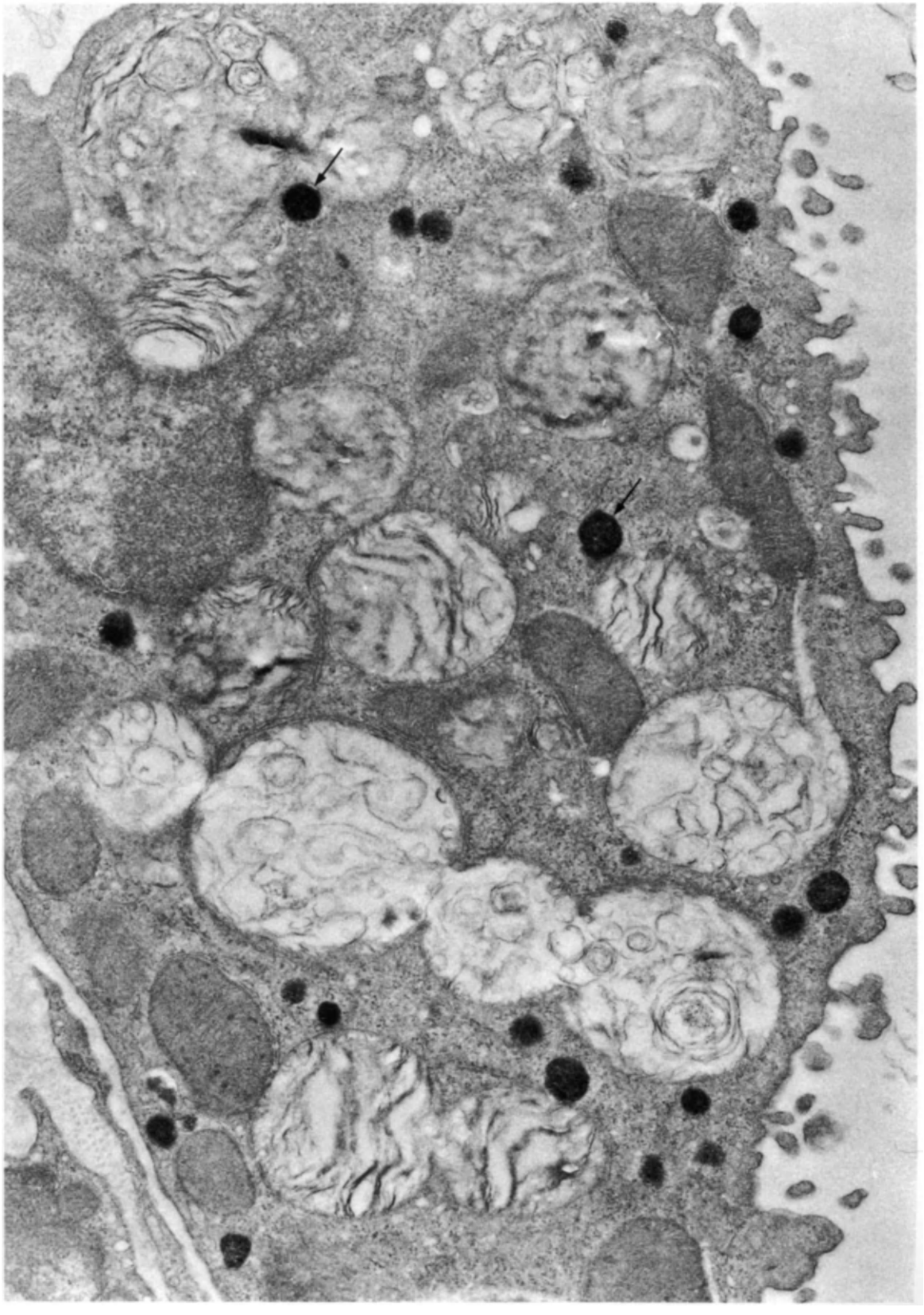
Studies on cytokinesis in onion (*Allium sativum*) root tip cells has provided evidence for the presence of an organelle (0.2 μm) containing catalase but not oxidases in the phragmoplast region of dividing cells. Since the appearance and disappearance of these organelles coincides with cytokinesis they are referred to as 'phragmosomes' to imply their role in cell plate formation (Porter and Caulfield, 1958; Hanzely and Vigil, 1975).

Thus it would appear that catalase-containing organelles with and without oxidases exist but sufficient details about the enzyme content of these organelles (in all sites) are not available to make such distinctions in many instances. Therefore, in keeping with current practice one may continue to use terms such as microbodies, peroxisomes and microperoxisomes to describe these organelles. I prefer the noncommittal term 'microbodies' and this is used most of the time in this text.

The function of microbodies in mammalian cells has not been fully elucidated. Their relative abundance in cells involved in cholesterol metabolism and synthesis of steroids (e.g. hepatocytes, and steroid secreting cells in ovary, testis and adrenals), and the dramatic increase in their numbers after administration of hypocholesterolaemic drugs suggest that they have a role in cholesterol metabolism (Fawcett, 1981). There is also evidence that along with other organelles, microbodies play important roles in lipid metabolism such as synthesis of plasmalogen precursors and oxidation of fatty acids (Lazarow and de Duve, 1976; Kindl and Lazarow, 1982; Masters and Crane, 1984). Several other functions have been ascribed to microbodies. These include (1) glyconeogenesis; (2) providing an ancillary site for carbohydrate oxidation; (3) participating in the oxidation of reduced nicotinamide dinucleotide; (4) protecting cells against oxygen toxicity; (5) thermogenesis; and (6) purine catabolism (for references *see* Goldfischer and Reddy, 1984).

Plate 327

This electron micrograph illustrates positive staining for catalase in microbodies of a type II pneumocyte in guinea-pig lung. Only the small round microbodies (arrows) contain electron-dense reaction product. Other organelles such as nucleus and mitochondria are negative for catalase. Stained *en bloc* with uranium. × 23 000 (*From Schneeberger, 1972b*)



Structure and normal variations

As pointed out in the introduction, initial work on microbodies created the impression that microbodies in vertebrates were present only in hepatic and renal tubular epithelial cells, but it was recognized that they were found also in some plant cells and protozoa. Later studies have established that small catalase-positive organelles acceptable as microbodies (usually referred to as microperoxisomes) are of ubiquitous occurrence.

It would be impossible to list in detail all the sites in which large and small microbodies have been found but a fairly comprehensive list can be presented.

Microbodies have now been found in: (1) algae, yeasts, plant cells and protozoa (Frederick and Newcomb, 1959, 1969; de Duve and Baudhuin, 1966; Frederick *et al.* 1968; Muller *et al.*, 1968; Tolbert *et al.*, 1968; de Duve, 1969a, b; Muller, 1969; Tolbert and Yamazaki, 1969; Vigil, 1969, 1970; Matsushima, 1971; Clandinin, 1972); (2) fat body of an insect (Locke and McMahon, 1971); (3) hepatocytes and renal tubular epithelial cells of a large number of vertebrates including mammals (for references, *see below* and Hurban *et al.*, 1972; Veenhuis and Wendelaar Bonga, 1977); (4) sinusoidal cells of rat liver (Fahimi *et al.*, 1976); (5) macrophages (Breton-Gorius and Guichard, 1975; Fahimi *et al.*, 1976; Eguchi *et al.*, 1979); (6) human erythroblasts (Breton-Gorius and Guichard, 1975); (7) myocardium of several species including primates (Herzog and Fahimi, 1974, 1975; Hicks and Fahimi, 1977); (8) brown adipose tissue of the rat (Ahlabo and Barnhard, 1971; Cannon *et al.*, 1982); (9) rat adrenals (Beard, 1972); (10) Clara cells and Type II alveolar cells of a variety of primates and rodents (Petrik, 1971; Schneeberger, 1972a, b); (11) interstitial cells (Leydig cells) of rodent testes (Reddy and Svoboda, 1972); (12) absorptive cells of mammalian small intestine (Novikoff and Novikoff, 1972); (13) parenchymal cells of various exocrine glands of the rat such as pancreas, parotid, submandibular, lacrimal, nasal mucosal and von Ebner's gland (Hand, 1973); and (14) neurons of catecholaminergic areas of the central nervous system (McKenna *et al.*, 1976).

The morphology of microbodies from hepatocytes and renal tubular epithelial cells has been studied extensively (Gansler and Rouiller, 1956; Rouiller and Bernhard, 1956; Rhodin, 1958; Ashford and Porter, 1962; Novikoff and Shin, 1964; Afzelius, 1965; Ericsson and Trump, 1966; Svoboda *et al.*, 1967; Tsukada *et al.*, 1968; Tisher *et al.*, 1968; Beard and Novikoff, 1969) and their enzyme content has also been the subject of many reports (Beaufay *et al.*, 1964; Afzelius, 1965; Allen and Beard, 1965; Baudhuin *et al.*, 1965a, b; Shnitka, 1966; Tsukada *et al.*, 1966).

These studies show that microbodies are round, oval or elongated structures bounded by a single membrane and that they contain a granular matrix. However, some microbodies have a nucleoid while others do not (*Plate 328*). If the nucleoid is present it may be either amorphous or

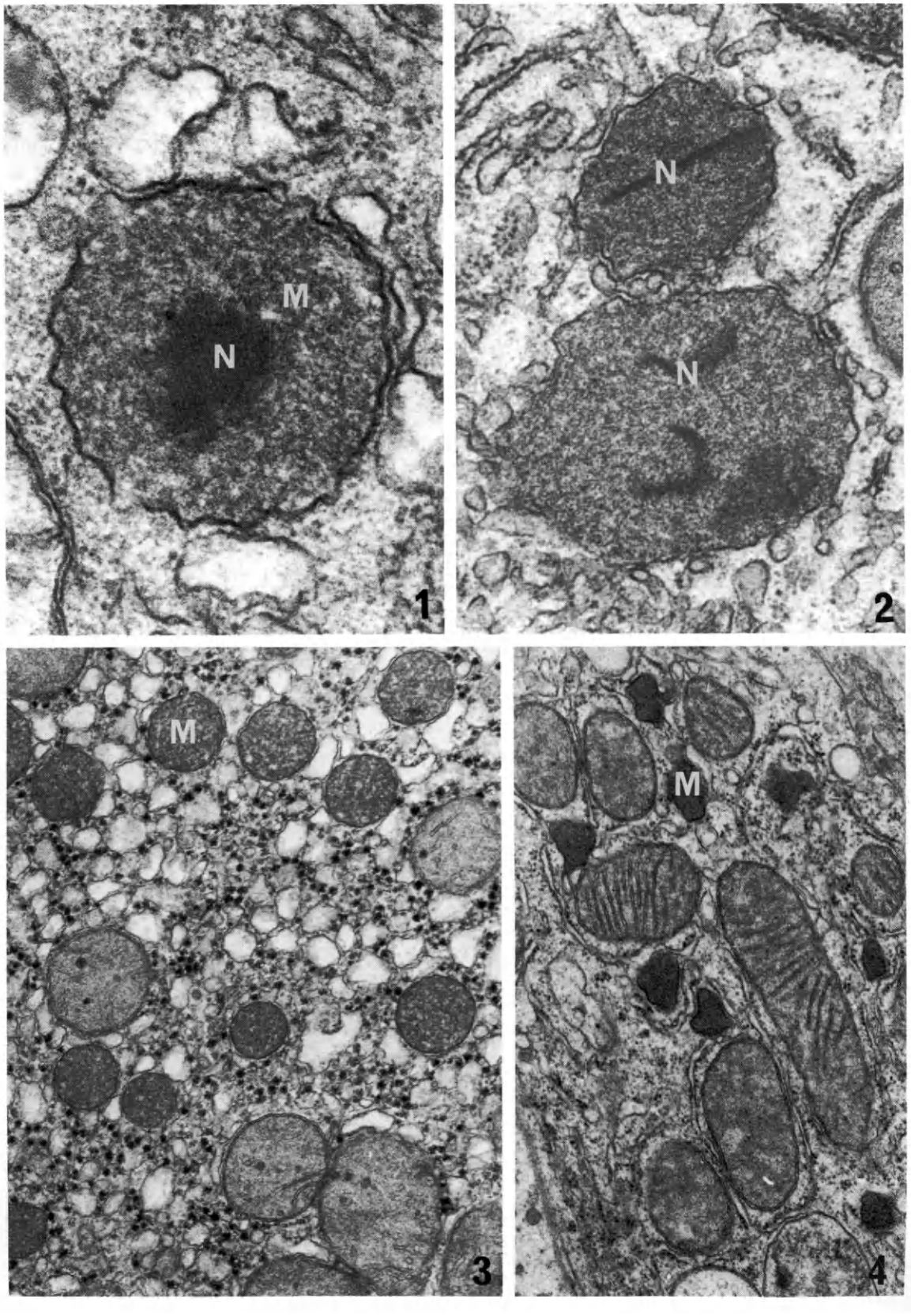
Plate 328

Fig. 1. Hepatic microbody from a rat, showing granular matrix (M) and a crystalline nucleoid (N). × 79 000

Fig. 2. Hepatic microbodies from a hamster. The lamellar nucleoids present rod-like and curved profiles in sectional material. × 50 000

Fig. 3. Microbodies (M) from human liver. Note the absence of nucleoids. × 27 000

Fig. 4. Microbodies (M) from the liver of a canary. Note their irregular shape, smaller size, denser matrix and the absence of nucleoids. × 29 000


M
N
1
N
N
2
M
3
M
4

crystalline. Rat hepatocyte microbodies contain catalase, urate oxidase, D-amino acid oxidase and α-hydroxy acid oxidase. The catalase and D-amino acid oxidase reside in the matrix while the urate oxidase is located in the crystalline cores* or nucleoids. In rat kidney microbodies, catalase, D-amino acid oxidase, and L-amino- and L-α-hydroxy acid oxidase have been demonstrated, but urate oxidase is absent.

A positive correlation between the presence of urate oxidase and nucleoids (particularly nucleoids with a crystalline structure) has now been reported for hepatic and renal microbodies of a large number and variety of vertebrates (Afzelius, 1965; de la Inglesia *et al.*, 1966; Shnitka, 1966; Hruban and Rechcigl, 1967). Thus the liver of man and birds does not possess urate oxidase activity and the hepatic microbodies lack nucleoids. However, some exceptions to such generalizations do seem to exist. For example, the kidney tissue of man, rat and Rhesus monkey (*Macaca mulatta*) lacks urate oxidase activity, but amorphous nucleoids have been found in the renal microbodies of man, amorphous and crystalline nucleoids have been described in the renal microbodies of the rat and crystalline nucleoids have been shown in Rhesus kidney microbodies (Tisher *et al.*, 1968). Nucleoids have not been noted in microbodies found in other sites such as brown adipose tissue, adrenals, alveolar epithelial cells and interstitial cells of the testis.

Many interesting species-related and other differences in the nucleoids of microbodies have been noted (*Plates 328–330*). Thus the nucleoids of the microbodies of rat hepatocytes are rounded or compact bodies while in the hamster they present in ultrathin sections as one or more rod-like or filamentous structures which are sometimes curved upon themselves or angulated. Their three-dimensional form probably resembles a thin plate or a folded sheet. In bovine microbodies, a linear density or marginal plate apposed against the membrane covering the microbody has been described, and this is thought to represent a filamentous nucleoid. We (Ghadially and Ailsby, unpublished observations) have seen such a plate or plates in hepatic and renal microbodies of the cow and rabbit (*Plates 329* and *330*). Hepatic microbodies of Rhesus monkey generally do not contain a nucleoid but a rare microbody with a small nucleoid is seen. However, in the squirrel monkey, microbodies with irregular, linear or angular nucleoids occur (Wattiaux-de Coninck *et al.*, 1965; Svoboda *et al.*, 1967). Although the hepatic microbodies of normal humans do not contain a nucleoid, Biempica (1966) found that in a complex disease state the microbodies developed a crystalline nucleoid.

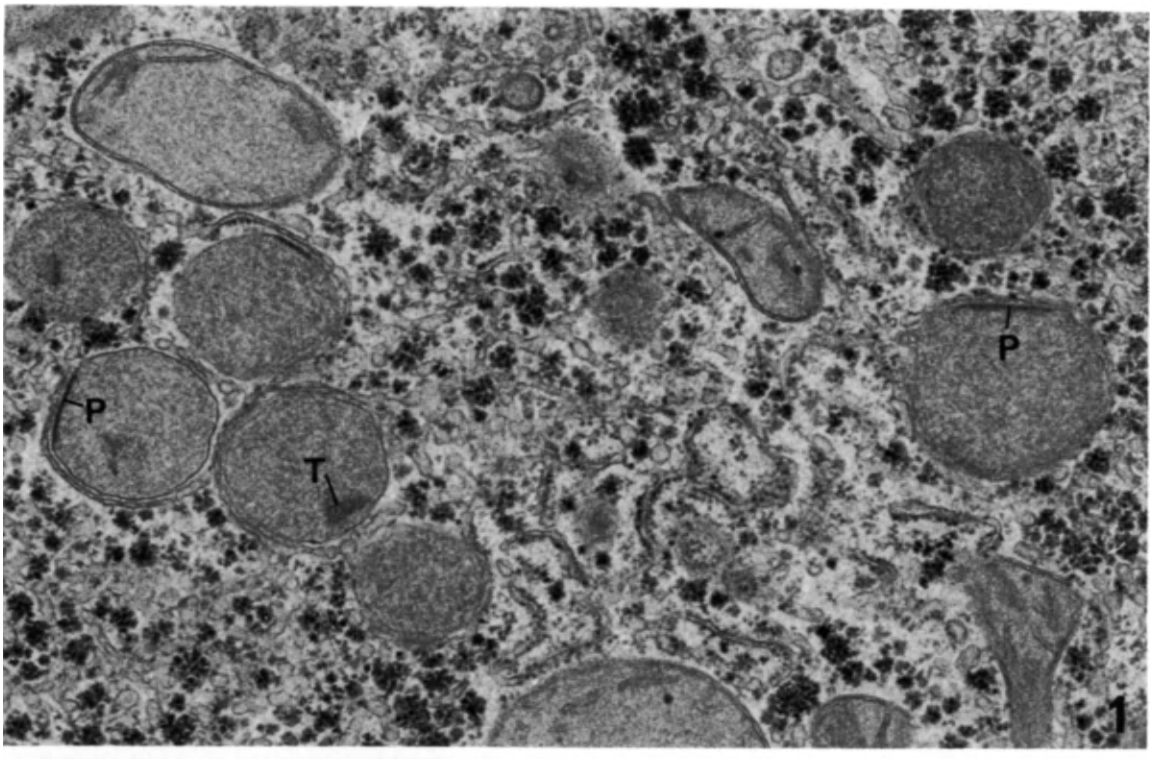
Newly formed microbodies lack a nucleoid because this structure is developed at a later stage of maturation of the microbody. Thus in the hepatocytes of fetal rats only rarely do microbodies contain nucleoids and the matrix is not as dense as in the adult rat (Tsukada *et al.*, 1968). Similarly, most of the abundant newly formed microbodies in the regenerating rat liver also lack a nucleoid (Svoboda *et al.*, 1967).

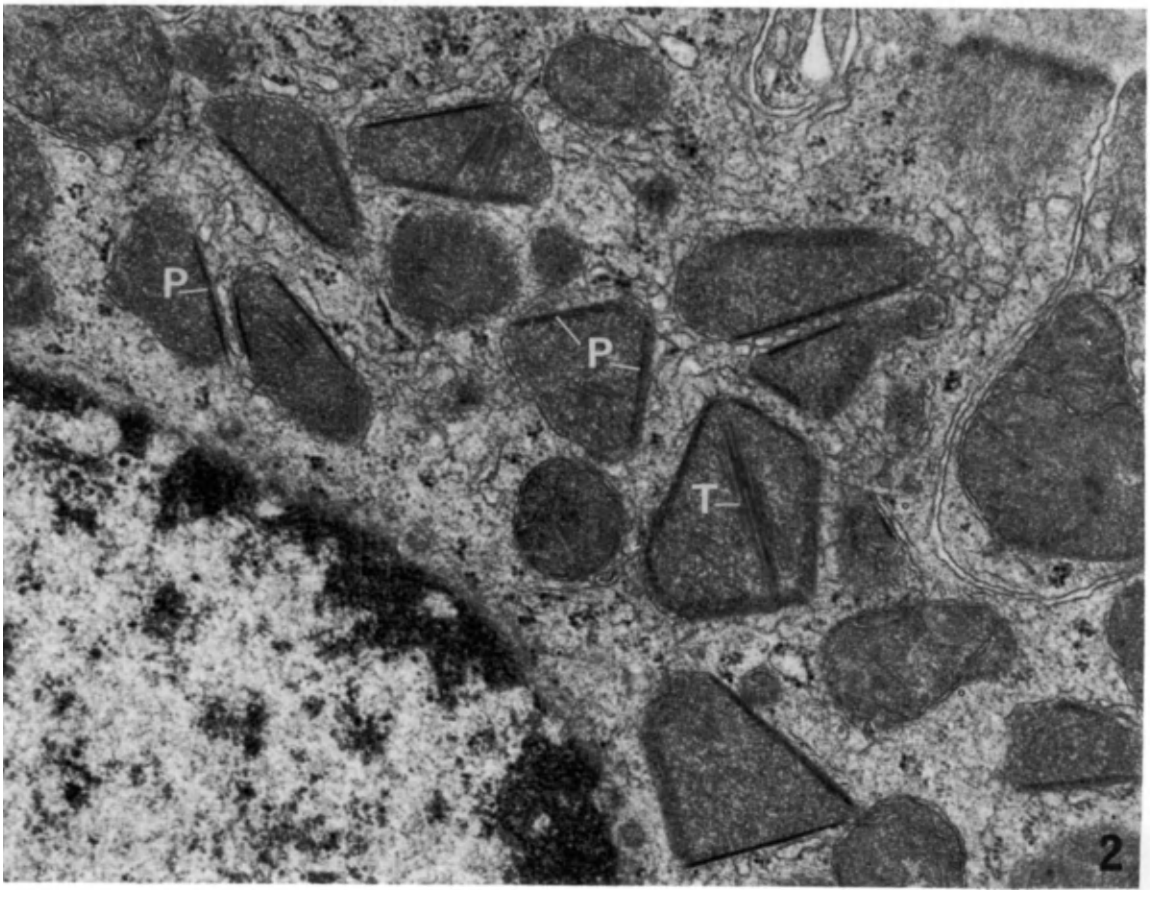
*The microbodies in some rat hepatomas have a rather large crystalline core. According to Hruban and Swift (1964), the fine structure of these cores matches quite precisely the purified urate oxidase (previously called 'uricase') crystals produced *in vitro*. These crystals are composed of hollow cylindrical subunits of two sizes (4.5 nm and 9.5–11.5 nm in diameter) which because of their small size are best referred to as microtubular subunits. (For differences between tubules and microtubules *see* page 938.)

Plate 329

Fig. 1. Microbodies from a cow hepatocyte showing linear densities which probably represent a normal section through a marginal plate (P). Also seen are structures which could either be conventional nucleoids or tangential (T) sections through marginal plates. × 37 000

Fig. 2. Microbodies from a renal tubular epithelial cell of a cow. Multiple marginal plates (P) are present as are crystalline bodies in the matrix which could be nucleoids or tangential (T) sections through plates. × 37 000


P
T
P
1


P
P
T
2

It would appear that the number and size of microbodies show many variations. Hepatic microbodies from various species range in size from 0.1-1 μm; but most measure approximately 0.5 μm in diameter. In type II alveolar cells, microbodies are much smaller and measure 0.1–0.2 μm in diameter. The ratio of microbodies to mitochondria is said to be approximately 1:4 in hepatocytes and between 1:1 and 1:2 in type II alveolar cells (Schneeberger, 1972a).

The site of origin and the manner in which microbodies are produced remain speculative. There is said to be a turnover of microbodies (Poole *et al.*, 1969) but how these organelles regress is not clear either. Suggested mechanisms for the removal of microbodies include: (1) transformation or incorporation into lysosomes; and (2) dissolution of the organelle into the surrounding cytoplasm (Svoboda *et al.*, 1967). The idea that microbodies may arise from or develop into mitochondria (Gansler and Rouiller, 1956; Rouiller and Bernhard, 1956; Engfeldt *et al.*, 1958; Pavel *et al.*, 1971) has been abandoned by most workers. A possible origin of microbodies from the following structures has been proposed: (1) smooth endoplasmic reticulum (Hagiwara *et al.*, 1961; Hruban *et al.*, 1963, 1965; Novikoff and Shin, 1964; Essner, 1966; Svoboda and Azarnoff, 1966; Tsukada *et al.*, 1966; Svoboda *et al.*, 1967); (2) Golgi complex and multivesicular bodies (Rouiller and Bernhard, 1956; Rouiller and Jézéquel, 1963; Bruni and Porter, 1965; Ericsson and Glinsmann, 1966; Dvorak and Mazanec, 1967); and (3) rough endoplasmic reticulum (Essner and Masin, 1967; Tsukada *et al.*, 1968; Mochizuki, 1968).

Regarding hepatic microbodies there was, at first, general agreement that the necessary enzymes were produced by the polyribosomes on the rough endoplasmic reticulum and that the material was then sequestrated in diverticula arising from the endoplasmic reticulum or travelled to the Golgi complex where it was parcelled off to form microbodies. Later cytochemical studies have, however, cast doubt on this thesis* and it is suggested by Wood and Legg (1970) that 'new microbodies may arise from pre-existing microbodies and that catalase after formation on the ribosomes of the rough endoplasmic reticulum, may be transferred directly into microbodies without passing through the cisternae of either the endoplasmic reticulum or Golgi apparatus'. Similar sentiments are expressed by Schneeberger (1972a), who found that in type II alveolar cells of the lung there is a threefold increase in the number of microbodies immediately before birth, and that microbodies at all stages of development are found in close proximity to the rough endoplasmic reticulum. However, physical connections between the two were not demonstrable and she states, 'It appeared that peroxisomes were formed *de novo* within the cytoplasm'.

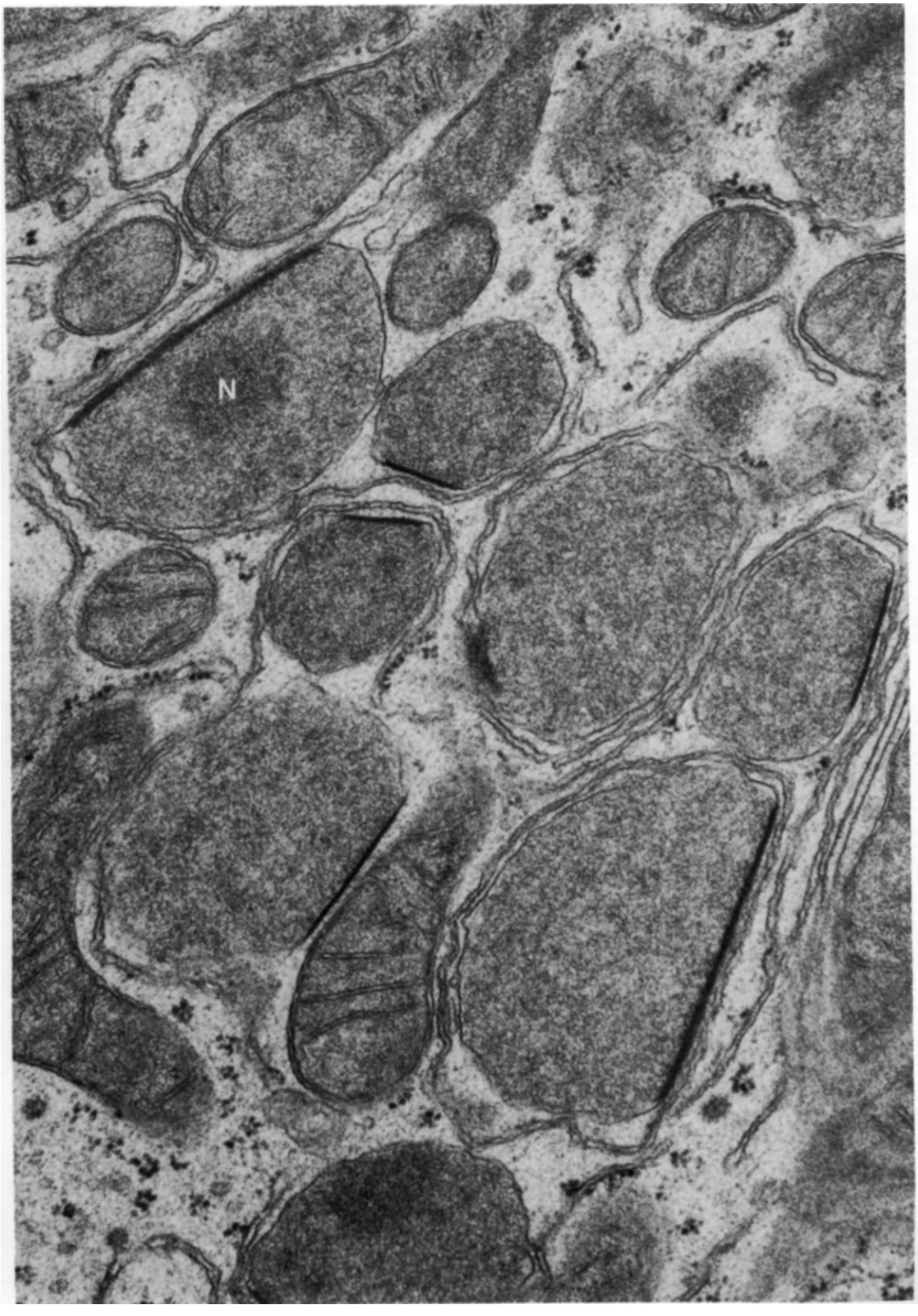
Yet another concept (Lazarow *et al.*, 1982) is that groups of microbodies are interconnected by narrow channels forming a microbody reticulum which is not continuous with the endoplasmic reticulum and it is claimed that the chemical composition of the membrane of the microbody reticulum is different from that of the membrane of the endoplasmic reticulum. It is further envisaged that the microbody membrane proteins and the enzymes it contains are synthesized by polyribosomes lying free in the cytoplasm, and that new microbodies form by budding from the microbody reticulum. Microbodies do not contain DNA or RNA, hence they are not regarded as self-replicating organelles like mitochondria.

The role of microbodies in cell physiology is poorly understood. Various suggestions on this point are summarized on page 768. Insights into their function derived from pathological states are dealt with in the next section.

*This idea has by no means been totally abandoned. Novikoff and Novikoff (1982) have recently presented some quite convincing electron micrographs of microbodies springing from endoplasmic reticulum.

Plate 330

Microbodies from renal tubular epithelium of a rabbit. Note the rigid marginal plate which distorts the spherical shape of the microbody. One of the microbodies also contains an amorphous nucleoid (N). It is difficult to believe that this is a tangential cut through a marginal plate. × 55 000


N

Pathological variations in size, shape and numbers

Several chemical agents and disease processes are known to produce an increase in the number of microbodies. Often this is associated with an increase in size and variations of morphology. A reduction in the number of microbodies is of less frequent occurrence, and rarer still is the condition (Zellweger's syndrome) where no microbodies are detectable in the liver and kidney. It is matters such as these that form the subject of this section.

A rapid and sustained increase in hepatic or hepatic and renal microbodies can be produced in some species by feeding clofibrate (ethyl chlorophenoxyisobutyrate). Such an increase may also be accompanied by an alteration in the size and shape of microbodies (*Plates 331* and *332*). This includes the formation of elongate and bipartite microbodies, and the appearance of tail-like or strap-like protuberances from them (Hess *et al.*, 1965; Svoboda *et al.*, 1967; Hartman and Tousimis, 1969). Clofibrate is a hypolipidaemic agent which lowers serum cholesterol and triglycerides in man, but hypolipidaemia in itself does not appear to be responsible for the increase, since some but not all hypolipidaemic agents produce this change. The response is also sex and species specific. For example, in the male rat, hepatic and renal microbodies are increased in number after clofibrate feeding but this is not so in the female. An increase in hepatic microbodies after clofibrate is seen in mice, dogs and hamsters but not in the guinea-pig, rabbit or squirrel-monkey (Svoboda *et al.*, 1967).

Besides clofibrate feeding an increase in hepatic microbodies has been seen after administration of nafenopin, methyl clofenapate and SaH 42–34 (1-methyl-4-piperidyl-bis [P-chlorophenoxy] acetate) which are hypolipidic analogues of clofibrate and also after the administration of a few other compounds structurally unrelated to clofibrate but which also induce hypolipidaemia (for references and details *see* Moody and Reddy, 1976).

Increased numbers of hepatocellular microbodies have also been reported to occur in a variety of infections and toxic conditions including: (1) various hepatitides (De Brito *et al.*, 1970; Canonico *et al.*, 1977); (2) alcoholic liver disease (Rubin and Lieber, 1967); (3) Reye's syndrome (fatty liver and encephalopathy) (Iancu *et al.*, 1977; Schubert *et al.*, 1979); (4) cholestatic jaundice of pregnancy (Van Haelst and Bergstein, 1970); (5) chronic passive congestion (Safran and Schaffner, 1967); (6) use of oral contraceptives (Larsson-Cohn and Stenram, 1967); and (7) administration of anabolic steroids (Orlandi *et al.*, 1965); and (8) vitamin E deficiency (Dabholkar, 1982).

It is not entirely certain that a true increase in the number of microbodies occurred in all the instances mentioned above. For example, Svoboda and Reddy (1975) did not find an increase in microbodies in six cases of Reye's syndrome, but they found non-crystalline cores in these microbodies. It will be recalled that normal human hepatocellular microbodies do not have a core, crystalline or otherwise.

The idea (*see also* page 767) that microbodies may have evolved as a protection against oxygen toxicity engendered by an increasing content of oxygen in primitive environment (de Duve and Baudhuin, 1966) led to a study of catalase and microbodies in type II alveolar cells of rats exposed to toxic concentrations of oxygen (Rosenbaum *et al.*, 1975). An increase in catalase and microbodies was found in animals made tolerant to oxygen toxicity by exposure to high but sublethal levels of oxygen concentration.

Reports on other drugs which have altered microbody population and/or morphology include: (1) alterations of microbody population after ethionine; (2) increase in number and size after salicylates; (3) increase in number and changes in matrix after thioacetamide; (4) enlargement of crystalloid core after azaserine and terephthalamilides; (5) extrusion of nucleoid

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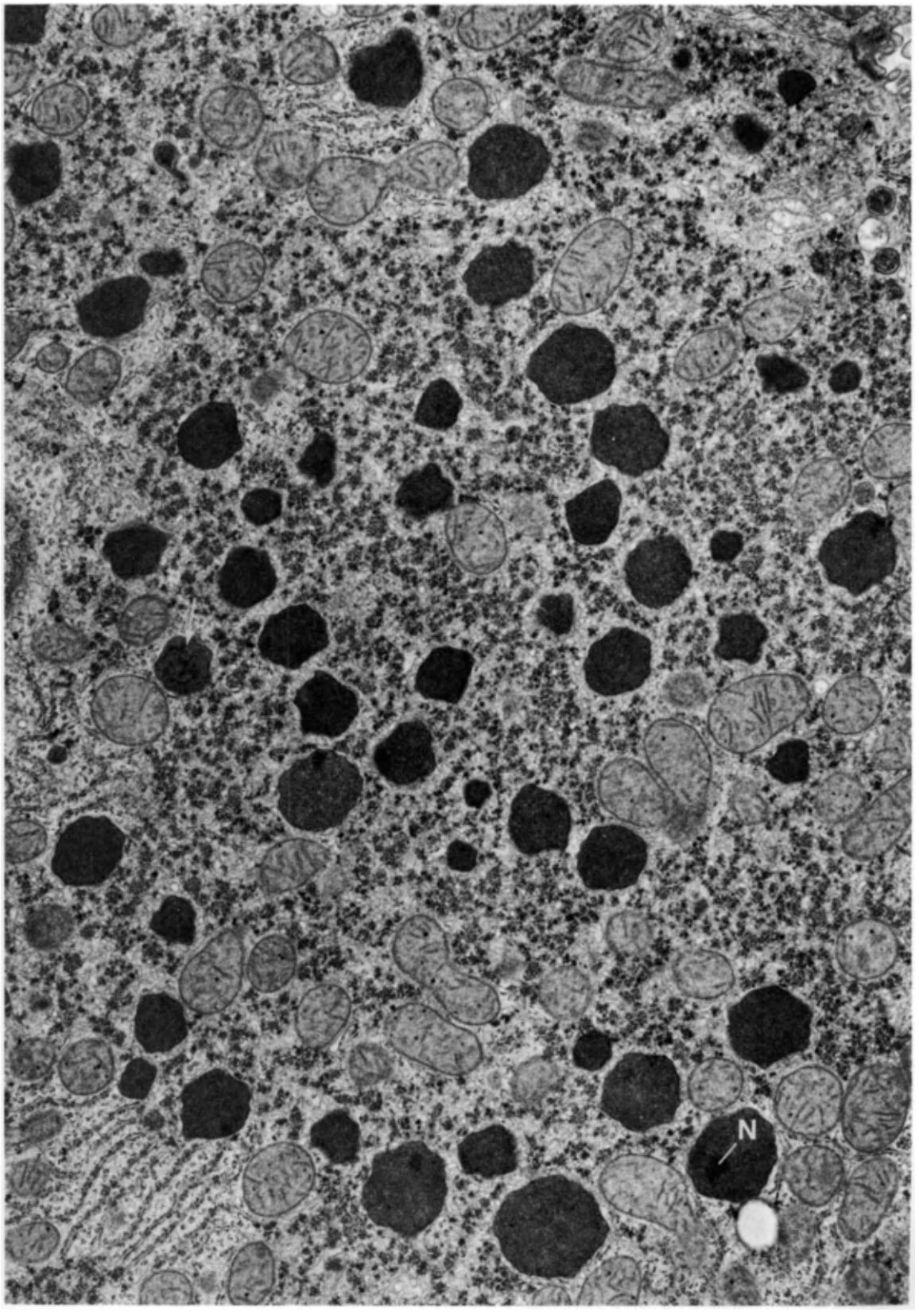
Plate 331

The marked increase in the number of microbodies produced in rat hepatocytes by feeding clofibrate is illustrated in this electron micrograph. Only a few of the microbodies contain a nucleoid (N) but some (arrow) contain multiple small nucleoids. × 15 500


N

after hexahydrosalicylic acid and sodium tungstate (for references to the above five items *see* Svoboda *et al.*, 1967); (6) development of a non-crystalline core where none existed before in Reye's syndrome (Svoboda and Reddy, 1975); and (7) development of a crystalline core in a complex disease state (Biempica, 1966).

Inclusions of various other types have been noted (Hruban *et al.*, 1974: Reddy *et al.*, 1982) in microbodies altered by various experimental procedures. These include: (1) fibrillar inclusions; (2) cavitation of matrix and membrane-bound vesicles; and (3) double-walled tubular inclusions about 110 nm in diameter.

In the hepatocytes (*Plate 333*) of a hamster treated with phenobarbitone we (Ghadially and Bhatnagar, unpublished observation) found: (1) some very large microbodies deserving to be called giant microbodies; (2) microbodies containing vesicles; (3) microbodies with pseudoinclusions containing cytoplasmic material; (4) a microbody containing a true glycogen inclusion; (5) cavitation of microbody matrix; and (6) numerous glycogenosomes (*Plate 319*). Since these changes were seen in only one animal and since such changes have not been reported to occur after phenobarbitone treatment, we are at a loss to explain the occurrence of these changes in this animal.

However, the most interesting anomaly affecting microbodies is the absence or relative absence (i.e. only a few small catalase reactive microbodies can be detected) of these organelles in some genetic disorders which are characterized by elevated plasma levels of very long chain fatty acids and in some instances of pipecolic acid, bile acid intermediates and phytanic acid. Perhaps the best known of these rare disorders is Zellweger's syndrome (severe hypotonia, hepatomegaly with cirrhosis, renal cysts, brain maturation defects, lipid accumulation in astrocytes, a characteristic facies and minor skeletal abnormalities) which usually proves fatal in under one year. Goldfischer (1979) reported that in five cases with this disorder no structures resembling microbodies were detected in the liver and kidney by cytochemical and ultrastructural studies. Several workers have confirmed the absence of hepatocellular microbodies in Zellweger's syndrome and stressed the importance of electron microscopy in establishing the diagnosis (Pfeifer and Sandhage, 1979; Müller-Höcker *et al.*, 1981; Mooi *et al.*, 1983*). However, a few small microbodies have been found in cultured skin fibroblasts derived from patients suffering from this condition (Arias *et al.*, 1985).

In adrenoleucodystrophy (Schilder's disease) which usually manifests in prepubertal boys (death occurs during adolescence in most cases), there seems to be a biochemical defect in microbodies, but there are no morphologically detectable changes in these organelles (Goldfischer and Reddy, 1984). However, in neonatal adrenoleucodystrophy (affects children who do not usually live longer than six years), the hepatocellular microbodies are reduced in size and numbers or are undetectable (Goldfischer and Reddy, 1984; Goldfischer *et al.*, 1985), but they are present in cultured fibroblasts derived from some of these patients (Beard *et al.*, 1986).

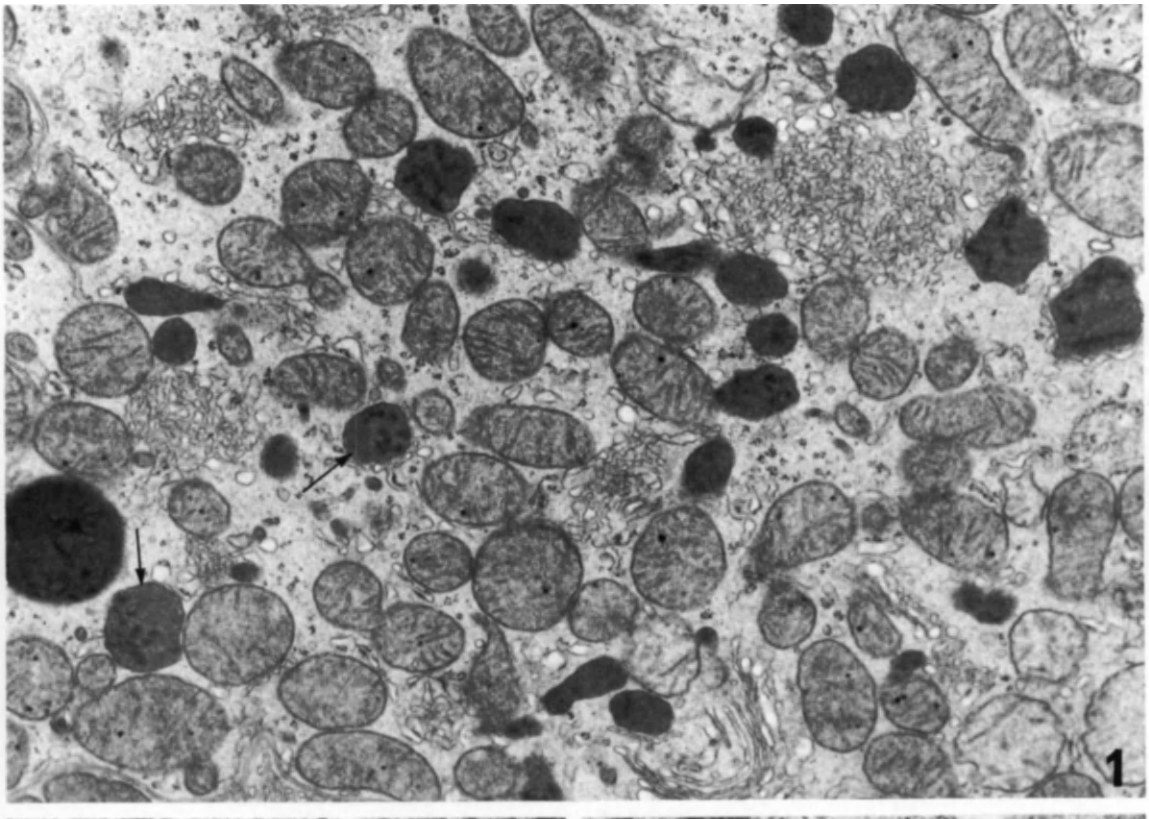
*Angulate lysosomes (*Plate 309, Fig. 1*) were consistently found in macrophages (i.e. Gaucher-like cells) by Mooi *et al.* (1983) in biopsy and autopsy specimens of liver from five cases of Zellweger's syndrome. They point out that although angulate lysosomes are not specific or diagnostic of this condition, in the correct clinicopathological setting they may well be of considerable diagnostic significance, especially when only poorly preserved liver tissue (e.g. postmortem material) is available in which the absence of peroxisomes cannot be assessed with certainty.

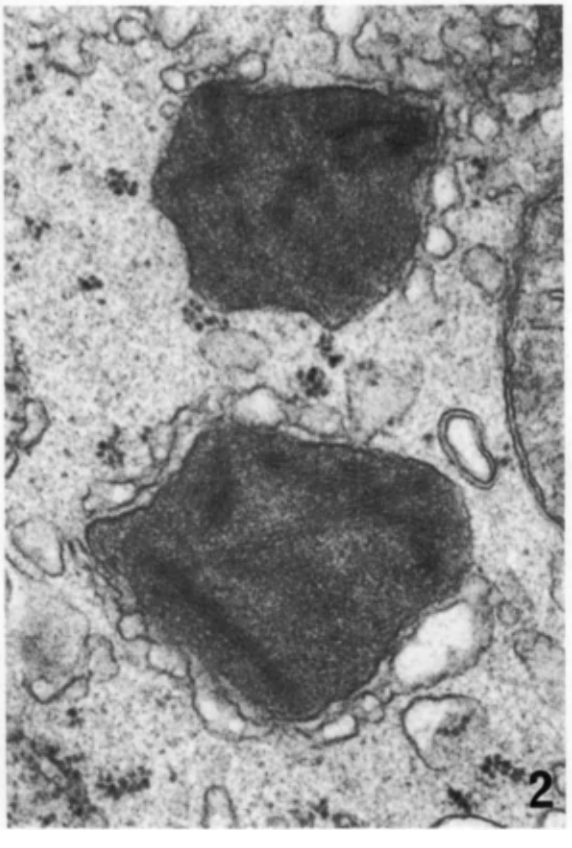
Plate 332

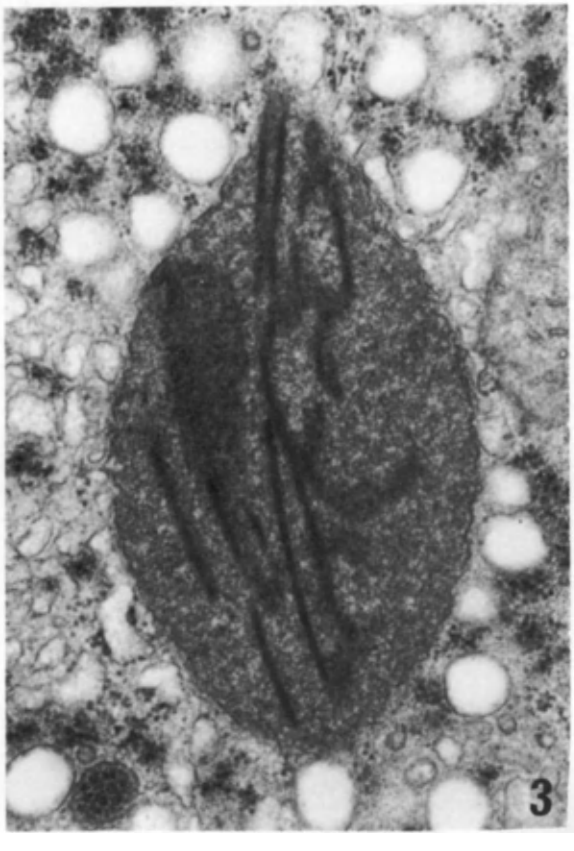
Fig. 1. The marked increase in the number of microbodies produced in renal tubular epithelial cells of the rat by feeding clofibrate is illustrated in this electron micrograph. Many microbodies (arrows) show multiple nucleoids. × 16 000 (*Ghadially and Ailsby, unpublished electron micrograph*)

Fig. 2. High-power view of two of the microbodies shown in *Fig. 1.* × 51 000 (*Ghadially and Ailsby, unpublished electron micrograph*)

Fig. 3. An unusually large microbody (approximately 2.4 μm long) found in the liver of a hamster that had received phenobarbitone. × 40 000 (*Ghadially and Bhatnagar, unpublished electron micrograph*)


1


2


3

The situation regarding the presence or absence of microbodies in phythanic acid storage disease is somewhat more complex. Genetic disorders affecting the metabolism of phytanic acid (a dietary fatty acid) include: (1) Refsum's disease affecting young adults; and (2) the condition called 'infantile Refsum's disease' or 'infantile phytanic acid storage disease'. In the former (item 1), hepatic microbodies are said to be absent (Ogier *et al.*, 1985), but normal numbers or perhaps even a slightly increased number of microbodies is seen in cultured skin fibroblasts (Beard *et al.*, 1985). In the latter (item 2) only small microbodies marginally reactive for catalase are found in the liver. However, in four of the six cases studied by Beard *et al.* (1986), skin fibroblast cell lines contained microbodies strongly reactive for catalase but not in the other two cases.

There is usually a reduction in the number of microbodies in tumours. It is said that the number of microbodies present is inversely proportional to the growth rate of rat hepatomas and that microbodies are absent in the rapidly growing Morris hepatoma No. 3683 (Dalton, 1964; De Duve and Baudhuin, 1966; Mochizuki *et al.*, 1971). Similarly, Sima (1980) found that microbodies 'appeared in a decreasing number in the slow growing, benign subependymal giant cell astrocytomas, astrocytomas and glioblastomas in that order'.

Perhaps the most constant and frequently studied biochemical change known to occur in animals bearing a variety of tumours is the reduction of liver catalase activity (Greenstein, 1954) and it would appear that there is also a decrease in the number of microbodies in the hepatocytes (Ghadially and Parry, 1965; Mochizuki, 1968). Since 80 per cent of the liver catalase activity is believed to be localized in microbodies and about 40 per cent of microbody protein can be ascribed to catalase (Beaufay *et al.*, 1964; Fujiwara, 1964; de Duve and Baudhuin, 1966), a reduction of microbodies in the liver of the tumour-bearing host may be expected. In keeping with this is: (1) the reduction in catalase staining in kidney and liver biopsies that has been described in three out of four patients bearing malignant tumours (Wittrin *et al.*, 1975); and (2) biochemical studies which have revealed diminished hepatic catalase in patients with liver metastasis (Blumenthal and Brahn, 1910).

The mechanism and specificity of catalase depression in the liver of the tumour-bearing host have been the subject of much dispute but there is now evidence that this change and the remarkable increase in hepatocellular lysosomes that occurs in the tumour-bearing animal is probably engendered by a toxic polypeptide (toxohormone) produced by viable tumour tissue (for references to the considerable literature on the subject, *see* Kampschmidt, 1965; Nakahara, 1967; Parry and Ghadially, 1970; *see also* page 694 for more details about the changes in the liver of the tumour-bearing host).

Plate 333

Atypical microbodies found in the hepatocytes of a hamster treated with phenobarbitone.

Fig. 1. A 3.8 μm long rod-shaped microbody. × 19 000

Fig. 2. A 3.2 μm long dumbbell-shaped microbody. × 21 000

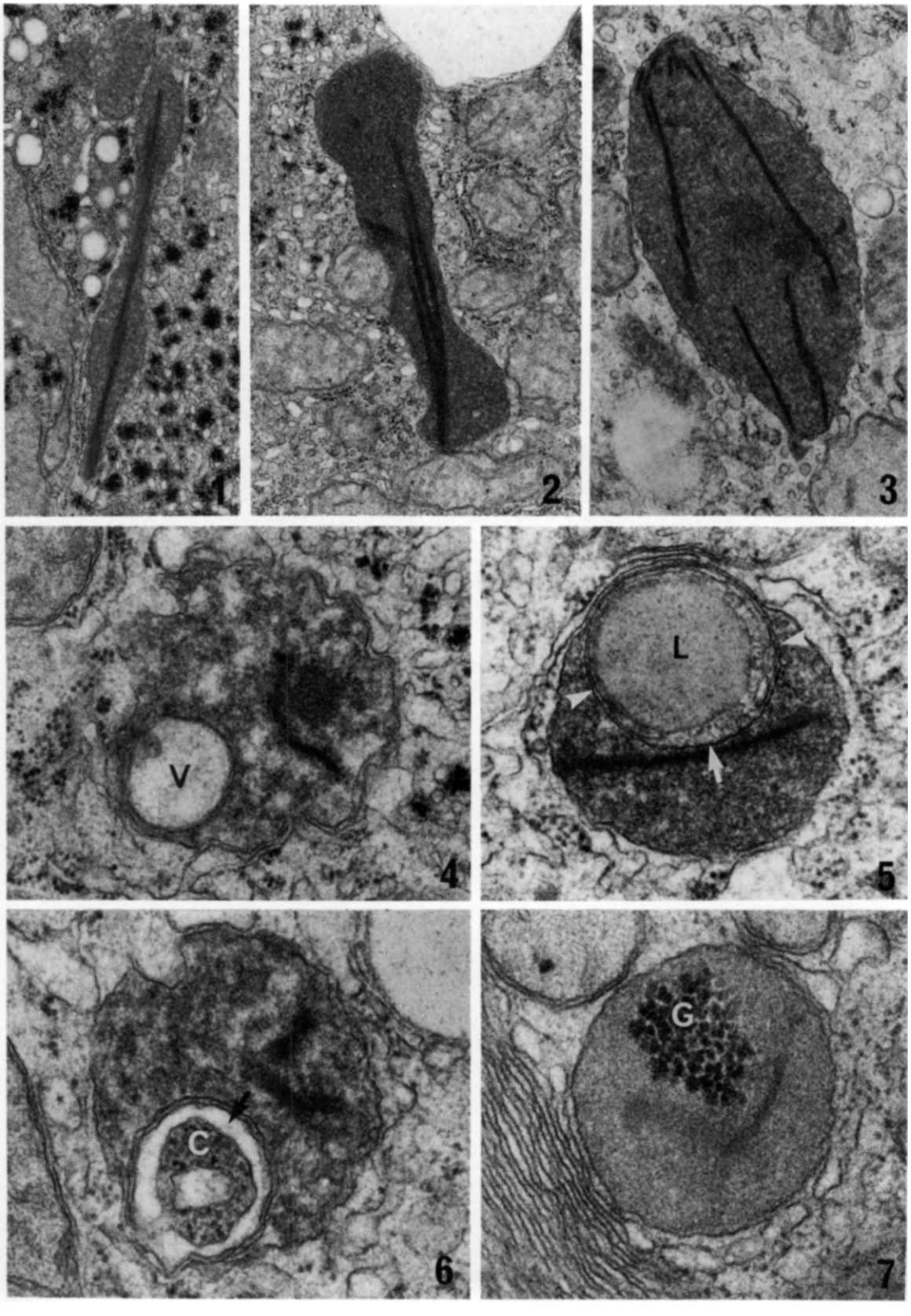
Fig. 3. A 2.6 μm × 1.3 μm rugby football-shaped microbody. × 27 000

Fig. 4. Cavitation of the matrix gives this microbody a moth-eaten appearance. Note also the vacuole (V) (presumably a pseudoinclusion) containing flocculent material. × 43 000

Fig. 5. A single-membrane-bound (arrow) pseudoinclusion in a microbody. The inclusion contains a small amount of cytoplasm and a lipid droplet (L). Microbodies are bounded by a single membrane, hence such inclusions are essentially single-membrane-bound (arrow). The double-membrane-bound profiles seen in some places (arrowheads) probably result from a juxtapositioning of an included cytomembrane aside the single membrane limiting the inclusion. × 52 000

Fig. 6. A pseudoinclusion in a microbody which is apparently bound by two membranes. The appearances seen here are interpreted as a pseudoinclusion containing cytoplasmic material (C) and dilated endoplasmic reticulum containing lucent material (arrow). × 62 000

Fig. 7. A microbody containing a true (i.e. non-membrane-bound) glycogen (G) inclusion. × 65 000


1
2
3
V
4
L
5
C
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G
7

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9

Melanosomes

Introduction

A melanosome may be defined as an organelle in which melanin is synthesized. The end-product of its action is the melanin granule (now called stage IV melanosome), into which it is ultimately transformed. Melanosomes are produced by melanocytes, and melanosomes in early stages of development are as a rule found only in these cells*. Later stages of development are found both in melanocytes (*Plate 334*) and other cells such as the basal and prickle cells of the epidermis (keratinocytes) and phagocytic cells in the dermis (melanophages). Masson (1948). recognized two types of melanocytes: continent and incontinent. An example of continent melanocytes is found in the pigment epithelium of the eye where the cells retain the synthesized melanin throughout their life. The incontinent melanocytes of the epidermis and hair follicles discharge their melanin (melanosomes) into epithelial cells by a process which Masson called 'cytocrine activity'.

In the past there was much confusion regarding the nomenclature of melanin-containing cells, and it was widely held that a variety of cells, such as the basal cells of the epidermis and also various mesenchymal cells, could all synthesize melanin. This confusion was reflected also in the concepts and nomenclature of malignant melanomas, which were called 'melanocarcinomas' or 'melanosarcomas'; and the benign melanoma (naevus) was generally thought to arise from the basal cells of the epidermis. Innumerable later studies have, however, clearly established the melanocyte as a specific cell type, and its origin from the neural crest has also been long established (Rawles, 1947, 1948).

In this era, however, melanocytes were often referred to as melanoblasts, and melanophages as melanophores. At the Third International Pigment Cell Conference it was decided (Gordon, 1953) to call the mature melanin-producing and melanin-containing cell a melanocyte and to reserve the term melanoblast for the embryonic precursor capable of transforming into a

*In the normal state the melanocyte is virtually the only cell that synthesizes melanosomes but on very rare occasions the Schwann cell may perhaps also do so. Virtually all melanomas are melanocytic melanomas where neoplastic melanocytes produce melanosomes. Rare but indubitable examples of melanotic schwannomas where neoplastic Schwann cells produce melanosomes are also known to occur. In some neuroendocrinomas, cells acceptable as melanocytes are found and perhaps on very rare occasions cells containing neuroendocrine granules and melanosomes may be found, but about this one cannot be too certain (*see* pages 812–821).

melanocyte. It was also pointed out that the term 'melanophore' had long been used to describe certain pigment-effector cells of lower vertebrates (now thought to be a variety of melanocyte in which dispersion or aggregation of pigment leads to rapid skin colour changes) and the principle of priority in scientific nomenclature dictated that this term should not be used to describe the functionally and morphologically different melanophages of man and higher vertebrates, which contained phagocytosed melanin.

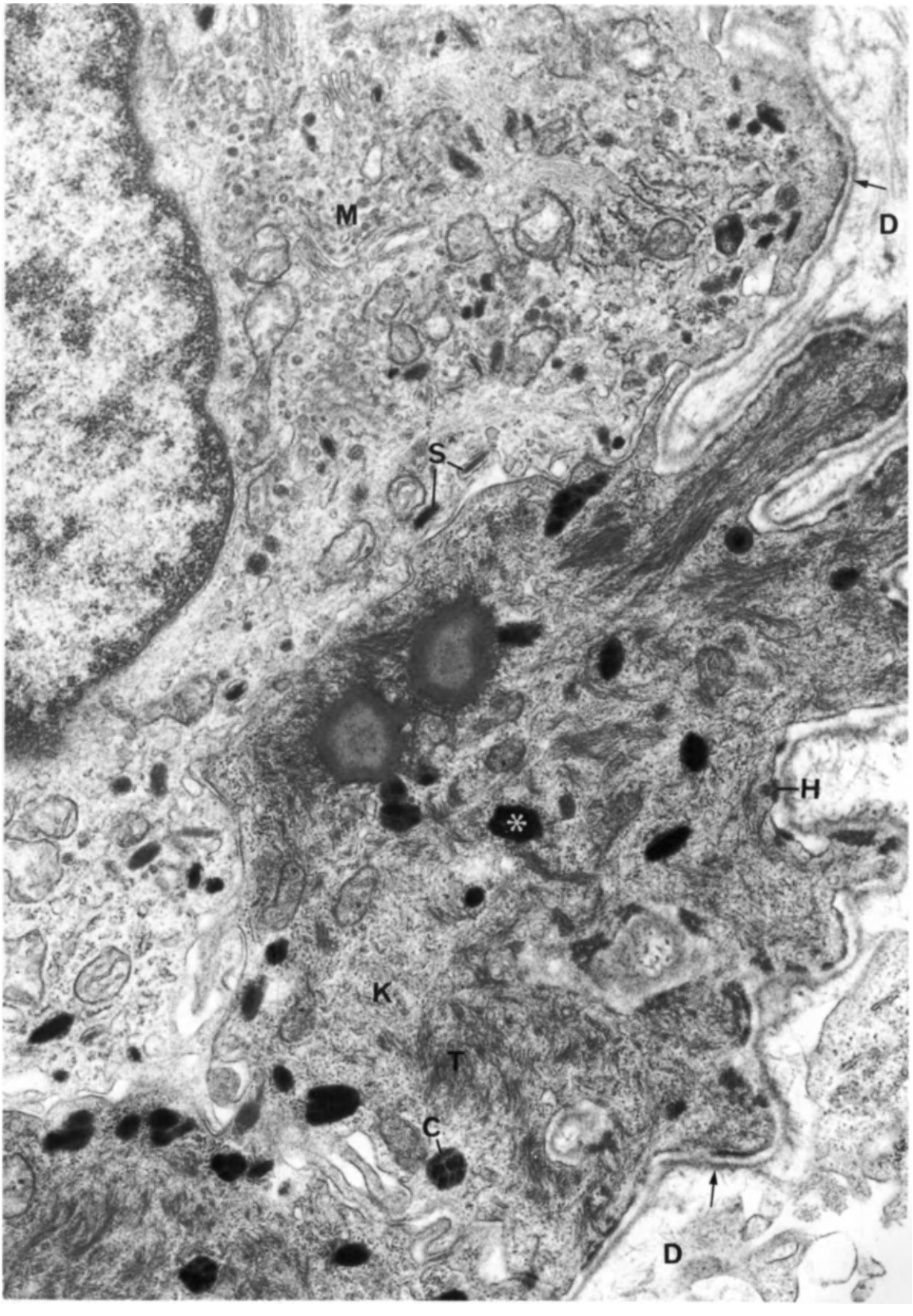
The melanocyte is now recognized as the basic pigment cell not only of human melanomas but of melanomas of all vertebrates. This view, first advocated by Gordon (1948) from the study of piscine melanomas produced by genetic methods in *Platypoecilus* × *Xiphophorus* hybrids (Gordon 1937; Grand *et al.*, 1941), was also found to be so for the *Lebistes* × *Mollienesia* hybrid (Ghadially and Gordon, 1957)* and the abundant melanotic tumours that arise in hamster skin painted with carcinogens (Ghadially and Barker, 1960; Illman and Ghadially, 1960; Ghadially and Illman, 1964, 1966; Ghadially, 1982).

The discovery of the melanosome by Seiji *et al.* in 1961 marks the last step in establishing unequivocally the melanocyte as a race of cells quite distinct and different from others. This advance led to a reconsideration of the nomenclature of pigment cells, and at the Sixth International Pigment Cell Conference the melanocyte was defined as 'A cell which synthesizes a specialized melanin-containing organelle, the melanosome' (Fitzpatrick *et al.*, 1966).

*According to the Rosen and Bailey (1963) classification, fishes of the genera *Lebistes* and *Mollienesia* are now classified under the genus *Poecilia*. The genus *Platypoecilus* has been absorbed into the genus *Xiphophorus*. (For colour illustrations and descriptions of these fishes, *see* Ghadially, 1969.)

Plate 334

Electron micrograph from normal skin (caucasoid) showing a melanocyte (M) and a keratinocyte (K) separated from the dermis (D) by basal lamina (arrows). The melanocyte has a paler appearance and contains numerous discrete melanosomes (S). The keratinocyte has a darker appearance and contains numerous bundles of tonofilaments (T) and hemidesmosomes (H) along its basal border. Numerous large compound melanosomes (⋆) (formed by the aggregation of solitary melanosomes transferred from the melanocyte to the keratinocyte) are present. In some (C), the melanosomes within the compound melanosomes can just be discerned. (*See also Plate 376*). × 26000


M
D
S
H
K
T
C
D

Morphology and normal variations

The concept that melanin synthesis occurs in a specialized organelle of the melanocyte called the melanosome was first proposed by Seiji *et al.* (1961). It is now clear that this organelle contains tyrosinase, an enzyme which is involved in the oxidation of the colourless amino acid, tyrosine, to the pigmented polymers we call the melanins (eumelanins are brownish-black, phaeomelanins are yellowish-red). Dopa (3,4-dihydroxyphenylalanine) is an intermediate compound formed during the first stage of this oxidative reaction. Thus, when either dopa or tyrosine is presented to cells containing tyrosinase, they show increased pigmentation. This is the basis of the dopa reaction (Bloch, 1927) and the tyrosinase reaction (Fitzpatrick *et al.*, 1950); both reveal the presence of the only known enzyme involved in melanin synthesis, namely tyrosinase.

The generally accepted classification of melanosomes (Fitzpatrick *et al.*, 1971) recognizes four stages of melanosome development. The first or earliest stage of development contains no melanin. The last stage is the fully mature melanin granule where the internal structure of the melanosome is totally masked or destroyed by melanin deposition (*Plate 335*). The earlier stages possess an active tyrosinase system; the last does not. The ontogeny of melanosomes has been the subject of excellent reviews (Toda *et al.*, 1968; Fitzpatrick *et al.*, 1971; Quevedo *et al.*, 1987). Several theories (at least four with many variations) have been advanced to explain in detail the genesis and development of melanosomes (*see* review by Quevedo *et al.*, 1987), but the basic idea as expressed in the classic theory is that tyrosinase is synthesized by the polyribosomes of the rough endoplasmic reticulum and transported via this organelle to the Golgi complex where it is parcelled off into small vesicles (melanosome stage I). The vesicles then enlarge and elongate* to form an oval organelle (melanosome stage II, previously called premelanosome) in which develops a characteristic patterned structure, the three-dimensional morphology of which is difficult to interpret. The internal structure has been regarded as a folded membrane, a concentric sheet, a collection of filaments, or a helical tubular structure with space of about 10 nm between each turn of the coil†. Deposition of melanin on the structure heralds the next stage (melanosome stage III, or partially melanized melanosome), and the completion of this process produces a uniformly electron-dense granule without discernible internal structure (melanosome stage IV, mature melanosome or melanin granule).

Variations in the size and shape of the melanosomes, depending on site and species, are known to occur. Breathnach (1969) reported that mature melanosomes of dark human skin measure 0.37 by 1.15 μm, that of the hair bulbs 0.3 by 0.7 μm while melanosomes from the pigmented epithelium in the eye can measure up to 1.4 μm in breadth and 1.9 μm in length.

*A variation on this idea is that at least in some sites and situations the Golgi-derived vesicles containing tyrosinase enter an elongated vacuole (presumably derived from the endoplasmic reticulum) containing a proteinaceous matrix. In such instances the early stages of melanosome development would resemble a multivesicular body. Certainly in malignant melanomas one occasionally finds oval melanosomes containing vesicles and they do resemble multivesicular bodies (*see Plate 337*). This sort of mechanism may also be operative in the production of granular melanosomes (page 804) and some giant melanosomes (page 810).

†In sectioned material various appearances are seen in stages II and III melanosomes. A common appearance is that of a series of parallel or concentric zig-zag or dotted lines. Another appearance seen is that of striated or banded material (periodicity of banding is usually about 10 nm) lying in the melanosome.

Plate 335

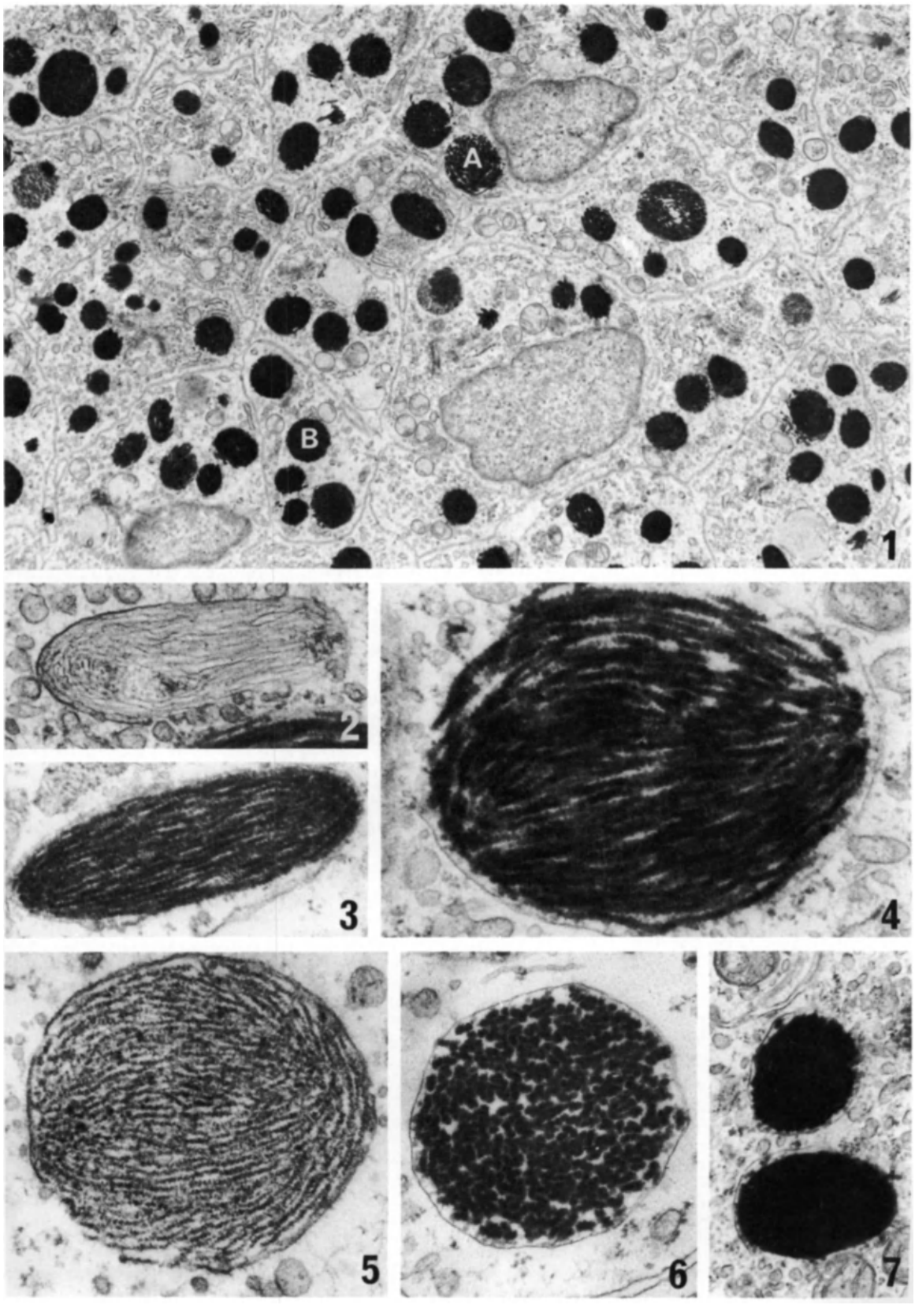
From the pigmented epithelium of the eye of a ten-week-old human fetus. (*Ghadially and Ailsby, unpublished electron micrographs*)

Fig. 1. Numerous state III (A) and IV (B) melanosomes are see in these cells. × 6500

Fig. 2. A stage II melanosome, showing membranous or filamentous formations in its interior. × 39 000

Figs. 3–6. Stage III melanosomes. The difference in appearances seen can be related to (1) the size and shape of the melanosome, (2) the plane of sectioning, and (3) the amount of melanin present. × 39 000; × 40 000; × 45 000; × 40 000

Fig. 7. Stage IV melanosomes or melanin granules are seen here. × 26 000


A
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1
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3
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5
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7

(Scanning electron microscope studies show that the last-named can be as much as 2.5 μm long). In red hair and in the relatively unpigmented skin between freckles in very pale caucasoids the melanosomes are more rounded (Birbeck and Barnicot, 1959; Breathnach, 1964). Differences in size, shape and internal structure, thought to be determined by genes, have been described in experimental animals such as mice (Moyer, 1961, 1963, 1966). Except in rare instances, melanosomes occur as discrete bodies in melanocytes, but in the keratinocytes* and melanophages they also occur grouped together within a single-membrane-bound bodies, which have at times been referred to as 'melanosome complexes' or 'compound melanosomes' (Drochmans, 1966). Because acid phosphatase activity had been demonstrated in these structures, they are regarded as lysosomes (Hori *et al.*, 1968). Fine granular material is also seen in these lysosomal bodies and this is regarded as material derived from the degradation of melanosomes and also perhaps other material incorporated within these lysosomes.

In causasoids and mongoloids, groups of melanosomes occur within lysosomes of the keratinocyte, but in the former they are not closely packed and fine granular material between them is more abundant (Szabo *et al.*, 1969). In negroids and Australian aborigines (Mitchell, 1968) the much larger melanosomes are dispersed individually within the keratinocyte, and characteristic 'complexes' with numerous melanosomes are not seen. Thus, a factor responsible for the more marked pigmentation of the negroid skin seems to be the persistence of pigment which, in the caucasoid, is destroyed by lysosomal activity.

Racial differences in pigmentation do not appear to be due to variations of population density of the melanocytes. The number of melanocytes varies with regions of the body, there being 2000 or more melanocytes per mm^2 on exposed skin such as that of the forearm and face but only about 100 per mm^2 on the rest of the body in caucasoids, mongoloids and negroids (Szabo *et al.*, 1969). However, quantitative and qualitative differences between the melanosomes in the melanocyte occur among the races. Melanosomes are larger and more numerous in negroids and virtually all are stage III and IV melanosomes; stage II melanosomes are rarely seen. In very pale caucasoids with blue eyes and red hair, very few melanosomes are seen in the melanocytes and few, if any, stage III or IV melanosomes are found. In darker caucasoids the stage IV melanosome is rarely seen, but melanosomes stage I, II and III are present (Fitzpatrick *et al.*, 1971).

Ultraviolet radiation leads to a general increase (particularly in keratinocytes†) in the number of melanosomes in all races. Qualitative changes are also noted; stage IV melanosomes develop in the skin of pale caucasoids and many more stage II and III melanosomes are found in negroids.

*Epidermal cells producing keratin are collectively referred to as keratinocytes. This includes basal cells and prickle cells.

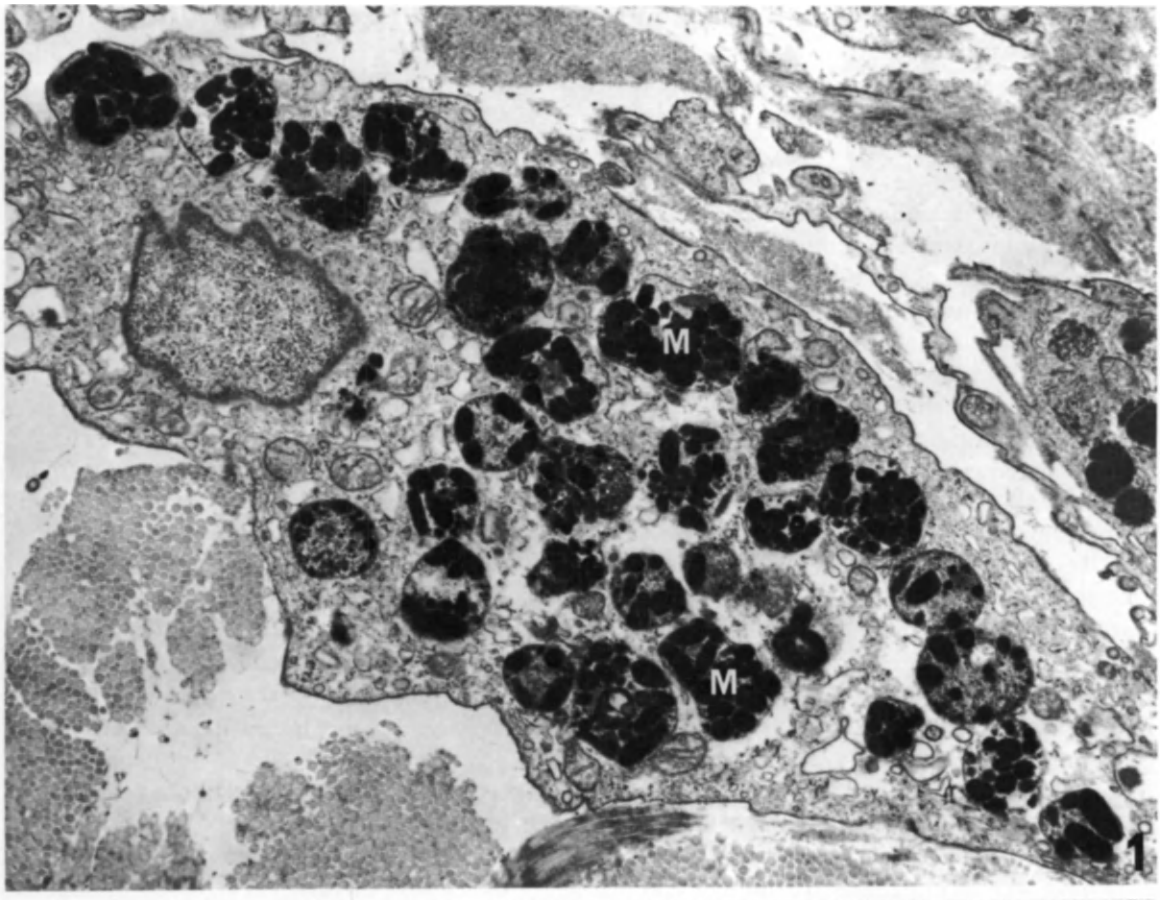
†A probable factor responsible for this increase may be a diminished rate of destruction of melanosomes in the heterolysosomes in the keratinocytes, presumably due to a disturbance of the lysosomal apparatus engendered by ultraviolet radiation. Whether there is an excessive production of melanosomes and/or a delayed destruction of melanosomes the net result is that the more superficially placed keratinocytes come to contain more or less intact melanosomes and it is this which produces the 'tan' in sunbathers during the summer months. Restoration of normal skin tone is achieved by desquamation (peeling off) of the pigmented keratinocytes after exposure to the strong summer sun ceases.

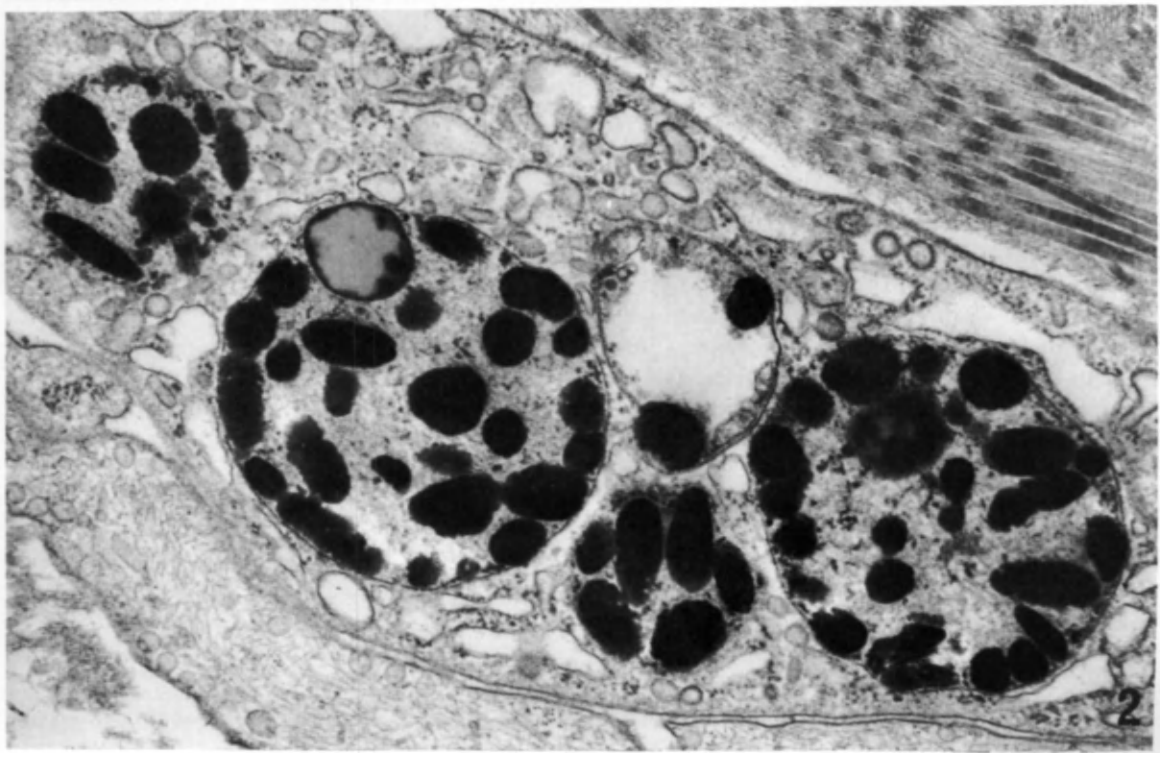
Plate 336

Skin rash from a case of cutaneous lymphoma.

Fig. 1. A dermal melanophage containing numerous so-called compound melanosomes (M) which are in fact heterolysosomes containing melanin granules. × 16 000 (*From Ghadially, 1985*)

Fig. 2. High-power view of compound melanosomes in a dermal macrophage (i.e. melanophage). The melanin granules in the interior of the compound melanosomes are easily recognized. × 33 000


M
M
1


2

Alterations in melanosomes in melanomas and pigmentary disorders

In addition to the normal variation in melanosome morphology (pages 790–793) there also occur quantitative and qualitative variations associated with melanomas* and pigmentary disorders. Mishima (1965, 1966) showed that considerable variations in the morphology of the melanosomes are seen in various disorders such as Dubreuilh's melanosis, amelanotic and melanotic melanoma, junction naevus, juvenile melanoma, naevus of Ota, blue naevus, vitiligo and albinism. Most of these morphological variations are not specific for any particular disorder but later workers (Curran and McCann, 1976; Drzewiecki, 1979) who have compared melanosomes in malignant melanoma with melanosomes in various types of naevi claim that melanosomes in malignant melanoma can be distinguished from all others by changes in melanosome structure and that this appears to be quite a sensitive and reliable indicator of malignant change, which may be of diagnostic value.

The melanosomes in malignant melanoma are very pleomorphic and show many structural derangements (*Plates 337–339* and *341–343*). Curran and McCann (1976) state that 'a basic fault was the failure of the membranous filaments to align properly in register so that the normal compact banded structure was replaced by loose coils and spirals of filaments'. Melanin is deposited on such filaments in an irregular (patchy) and, apparently, often incomplete fashion. It is thought that when such melanosomes (*Plate 337*) are found it is safe to assume that the lesion is malignant. Conversely, the presence of compact cigar-shaped or elliptical melanosomes with a banded internal structure and uniformly melanized melanosomes would suggest a benign lesion.

The idea that atypical or abnormal melanosomes are made by malignant cells while 'normal-looking' ones are made by benign cells is attractive for it fits in with our general concepts of neoplasia and one may further speculate that the more malignant the tumour, the more atypical the melanosomes are likely to be.

I am in general agreement with the sentiments expressed above because they fit in with what I have seen in malignant melanomas and because in our study (Ghadially *et al.*, 1986) on human and hamster blue naevi we did not find a single atypical melanosome of the type seen in malignant melanoma (*Plate 337*). However, it is worth noting that not all melanosomes in malignant melanomas are atypical and that normal-looking melanosomes are also found.

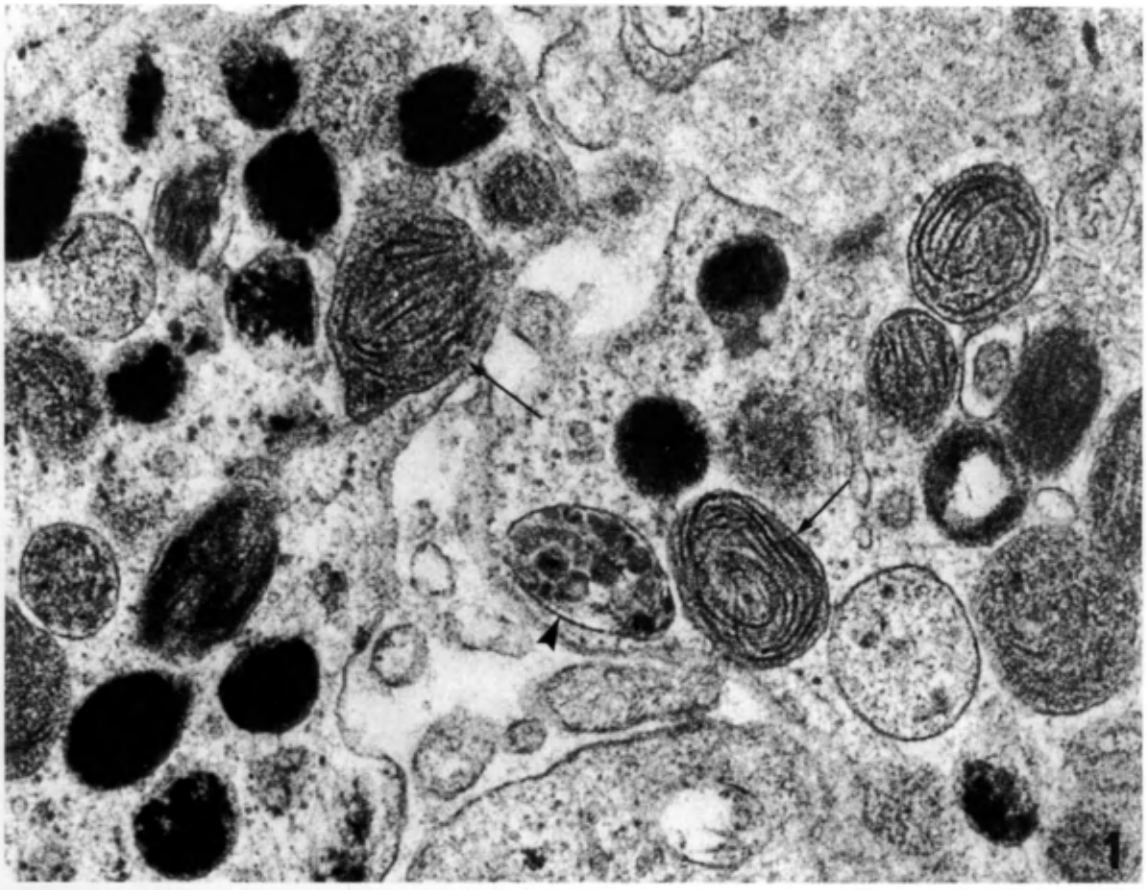
A review of published electron micrographs of melanosomes in naevi and in malignant melanoma also supports the idea expressed above. However, I am aware of one report (Bhawan *et al.*, 1980) where melanosomes with a 'deranged internal structure' are reported to occur in blue naevi, but this 'derangement' (their *Fig. 6*) is somewhat different from that seen in malignant melanoma in that opened-out spirals of filaments are absent. The tumours described by these authors are unusual in another respect, in that the cells are ensheathed by a well-formed external lamina (*see* pages 812 and 821 for further comments).

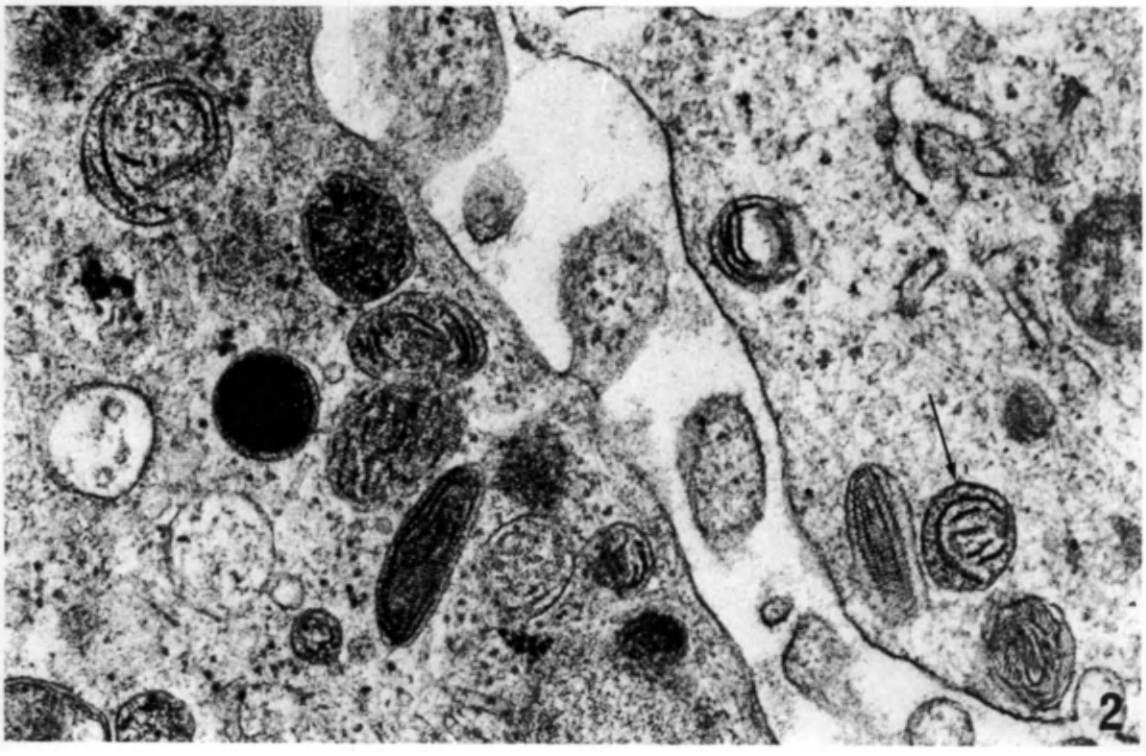
Be that as it may, the bulk of the evidence supports the idea that melanosomes in malignant melanoma are as a rule different from those seen in naevi and that this point may be of diagnostic value. Like so many of our newly discovered diagnostic criteria, only time will show how reliable they are and what weight one can put on them in arriving at a diagnosis.

*In this section of the text we deal with melanosomes found in a tumour which may more fully be described as the 'malignant melanocytic melanoma' but which is usually spoken of as a 'malignant melanoma' or just 'melanoma' even though benign melanomas (pigmented naevi) are very common. There has been much controversy regarding the nature of the cells in certain other melanin-producing tumours, such as blue naevi and pigmented schwannoma. These matters are dealt with on pages 812–821, where a general discourse on pigment producing cells and tumours is also presented

Plate 337

Figs. 1 and 2. Pleomorphic melanosomes from malignant melanoma of skin. Atypical melanosomes of the type indicated by arrows support the idea that this lesion is malignant. Note also the melanosome (arrowhead) with vesicles in its interior. × 55 000, × 56 000





Mishima's (1967) contention that melanosomes in malignant melanoma arising from lentigo maligna (Dubreuilh's precancerous melanosis) tend to be ellipsoids which present as 'rings' in sections, while those in malignant melanoma originating from a junctional naevus are cigar-shaped has not been borne out by later studies, nor has the claim by Kluğ and Gunther (1972) that two types of melanosomes exist*, one (Type A) which is cigar-shaped and shows periodic banding internally and another (type B) which is spherical in which an internal filamentous structure can be detected. Several intermediate and aberrant forms of melanosomes are found in melanomas, including spherical melanosomes with a banded interior and elliptical ones which contain scattered filaments and/or vesicles (*Plate 337*). To this one may add granular melanosomes (pages 804 and 805), balloon melanosomes (pages 806 and 807) and giant melanosomes (pages 808–811) which have been found in benign (i.e. melanotic naevi) and malignant melanoma.

It is well known that the amount of melanin in melanomas can vary markedly, and the naked-eye appearance of these tumours can range from black, through various shades of grey, to pink. This has been found to be so for melanomas of various animals including man and it is customary to refer to such tumours as melanotic, hypomelanotic or amelanotic melanomas. In the last instance melanin cannot be demonstrated unequivocally by histological examination of the tumour and the diagnosis of the lesion may become difficult or impossible (*Plate 338*). The amelanotic state indicates a loss of functional activity or dedifferentiation, and as such one may expect amelanotic melanomas to be more aggressive than melanotic melanomas (Illman and Ghadially, 1960; Ghadially and Illman, 1966). While this is, to some extent, correct, in man at least both well pigmented and non-pigmented malignant melanomas show a devastating capacity to metastasize.

*However, on the basis of overall shape at least two, probably three varieties of normal ocular melanosomes are recognized: (the internal structure, however, is similar) (1) spherical melanosomes; (2) cigar-shaped melanosomes; and (3) rod-like melanosomes. Intermediate forms do occur but even so the spherical melanosomes are quite distinct and different from the elongated melanosomes (i.e. the cigar-shaped and rod-like melanosomes).

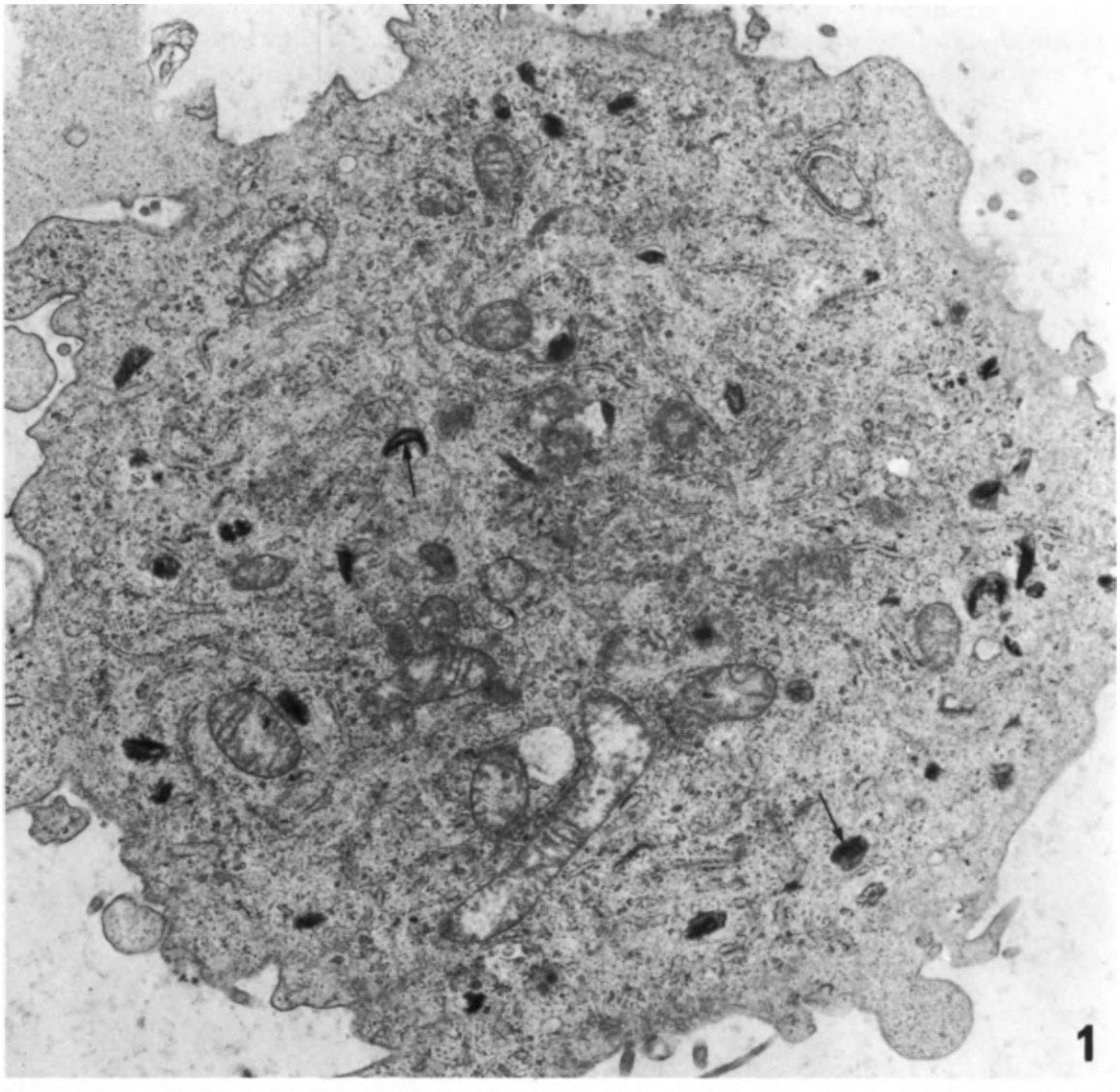
Plate 338

Malignant melanoma of human ski. Histologically the primary tumour and numerous secondary deposits in grossly enlarged lymph nodes and elsewhere showed no pigment, but later at autopsy a small secondary deposit containing a little melanin was found in the brain. Light microscopy of the malignant effusion that developed in the patient showed numerous tumour cells but no melanin. At electron microscopy some of the tumour cells were found to contain melanosomes.

Fig. 1. A malignant melanocyte from peritoneal effusion showing numerous small pleomorphic melanosomes (arrows). × 17 500

Fig. 2. High-power view of atypical melanosomes. Some, however, show a characteristic internal pattern (arrows). × 70 000

Fig. 3. A rather large melanosome found in one of the tumour cells. A striated structure can just be discerned in its interior. × 112 000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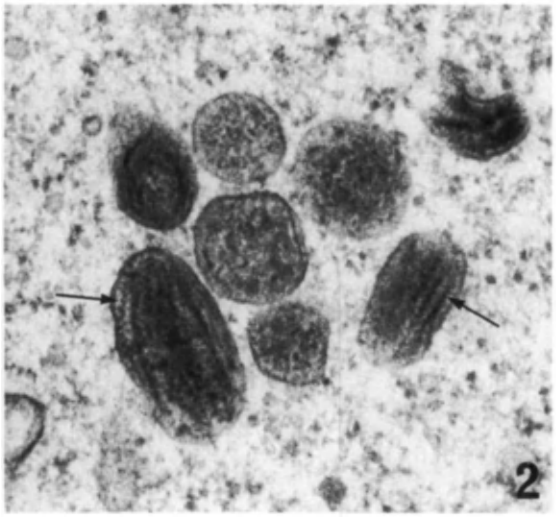
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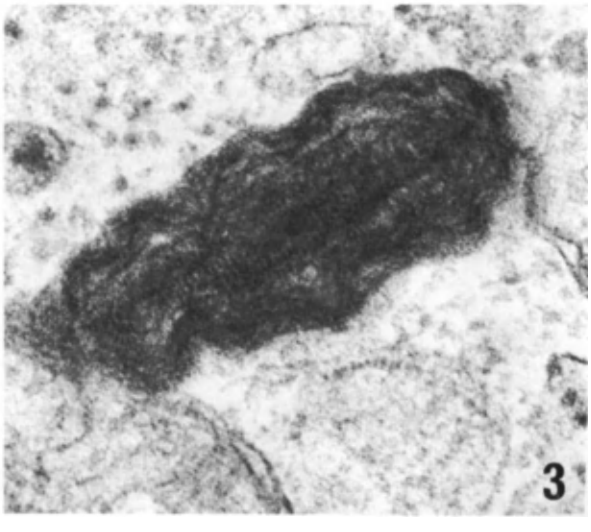
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It has already been noted (page 787) that the basic pigment cell of melanomas is the melanocyte. As well as the neoplastic melanocytes, however, there also occur numerous melanophages in the melanoma, and it is these cells which contain most of the pigment (*Plate 339*). Here as in the normal state, the malignant melanocytes contain solitary or discrete melanosomes* (many too small to be visualized by light microscopy) while the melanophages contain large compound melanosomes composed of numerous melanosomes derived from the malignant melanocytes. Such compound melanosomes contain recognizable melanosomes and also much granular material derived from their breakdown. Acid phosphatase activity has been demonstrated in such compound melanosomes, and they are thought to be a variety of heterolysosome. The melanosomes in the malignant melanocyte tend to retain certain characteristic features of internal structure which permit their identification, but these organelles also show much variation in size, shape and internal organization as noted earlier (page 794).

Pigment-containing melanomas are easily diagnosed histologically, but when pigment production is sparse or absent this may be difficult (*Plate 338*). In such instances, electron microscopy may establish the diagnosis unequivocally by demonstrating small, poorly pigmented or unpigmented melanosomes (i.e. stage II and stage III melanosomes) in the tumour cells†.

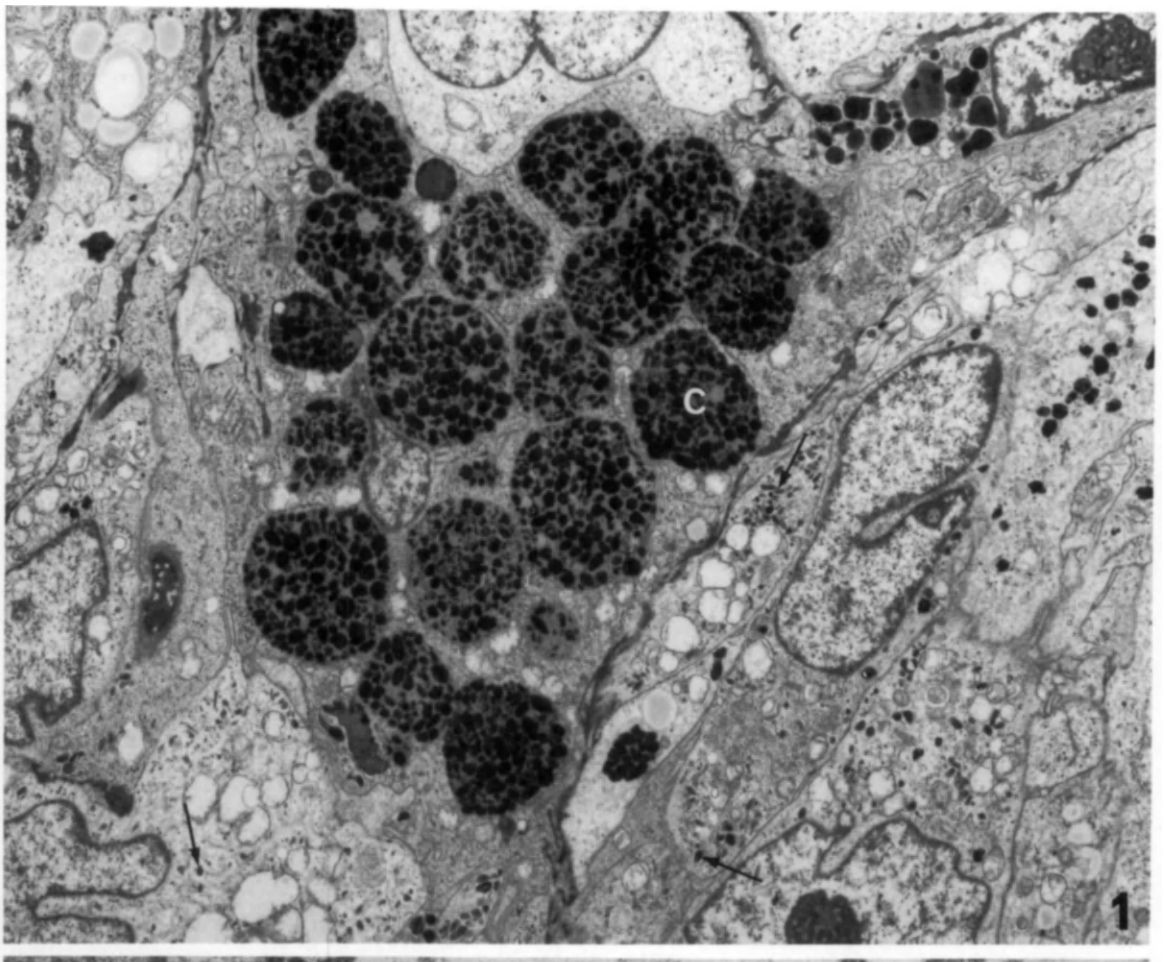
*Small compound melanosomes may on rare occasions be found in normal melanocytes. These arise by a process of autophagy whereby a few melanosomes and at times also other organelles suffer sequestration in autophagosomes and autolysosomes. A similar phenomenon is seen more often in malignant melanocytes but again usually only one or two compound melanosomes of modest dimensions are present in a few melanocytes but the majority of melanosomes are solitary. This phenomenon has led some to speculate that melanophages do not occur in melanomas and that cells containing compound melanosomes are effete malignant melanocytes. This view is in my opinion untenable. A hypothetical melanocyte containing compound melanosomes derived from its own melanosomes would be about the same size as a melanocyte. Melanophages are often very much bigger than melanocytes: (*see* for example *Plate 339*) a state which they achieve by ingesting innumerable melanosomes no doubt derived from many melanocytes.

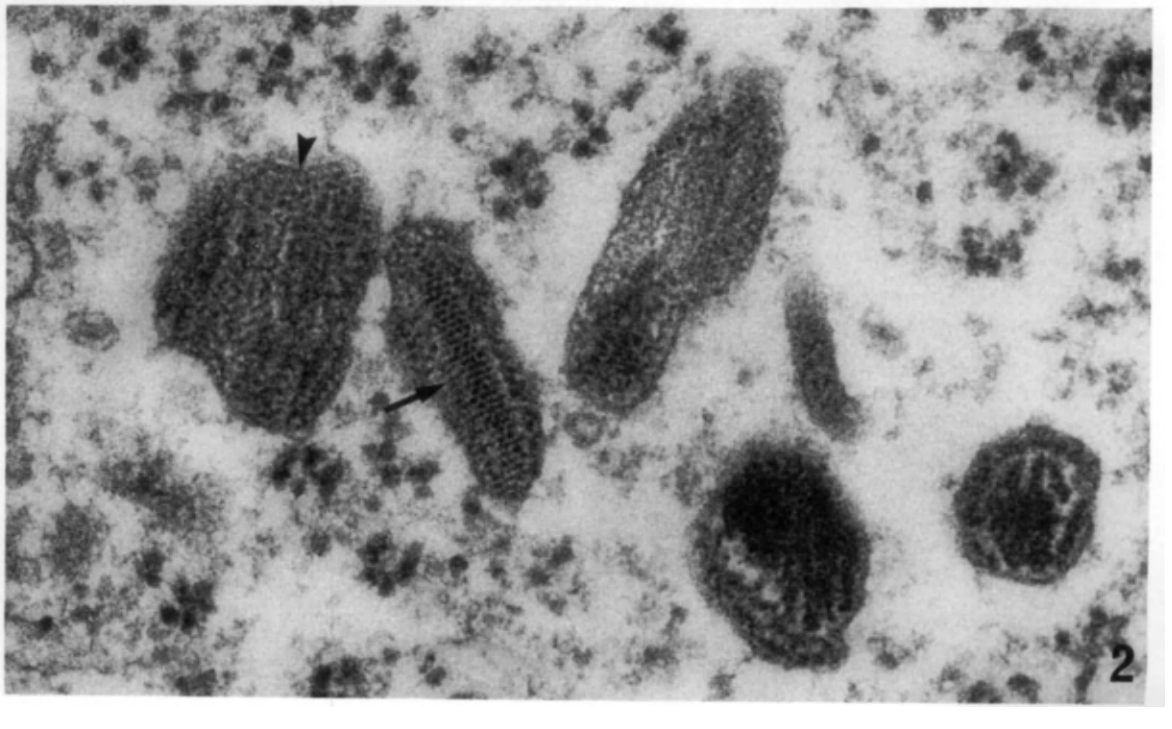
†The problems and pitfalls of identifying poorly differentiated melanosomes and establishing the diagnosis of melanoma have been dealt with elsewhere (Ghadially, 1985). *See also* page 488 where the value of straight microtubules in the endoplasmic reticulum in diagnosing malignant melanoma is discussed.

Plate 339

Fig. 1. Melanoma of the eye. The melanocytes containing solitary melanosomes (arrows) are dwarfed by the huge melanophage containing large compound melanosomes (C). × 6200

Fig. 2. High-power view of melanosomes from tumour shown in *Fig. 1.* Note the characteristic transverse striations (arrow) in one melanosome and the zig-zag pattern in others (arrowhead). Two melanosomes (M) show patchy melanin deposition. × 112000


C
1


2

Let us now look at some disorders of cutaneous pigmentation affecting humans and other animals. A comprehensive review is beyond the scope of this book. Only a brief summary of some interesting conditions is warranted.

In hypomelanotic conditions such as human piebaldism and in recessively spotted guinea-pigs, melanocytes are absent from the 'white forelock' or the white skin areas (Breathnach *et al.*, 1965; Breathnach and Goodwin, 1965; Mosher *et al.*, 1987). In albino skin and hair follicles melanocytes and melanosomes are present, but they contain little or no melanin. In certain albino animals such as mice and rabbits there may be a virtually total absence of melanin in the cutaneous and ocular melanosomes (i.e. only stage I and II melanosomes are present) but in others (e.g. rats) a few stage III melanosomes with a minimal amount of melanin content also occur (*Plate 340*). In human oculocutaneous albinism* most melanosomes are stage II melanosomes but a few stage III melanosomes showing minimal melanization are also present. The transfer of melanosomes from melanocytes to keratinocytes occurs as in the normal state. The basic defect in the human albino is subnormal melanogenesis, and this is determined genetically. The subnormal melanogenesis is thought to be due to deficient tyrosinase synthesis but the unavailability of free tyrosin or the presence of tyrosinase inhibitors may also be involved (Witkop *et al.*, 1963; Fitzpatrick and Quevedo, 1966; Chian and Wilgram, 1967).

In the low level of pigmentation seen in areas of chronic eczematous dermatitis (Pinkus *et al.*, 1959) the melanocytes become loaded with melanosomes, but few melanosomes are transferred to the keratinocytes. It is believed that the defect lies in the keratinocytes which are unable to accept the melanosomes. Such a mechanism also probably explains the hypomelanosis in lupus erythematosus (Papa and Kligman, 1965). In psoriasis and verruca vulgaris there is also a low level of pigmentation which is believed to be due to an accelerated production of keratinocytes. This results in poor or brief contact with the dendrites of melanocytes, so that the normal complement of melanosomes is not transferred from melanocyte to keratinocyte (Fitzpatrick and Mihm, 1971).

However, failure of melanosome transfer may also be due to an abnormality of the dendritic processes of the melanocyte. Thus in certain mouse genotypes (Silvers, 1961; Quevedo and Smith, 1963) and in chronically solar-irradiated human skin (Mitchell, 1963) poor pigmentation is associated with poorly developed or short and stumpy melanocytic dendrites, presumably incapable of transferring melanosomes to the keratinocytes.

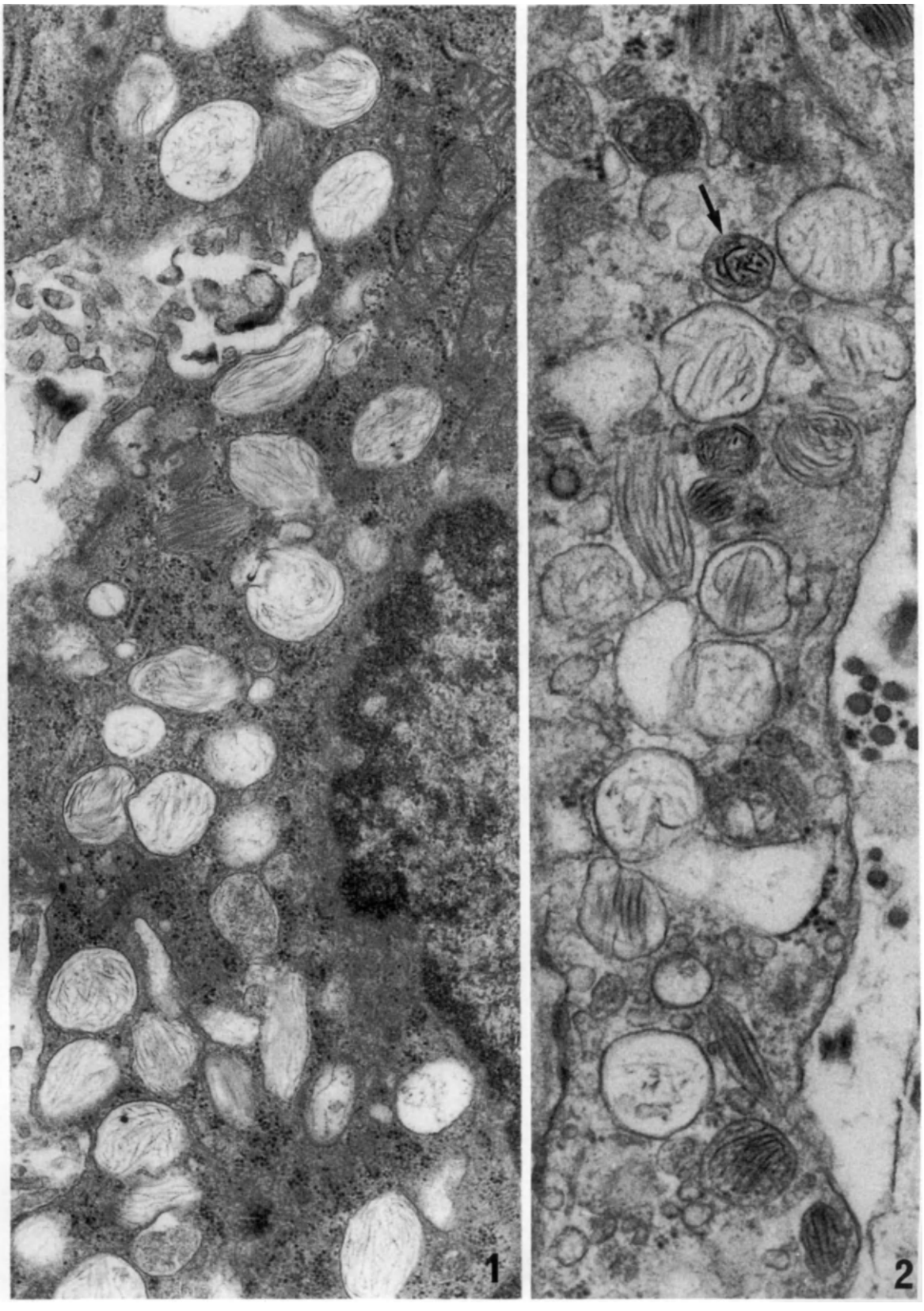
White macules or 'depigmented naevi' occur in 90 per cent of patients with tuberous sclerosis. Since this may be the earliest sign of the disease (Gold and Freeman, 1965; Fitzpatrick *et al.*, 1968; Fitzpatrick and Mihm, 1971; Mosher *et al.*, 1987), it is important to distinguish these lesions from the white macules seen in other conditions such as vitiligo. In vitiligo, there is a marked reduction or absence of melanocytes from the depigmented areas but this is not the case with the white macules of tuberous sclerosis where there is a normal or only slightly reduced complement of melanocytes. However, when melanocytes from normal surrounding skin are compared with those in the lesion, it is found that there is a marked reduction in the amount of melanin in the melanosome and there is also a marked decrease in the number and size of these organelles.

*Giant melanosomes occur in the skin of individuals affected with X-linked ocular albinism (*see* page 808).

Plate 340

Fig. 1. Stage II melanosomes from the angle of the eye of an albino rabbit. Note the characteristic filamentous profiles in the melanosomes and the absence of indubitable melanization. × 30 000

Fig. 2. Melanosomes from the eye of an albino rat. Most of the melanosomes are stage II melanosomes, but some stage III melanosomes (arrow) with a little melanin deposition are also present. × 53 000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The mechanism behind this defect is not understood, but Fitzpatrick and Mihm (1971) have suggested that 'the synthetic mechanisms for melanin are probably dampened'. It would appear that electron microscopy of these lesions in conjunction with clinical observations and histochemistry could play an important role in the early diagnosis of tuberous sclerosis. Enumeration of the number of melanosomes per dendrite cross-section may be of further help in diagnosis. According to Mosher *et al.* (1987) 'less than 0.76 being pathognomonic and over 1.81 making tuberous sclerosis unlikely'.

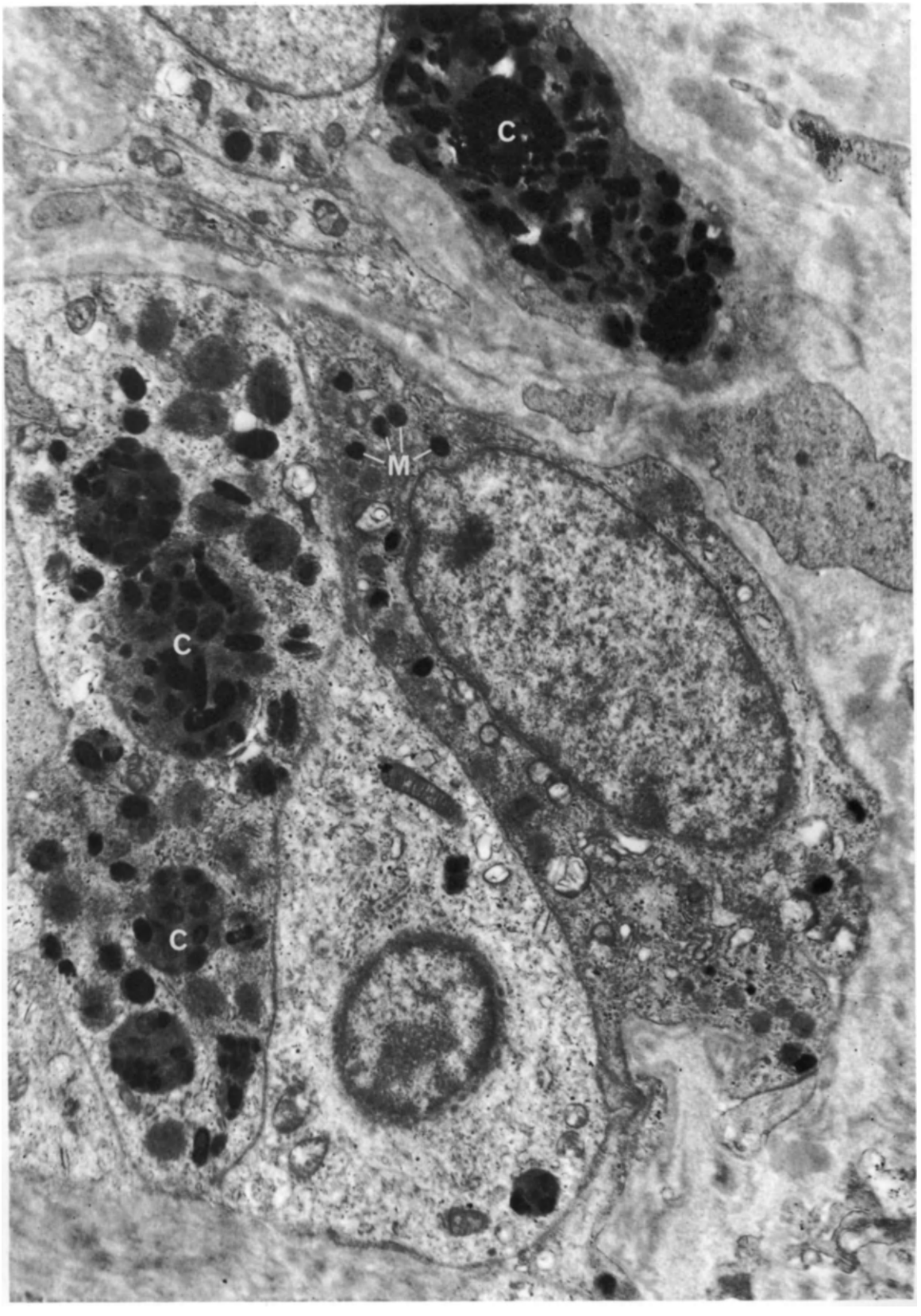
A well recognized (for over 175 years), slowly progressive disease of ageing grey or white horses* characterized by the presence of nodules of melanin-laden cells in the skin (and later in various other organs also), has been considered by some to be a type of malignant melanoma and by others to be a variety of melanosis or melanotic disease (for references *see* Levene, 1966). It is estimated that 80 per cent of grey horses surviving to old age will develop this disease. The slow progress of the disease (over a period of many years), the massive deposits of pigment (at times several kilograms) in the multiple cutaneous nodules and internal organs which at least until the terminal stages cause little distress to the animal, and the small proportion of cases which are fatal or reach a stage necessitating slaughter, is not the kind of clinical history one associates with malignant melanoma. Histologically also the lesions have a benign appearance in that cellular and nuclear pleomorphism are absent and mitoses are hard to find.

It has therefore been suggested (Levene, 1966) that equine melanosis may be a 'storage phenomenon' engendered by the release of melanin from cutaneous lesions and its subsequent storage in histiocytes and other cells in various organs. However, the idea that this might be a neoplastic condition is not totally abandoned and Levene (1966) concludes that 'its nature whether tumour or a melanin storage phenomenon is an open question'. Electron microscopy of a cutaneous lesion (*Plate 341*) supplied by Levene showed that it is composed of well differentiated melanocytes containing solitary melanosomes in various stages of development and many melanophages containing compound melanosomes. This does not help to resolve the controversy. However, examination of visceral lesions might, for if only melanophages are found (i.e. no melanocytes) one would have to accept that this is indeed a melanin-storage disease, but if melanocytes are also found one would have to concede that it is a neoplastic disorder.

*This is a disease of Equidae. While it is seen predominantly in ageing white and grey horses, it is occasionally seen in other varieties of horses, and also ponies and mules.

Plate 341

From a cutaneous nodule (perianal) of a grey horse with melanoma or melanotic disease. Seen here is a melanocyte containing solitary melanosomes (M) and melanophages containing compound melanosomes (C). × 13 500 (*From tissue supplied by Dr A. Levene*)


C
M
C
C

Granular melanosomes

The term 'granular melanosome' is used to describe a melanosome which presents as a single-membrane-bound rounded structure, containing electron-dense granules and particles (*Plate 342*). At no stage of its development does the granular melanosome contain the characteristic striated structure seen in some stage II and III melanosomes or the arrays of zig-zag or spiralled filaments seen in others.

The manner in which granular melanosomes develop is not clearly established, but one may speculate that the stage I granular melanosome has vesicles in its interior (i.e. it resembles a multivesicular body) and that melanin deposition in these vesicles produces the granular melanosome.

Granular melanosomes have been seen in: (1) human blue naevi ('ordinary' and cellular) (Bhawan *et al.*, 1980; Ghadially *et al.*, 1986); (2) experimentally produced hamster blue naevi (Ghadially *et al.*, 1986); (3) human malignant melanoma (*Plate 342*); (4) Harding-Passey mouse melanoma (Birbeck and Barnicot, 1959); (5) café-au-lait spots of neurofibromatosis (Jimbow *et al.*, 1973); (6) lentigines of leopard syndrome (Bhawan *et al.*, 1976); and (7) basal cell carcinoma (Bhawan, 1979).

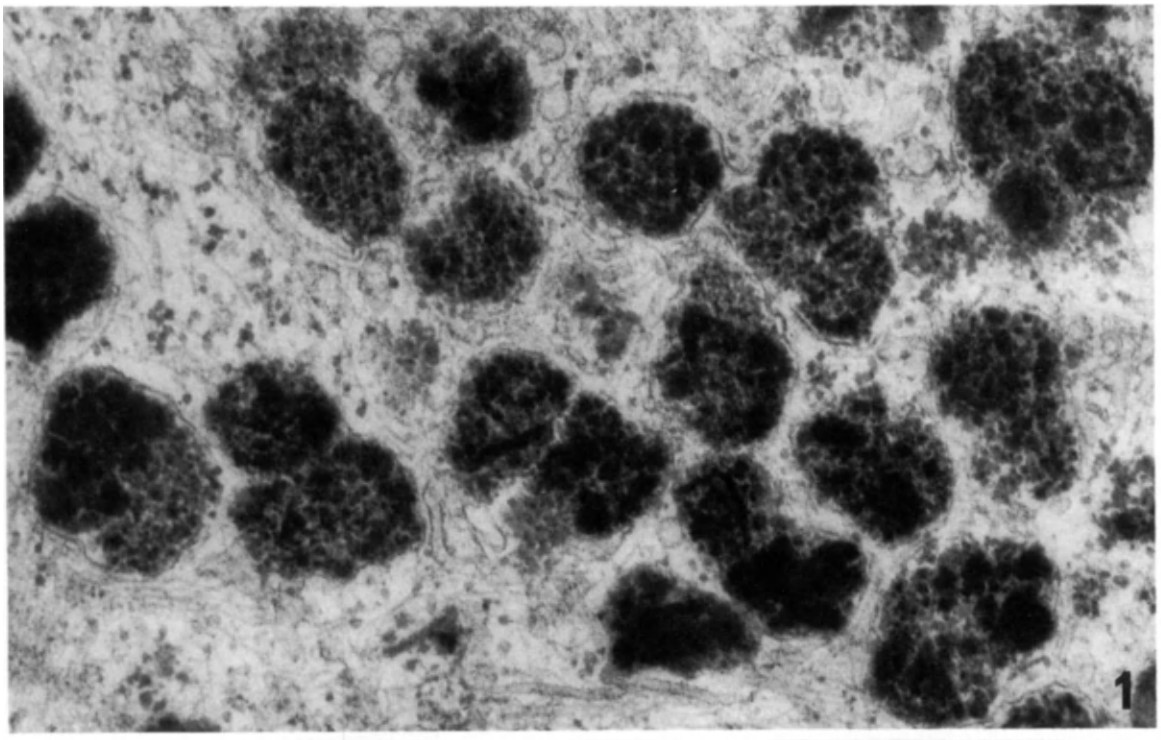
As noted before (page 798) the ultrastructural diagnosis of malignant melanoma is often established by the finding of melanosomes in cells that are obviously malignant. This is possible because classic melanosomes and their pathological variants have a distinctive morphology which permits us to distinguish them from other organelles. Identification of the granular melanosome is not so straightforward because a single-membrane-bound structure containing electron-dense granules could equally well be a lysosome. In actual practice, however, this does not, as a rule, create too great a difficulty, because the contents of lysosomes are far more pleomorphic than the granules of limited size range seen in granular melanosomes. Thus, in the correct histopathological setting when at least a few structures acceptable as granular melanosomes are present one can at least support the diagnosis of malignant melanoma.

In passing, one may note that the granules of basophil leucocytes also contain electron-dense granules (*Plate 178*), and are hence at times mistaken by the novice for granular melanosomes. Some profiles of ocular melanosomes (*Plate 335, Fig. 6*) resemble granular melanosomes, but the 'granules' in ocular melanosomes are in fact profiles produced by transverse sections through collections of melanin-laden filaments.

Plate 342

Fig. 1. Malignant melanoma of iris. All the melanosomes in this neoplastic melanocyte are granular melanosomes. They present as single-membrane-bound structures containing electron-dense granules. × 55 000

Fig. 2. Metastatic malignant melanoma. Several granular melanosomes (arrows) can be easily identified. However, there are others (arrowheads) where the contents are more or less homogeneously electron-dense. They probably represent a more advanced state of melanization in granular melanosomes. × 71 000 (*From a block of tissue supplied by Dr B. Mackay*)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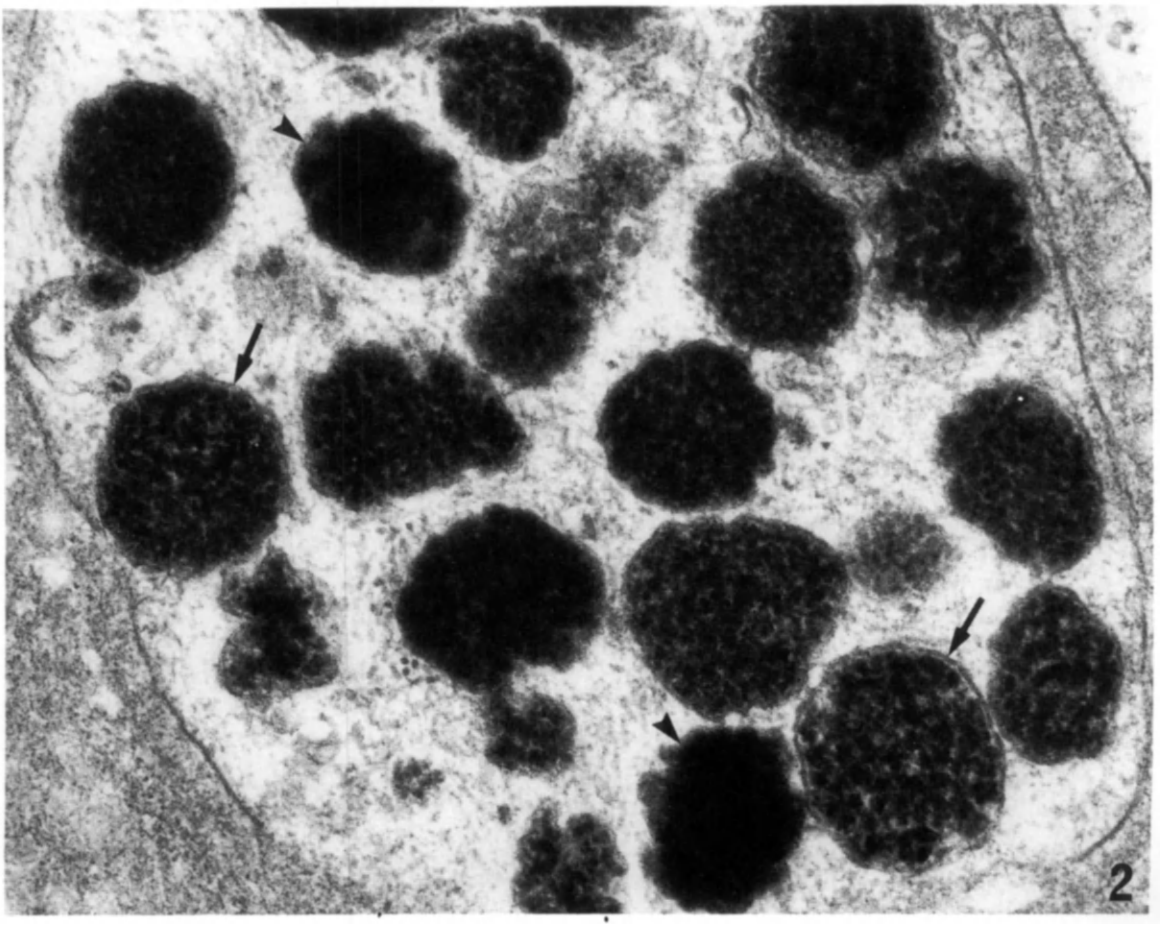

2

Balloon melanosomes

Balloon melanosomes are swollen, hydropic, presumably degenerating melanosomes which occur in cells called balloon melanocytes. Balloon melanocytes present as clear cells with the light microscope and as multivacuolated cells with the electron microscope (*Plate 343*). A few such cells may be found in melanocytic naevi and in malignant melanoma but when balloon cells predominate, the tumour is called a balloon cell naevus or balloon cell melanoma. Transitional forms between balloon cells and conventional melanoma cells have been seen with the light microscope, and hence it has been suggested that the balloon cells are altered melanocytes (Gardner and Vazquez, 1970). Ultrastructural studies (Hashimoto and Bale, 1972; Sondergaard *et al.*, 1980) support such an idea and show that the vacuoles probably derive from degenerating and coalescing melanosomes, for at times striated rod-shaped structures or coils of zig-zag filaments (similar to those seen in conventional melanosomes) are seen in these vacuoles. Such a structure is also depicted in a paper by Ranchod (1972) describing 'a metastatic melanoma with balloon cell changes' but he considers that the vacuoles represent dilated endoplasmic reticulum.

In a study on a balloon cell naevus, Okun *et al.* (1974) show rod-shaped striated structures (as seen in conventional melanosomes) lying in vacuoles, but they suggest that the vacuoles develop by what they call 'microvacuole formation in nevus granules'. However, their illustrations suggest that the 'nevus granules' are lysosomes in macrophages. These authors seem to have confused naevus cells with macrophages, mast cells and/or basophils and they come up with the fanciful suggestion that 'mast cells and nevus cells (as well as melanocytes) are histogenetically related'.

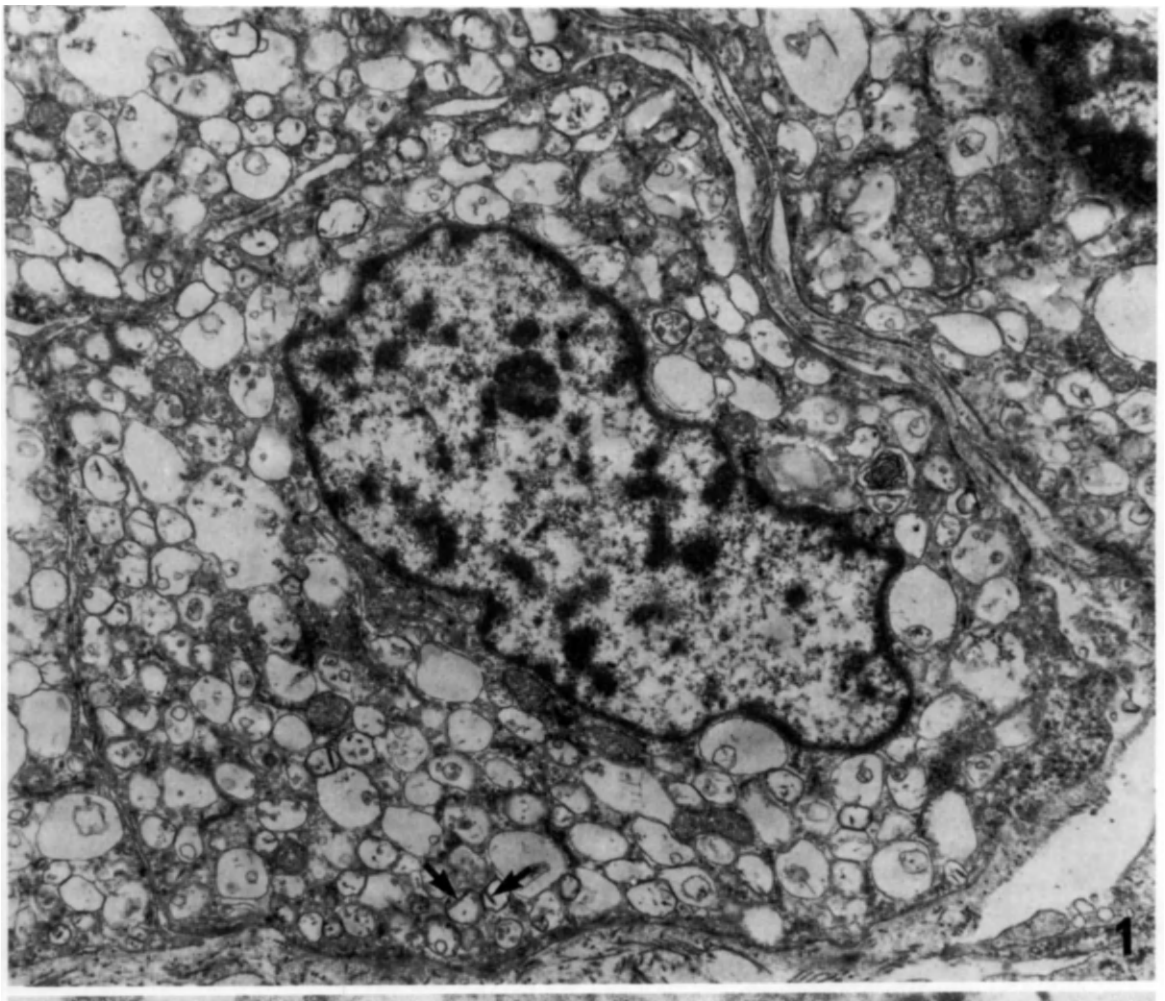
Be that as it may, the diagnosis of balloon cell melanoma can be established with the electron microscope by demonstrating the characteristic striated rod-shaped structure or coils of characteristic filaments in the 'vacuoles', or by the presence of more or less conventional melanosomes in adjacent not ballooned or not too ballooned cells. Often metastatic clear cell tumours are difficult to classify. Electron microscopy of such tumours can help in many ways, one of these is by identifying balloon cell melanoma.

Plate 343

From a formalin-fixed metastatic (in inguinal lymph node) balloon cell melanoma (primary in skin of thigh).

Fig. 1. Seen here are balloon melanocytes containing numerous vacuoles. Most of the vacuoles contain non-specific membranous, vesicular and particulate matter, but some contain rod-like structures (arrows), two of which are shown at higher magnifications in *Fig. 2.* × 9400 (*Sondergaard, Henschel and Hou Jensen, unpublished electron micrograph*)

Fig. 2. Balloon melanosomes. High-power view of 'vacuoles' (i.e. balloon melanosomes) containing rod-like structures indicated by arrows in *Fig. 1.* One of the rods shows the characteristic striated pattern one expects to see in stage II melanosomes. × 195 000 (From Sondergaard, Henschel and Hou Jensen, 1980)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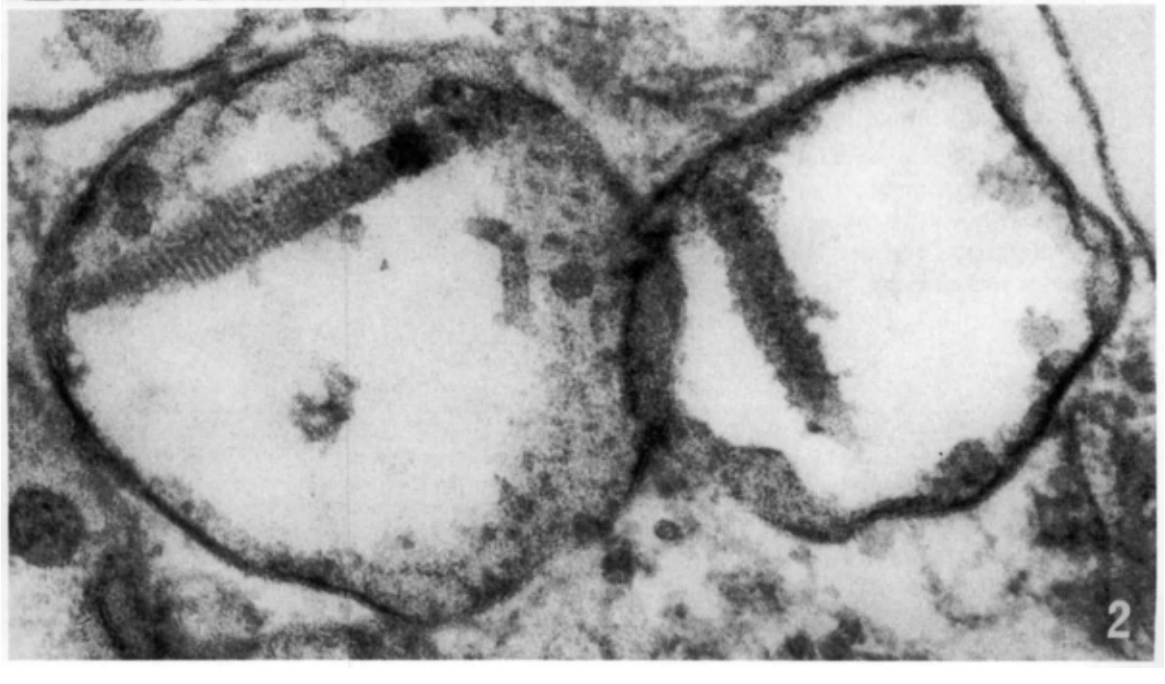
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Giant melanosomes

Giant melanosomes measuring up to about 6 μm (in skin) or 8 μm (in eye) in diameter have been seen in a variety of situations (*Plates 344* and *345*). Large osmiophilic lipid droplets could be mistaken for giant melanosomes. The distinction here is that lipid is not membrane-bound but giant melanosomes are, even though in some instances the membrane may be fragmented and difficult to demonstrate. However at times, the limiting membrane is easily discerned because it is 'loose fitting' so that a relatively clear area separates the electron-dense contents from the membrane.

The giant melanosome may be uniformly electron dense or within the intensely electron-dense mass one may discern a few or many small electron-lucent spherical bodies. These have been described as 'vesicles' by some authors although no membrane limiting such clear spaces is visualized. At times an electron-dense core may be discerned in the giant melanin granules (*Plate 345, Fig. 1*).

Giant melanosomes have been seen in the skin (melanocytes and/or keratinocytes and/or melanophages) in: (1)Aleutian Mink (Lutzner *et al.*, 1966); (2) Chediak–Higashi syndrome (Windhorst *et al.*, 1968); (3) beige mouse (*bgbg*) (murine equivalent of Chediak–Higashi sydrome) (Lutzner and Lowrie, 1972); (4) xeroderma pigmentosum (Guerrier *et al.*, 1973; Perrot and Ortonne, 1980); (5) café-au-lait spots of neurofibromatosis (Jimbow *et al.*, 1973; Takahashi, 1976); (6) X-linked ocular albinism (O'Donnell *et al.*, 1976, 1978; Cortin *et al.*, 1981); (7) melanocytic naevi (junctional and compound) (Konrad *et al.*, 1974a; Eady *et al.*, 1975; Konrad and Honigsman, 1975); (8) blue naevi (ordinary and cellular) of humans, and carcinogen-induced blue naevi in hamsters (*Plate 309, Fig. 3*) (Ghadially *et al.*, 1986); (9) leopard syndrome (Bhawan *et al.*, 1976); (10) generalized lentigines and multiple lentigines (Selmanowitz, 1975; Bhawan *et al.*, 1976; Weiss and Zelickson, 1977); (11) vitiligous achromia with malignant melanoma (Ortonne and Perrot, 1978); (12) Becker's melanosis (Bhawan and Chang, 1979); (13) carcinogen-painted skin of the mongolian gerbil (Quevedo *et al.*, 1980); (14) malignant melanoma with melanosis (Konrad *et al.*, 1974b; Rowden *et al.*, 1980); and (15) rarely in normal human skin (Konrad and Honigsmann, 1975).

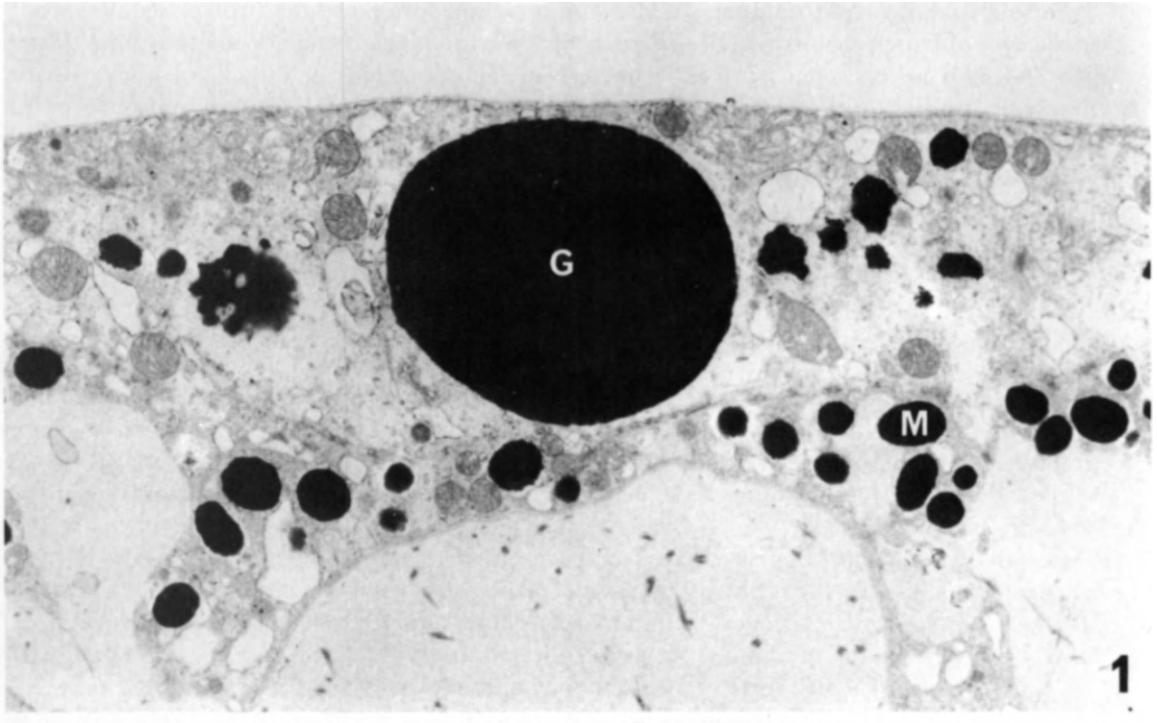
Some comments about item 6 are worth making. Currently, ten types of oculocutaneous albinism and five types of ocular albinism are recognized (Mosher *et al.*, 1987); one of them being X-linked ocular albinism. This condition can usually be detected in white affected males and carrier females on the basis of iris transillumination and a mosaic pattern to the fundus, but in blacks this is usually not possible. O'Donnell *et al.*, (1976, 1978) drew attention to the fact that black and white affected individuals and carriers have giant melanosomes in their skin and that this could be of diagnostic value, if other conditions where giant melanosomes occur can be ruled out. Cortin *et al.* (1981) confirm these findings and state that a search for giant melanosomes in the skin 'gave positive results more often than iris transillumination and funduscopy', and that 'skin biopsy should significantly improve the detection of both affected males and carriers'.

Plate 344

From an eye that had contained an iron foreign body for one year.

Fig. 1. A giant melanosome (G) measuring about 5.1 μm in diameter. Compare this with normal-sized melanosomes (M) which are also present. An eccentrically placed electron-dense core was present in this giant melanosome, but this is not recorded in this electron micrograph which was exposed to record overall cellular details rather than details within the electron-dense giant melanosome. Compare with *Plate 245, Fig. 1* where such details are recorded by correctly exposing for the melanosome and ignoring the cellular details. × 9000

Figs. 2–4. Images seen here suggest that some giant melanosomes may be derived by fusion of smaller melanosomes. The limiting membrane of the melanosomes is evident in some places (arrowheads). × 21 000; × 18 000; × 17 000


G
M
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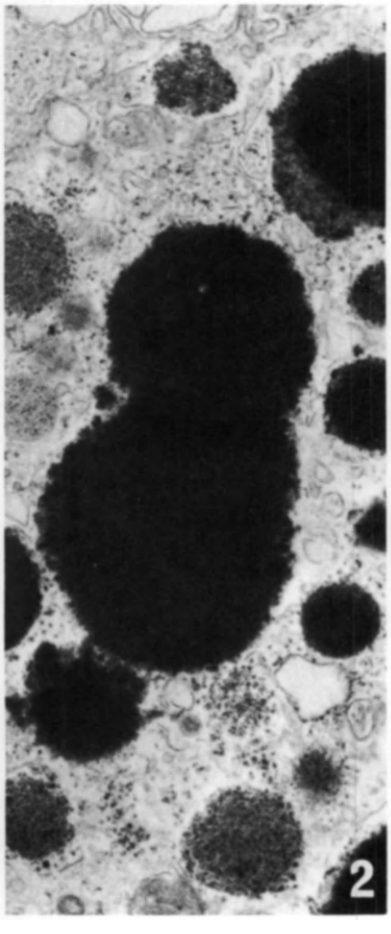
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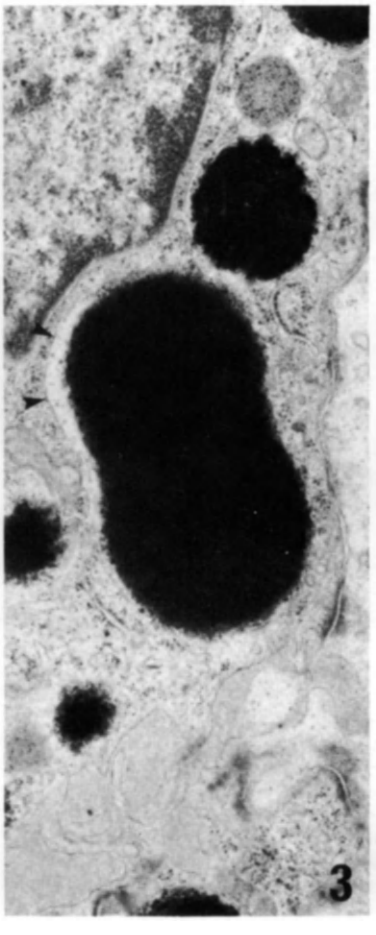
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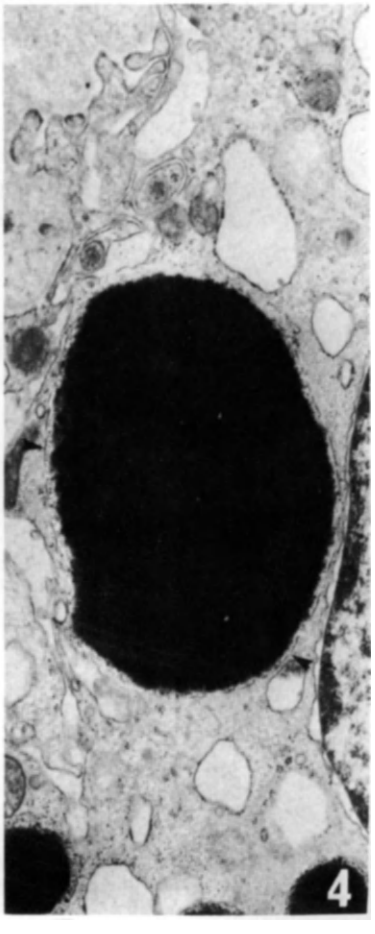
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During the course of studies on the effect of implanted foreign bodies (iron or stainless steel) in the eyes of Dutch rabbits we (Ghadially *et al.*, 1982) found giant stage IV melanosomes (*Plates 344* and *345*) up to about 8 μm in diameter* in the epithelium of the ciliary body, ciliary processes and root of the iris. Similar but somewhat smaller stage IV giant melanosomes up to about 2.7 μm in diameter were also found in the normal eye of a Dutch rabbit. Normal sized spherical or slightly oval melanosomes in this species rarely attain a diameter of 1 μm or slightly more.

The giant melanosomes presented round or oval profiles. Some of them were homogeneously electron dense but in others one could with some difficulty discern a dense core and also at times spherical or oval electron-lucent zones or bodies such as those shown in *Plate 345, Fig. 1.* However the dense core was not always difficult to demonstrate. It was easily seen in some giant melanosomes measuring about 2–3 μm in size (*Plate 345, Fig.2*). These presented as single-membrane-bound structures with a dense core surrounded by a fairly lucent zone peppered with particles. Smaller membrane-bound structures containing electron-dense granules and particles were also seen adjacent to such melanosomes. We also saw images which suggest that giant melanosomes were being formed by fusion of smaller melanosomes (*Plate 344, Figs. 2–4*).

Regarding the genesis of giant melanosomes (in the skin and eye) several possibilities have to be considered. The idea that giant stage IV melanosomes develop from giant stage II and stage III melanosomes with characteristic internal structure similar to that found in normal-sized melanosomes receives only limited support from our observations, because the largest stage III melanosome with characteristic filamentous structure that we have found measured only 2.6 μm in diameter. Thus while some giant melanosomes no doubt form in this fashion it is unlikely that the really large melanosomes measuring up to about 8 μm in diameter are derived in this manner.

Konrad *et al.* (1974a, b) suggested that the giant melanosomes they found in naevoid pigmented lesions of the skin resulted from a deranged morphogenesis, and that melanin was deposited in a centrifugal fashion by the agency of vesicles. Although the so-called 'vesicles' or lucent bodies are seen in giant melanosomes (and also in normal-sized melanosomes) there is as yet no clear evidence of vesicles (say for example from the Golgi complex containing tyrosinase or melanin) entering giant melanosomes and thus increasing their size. Even so this is an attractive possibility which may be operative in some instances.

For giant melanosomes seen in xeroderma pigmentosum, it has been suggested (Guerrier *et al.*, 1973; Perrot and Ortonne, 1980) that they are the product of autophagy, i.e. compaction of smaller melanosomes to form giant melanosomes in autophagic vacuoles. In our material, we could not find evidence to support such a contention but we did find images which can be regarded as fusion of smaller melanosomes to form larger melanosomes. On the basis of our study and a review of the past literature one may conclude that giant melanosomes develop in several different ways in different sites and situations.

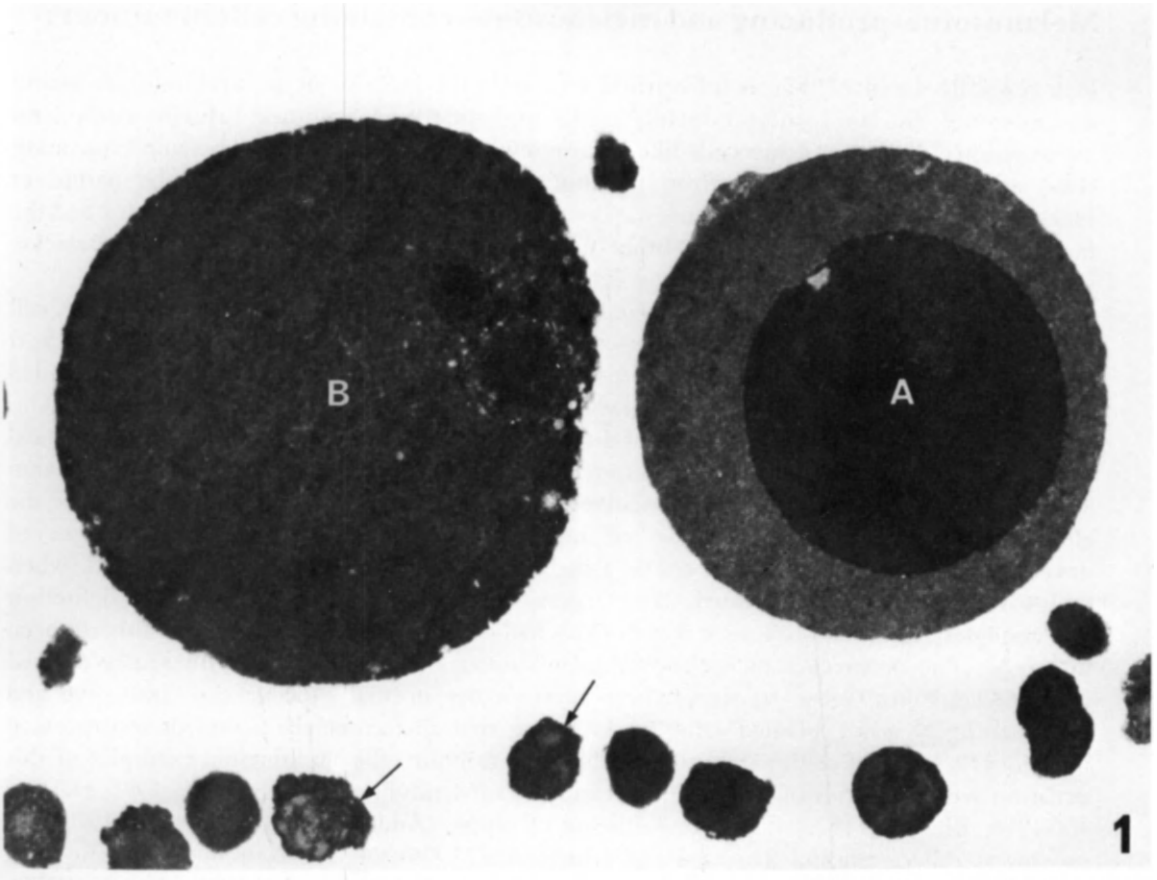
*The largest melanosome we found in this material was a slightly oval melanosome about 8.25 μm in diameter. It is shown in *Fig. 112*, in Ghadially (1985).

Plate 345

From an eye that had contained a stainless steel foreign body for five months.

Fig. 1. Two giant melanosomes, one (A) with a dense core and another (B) containing electron-lucent bodies. Electron-lucent bodies are also present in some of the normal-sized melanosomes (arrows). In order to reveal such details in the electron-dense melanosomes a prolonged exposure of the negative is needed. This grossly overexposes the rest of the cell (pitch black in the negative) so that this does not appear in the electron micrograph presented here. Compare with *Plate 344, Fig. 1.* × 16 500

Fig. 2. Two membrane-bound structures which can be regarded as early stages of development of giant melanosomes are seen here. One measuring about 2.4 μm (A) has a central well melanized core surrounded by electron-dense particles; the other (B) shows an irregular melanized area adjacent to which lie electron-dense particles. Smaller membrane-bound structures (arrows) containing electron-dense particles and granules are also present. It seems likely that they represent early stages in the development of some of the smaller melanosomes. × 21 000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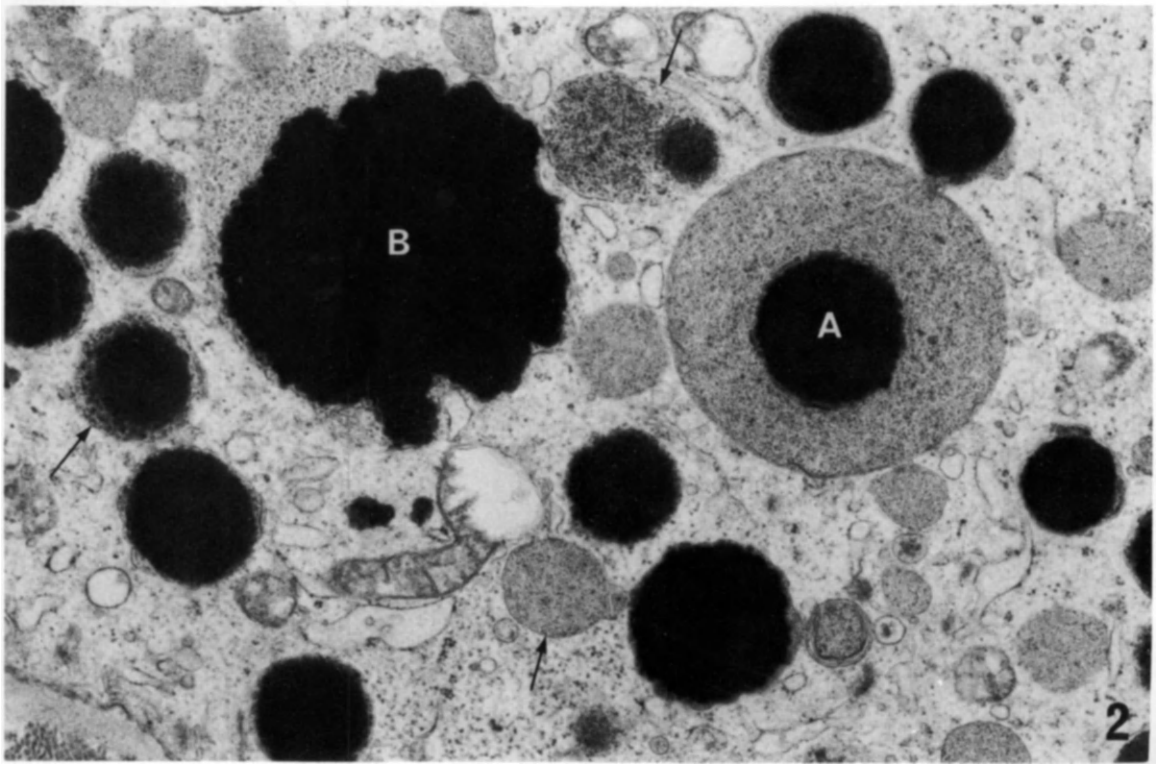
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Melanosome-producing and melanosome-containing cells in tumours

It is generally accepted that in the normal state only the melanocyte is capable of producing melanosomes (melanosome-producing cells), and that melanosomes (usually compound melanosomes) found in other cells like keratinocytes and melanophages (melanin-containing cells) are secondarily derived from melanocytes. The situation regarding the nature of melanosome-producing and melanosome-containing cells in tumours is more complex and the burning question has been — can other neoplastic cells besides the neoplastic melanocyte produce melanosomes?

At the Sixth International Pigment Cell Conference the melanocyte was defined as 'A cell which synthesizes a specialized melanin-containing organelle the melanosome' (Fitzpatrick *et al.*, 1966). Later students of the subject insisted that all melanosome-producing cells be called 'melanocytes' and that only melanocytes were capable of producing melanosomes. Those who believed in the above-mentioned concept disregarded light microscopic studies on controversial pigmented tumours, arguing (and with much justification) that histochemical tests for melanin are not specific, and that in such a controversial issue, a minimum requirement would be the electron microscopic demonstration of indubitable melanosomes in various stages of development* before accepting a cell as a melanin-producing cell. On the other hand, when melanosomes were demonstrated, they argued that the cells were therefore by definition 'melanocytes', and that obviously melanocytes had a wider distribution than had hitherto been imagined. The occurrence of melanosomes in tumours (other than those obviously derived from melanocytes) close to sites where melanocytes normally occur (i.e. skin, eye and leptomeninges) was explained away by assuming that adjacent melanocytes or incorporated melanocytes must have donated melanin to these tumour cells. Indubitable examples of this situation are some cases of: (1) basal cell carcinoma (Mandybur, 1974; Bleehen, 1975; Hahn *et al.*, 1976; Bhawan, 1979); (2) Paget's disease of nipple (Anderson and Robertson, 1979); (3) squamous .cell carcinoma (Jauregui and Klintworth, 1976); (4) adenocarcinoma of anorectal junction (Chumas and Lorelle, 1981); and (5) carcinoma of breast (Azzopardi and Eusebi, 1977).

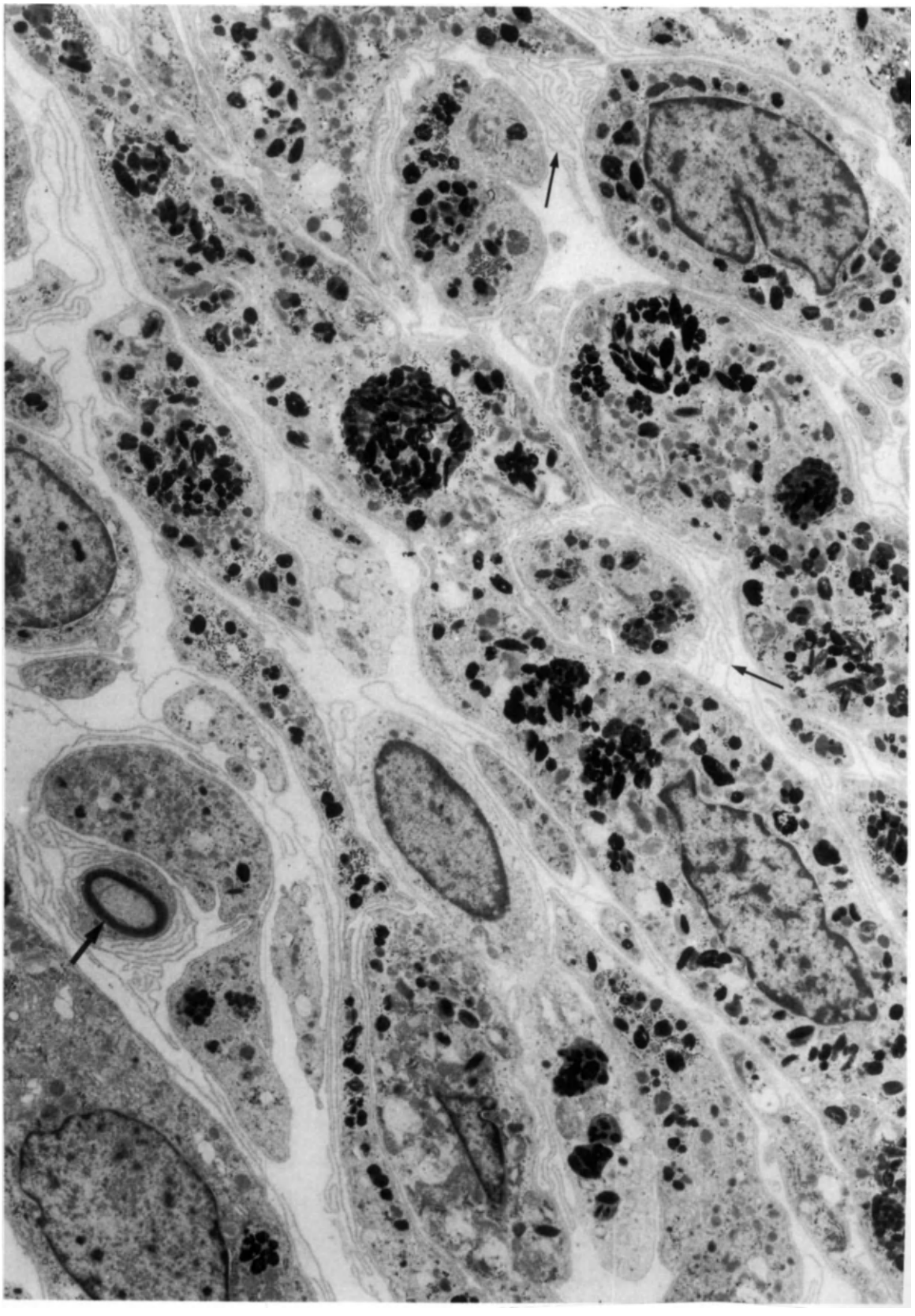
Today all melanin-producing cells are thought to be derived from the neural tube and neural crest (i.e. neuroectoderm). In normal humans, the only melanin-producing cell† of neural tube origin is the pigmented epithelial cell of the eye, and the only tumours of postulated origin from neural tube-derived cells are melanomas of the ocular pigment epithelium and the rare pigmented ependymoma (McCloskey *et al.*, 1976). All other pigment-producing cells in humans (including uveal stromal melanocytes in the eye) are derived from the neural crest. In the normal state, probably the only neural crest-derived cell which produces melanosomes and melanin is the melanocyte, but it is sometimes claimed that the normal Schwann cell may also at times produce melanosomes; certainly neoplastic Schwann cells can at times do this (*Plates 346–348*). This is not surprising since both cells have a common ancestry from the neural crest.

*It would not be good enough to show compound melanosomes or an occasional solitary melanosome in the tumour cells because these could be endocytosed melanosomes, that is to say secondarily derived from a melanocyte and not produced by the tumour cell itself. Further one would have to demonstrate that early stages of melanosome formation are present.

†We must ignore here cells containing the so-called 'neuromelanin' which is said to have melanin-staining properties but which has the ultrastructural morphology of lipofuscin. Certainly, no melanosomes of characteristic internal structure are involved in the production of 'neuromelanin'.

Plate 346

Melanotic schwannoma. The tumour is composed essentially of neoplastic Schwann cells and their cell processes; one of which infolds a myelinated axon (thick arrow). Note the external lamina ensheathing these cells and also the redundant folds (thin arrows) of external lamina. Solitary melanosomes and aggregates of melanosomes are seen in these cells. ×7100 (*From Robertson and Ghadially, 1983*)



Melanin-producing tumours of postulated origin from neural crest-derived cells include: (1) naevi and melanomas of skin, mucous membranes, uveal tract of the eye and leptomeninges (pages 794–799); (2) 'ordinary' (also called 'common') and cellular blue naevi in skin and other sites (*see below*); (3) melanotic neuroectodermal tumour of infancy (Taira *et al.*, 1978; Dehner *et al.*, 1979; Navas Palacios, 1980; Cutler *et al.*, 1981); (4) melanotic medulloblastoma (Boesel *et al.*, 1978); (5) malignant melanomas of sympathetic ganglia (Fu *et al.*, 1975); (6) melanotic schwannoma (*see below*); (7) clear cell sarcoma of tendons and aponeuroses which is now thought to be a variety of malignant melanoma and is at times referred to as 'malignant melanoma of soft parts' (Bearman *et al.*, 1975; Toe and Saw, 1978; Tsuneyoshi *et al.*, 1978; Navas and Garzon, 1979; Choux *et al.*, 1980; Azumi and Turner, 1983; Kindblom *et al.*, 1983); (8) 'spinal melanotic clear cell sarcoma' which appears to be a melanotic schwannoma resembling clear cell sarcoma of tendons because it contains an unusually large amount of glycogen (Parker *et al.*, 1980); and (9) some rare neuroendocrinomas which contain both neuroendocrine cells and cells acceptable as melanocytes. On even rarer occasions (perhaps two cases) it is claimed that neuroendocrine granules and melanosomes may be found in a single cell (Ho and Ho, 1977; Cebelin, 1980; Gould *et al.*, 1981; Grazer *et al.*, 1982; Marcus *et al.*, 1982).

We will now look at the melanotic schwannoma because it is here more than anywhere else that one finds proof of the fact that neoplastic cells other than neoplastic melanocytes can produce melanosomes.

Melanotic schwannomas have long been known to pathologists, but doubt remained as to whether it was the Schwann cell which actually synthesized melanosomes and melanin, because of the non-specificity of histochemical tests for melanin and because several past ultrastructural studies were far from totally convincing (for a critique *see* Ghadially, 1985). However, there are now some quite convincing studies (e.g. McGavran *et al.*, 1978; Mennemeyer *et al.*, 1979; Janzer and Makek, 1983; Robertson and Ghadially, 1983), which clearly show that the neoplastic Schwann cell is capable of producing melanosomes.

In the tumour we (Robertson and Ghadially, 1983) studied*, essentially only two types of cells (*Plates 346–348*) were found: (1) Schwann cells; and (2) melanophages. The neoplastic Schwann cells presented as elongated cells with numerous cell processes, fairly well developed Golgi complex and rough endoplasmic reticulum, several mitochondria and a few to numerous discrete melanosomes in various stages of development. Some of these cells also contained groups of melanosomes around which a limiting membrane could at times be discerned (i.e. compound melanosomes) but more often than not no membrane could be discerned; the impression created being that a group of melanosomes had fallen together to form an aggregate lying free in the cytoplasmic matrix. These cells were ensheathed in an external lamina (composed of a lamina densa and lamina lucida). Copious amounts of redundant external

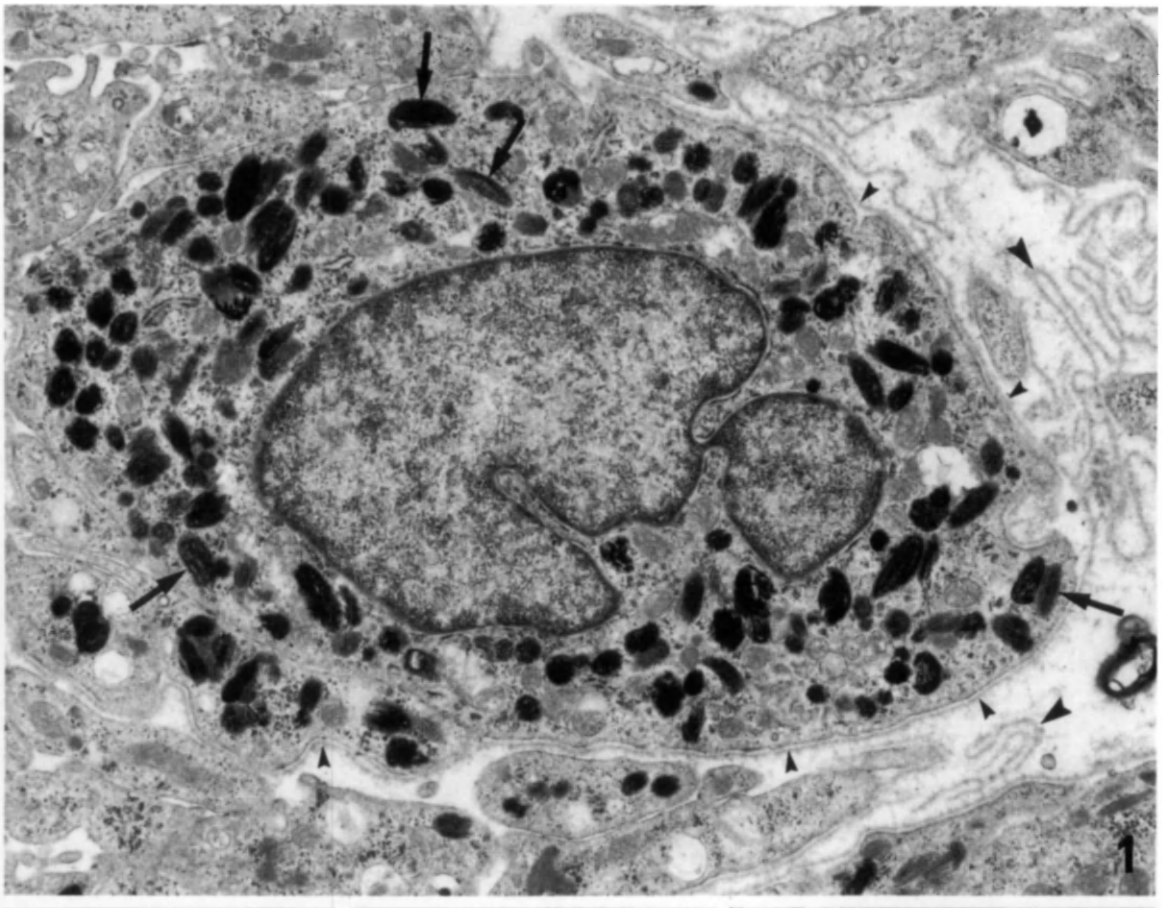
*This tumour occurred in a 23-year-old man involving the second sacral nerve root. Histologically it was composed of spindle cells containing pigment granules.

Plate 347

Melanotic schwannoma. Same case as *Plate 346*. (*From Robertson and Ghadially, 1983*)

Fig. 1. A neoplastic Schwann cell containing numerous melanosomes in various stages of development (arrows). Note also the external lamina (small arrowheads) and redundant folds (big arrowheads) of the external lamina. × 13 000

Figs. 2–4. Melanosomes in various stages of development found in neoplastic Schwann cells. *Fig. 2* Shows a stage II melanosome. The characteristic striated internal structure (periodicity 10 nm) is well visualized but there is little evidence of melanin deposition. × 123 000. *Fig. 3* shows a stage III melanosome. Melanin deposition is evident but the internal structure is not completely obliterated. × 92 000. *Fig. 4* shows a stage IV melanosome (i.e. a melanin granule). Melanin deposition has completely obliterated the internal structure in this melanosome. × 118 000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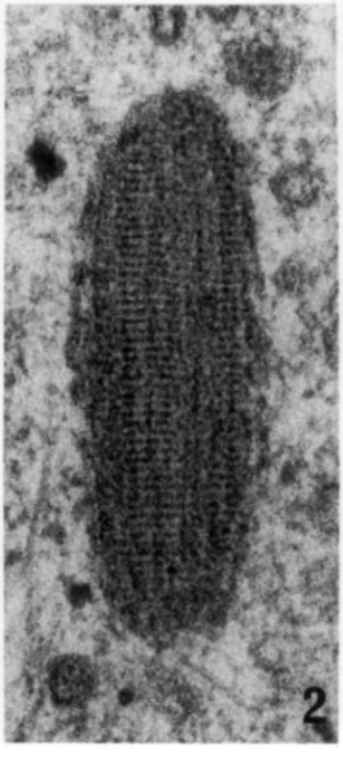
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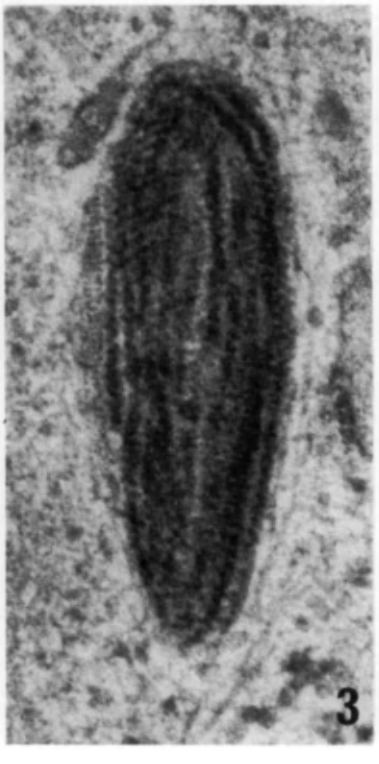
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4

laminae were present in several places between the tumour cells and a reduplicated basal lamina was seen around the blood vessels (*Plate 348, Fig. 1*). The morphology of these cells clearly establishes them as Schwann cells and the presence of solitary melanosomes in various stages of development (*Plate 347*) shows that they are producing melanin.

The melanophages were characterized by the presence of compound melanosomes and the absence of an external lamina*. The idea that Schwann cells in our melanotic schwannoma derived their melanosomes from melanocytes which had migrated from other sites, or from melanocytes arrested along the course of nerves during development is clearly not tenable, for melanocytes were not found in this tumour. Melanocytes are not ensheathed by an external lamina and certainly the excessive or redundant external lamina found in this tumour is seen in schwannomas but never in melanocytic melanomas. The abundant cell processes (covered by an external lamina) and the occasional myelinated axon within them (*Plate 346*) also unequivocally establish these as neoplastic Schwann cells.

Let us now look at blue naevi where there is no doubt that the naevus cells produce melanosomes but there has been a long standing debate as to whether the naevus cells are of schwannocytic or melanocytic lineage.

When a collection of cells containing melanin occur in a superficial plane in the skin (as in the common naevus near the dermoepidermal junction), they produce a blackish-brown lesion, but if such cells occur deep in the dermis the lesion has a bluish tint. The blue coloration is an optical effect produced by deeply sited melanin viewed through the overlying epidermis and superficial dermis. Quite large diffuse collections of dermal pigment cells are common in higher apes and its contracted vestigial form in humans is the so-called 'monogolian spot' or 'blue spot'. The term 'blue naevus' coined to designate these lesions has now been extended to include collections of extra-epithelial pigment cells in various other sites such as the prostate, lymph node, endocervix and middle ear (Jao *et al.*, 1971; Rios and Wright, 1976; Levene, 1980; Goldman, 1981). Adequate ultrastructural studies on extracutaneous blue naevi are few, but what evidence there is favours the idea that they are composed of melanocytes. We will now confine our discussion to the better studied blue naevi of the skin.

Two varieties of cutaneous blue naevi are recognized by histopathologists; the ordinary blue naevus (also called 'common blue naevus') and the cellular blue naevus. A review of published work and our own studies (Ghadially *et al.*, 1986) show that human ordinary blue naevi are composed of varying proportions of naevus cells and melanophages. Usually naevus cells predominate but on rare occasions many more melanophages than naevus cells occur. Such differences could be real or they may be due to sampling problems. The ordinary blue naevus is distinguished from the cellular blue naevus by the relative paucity of pigment cells which are

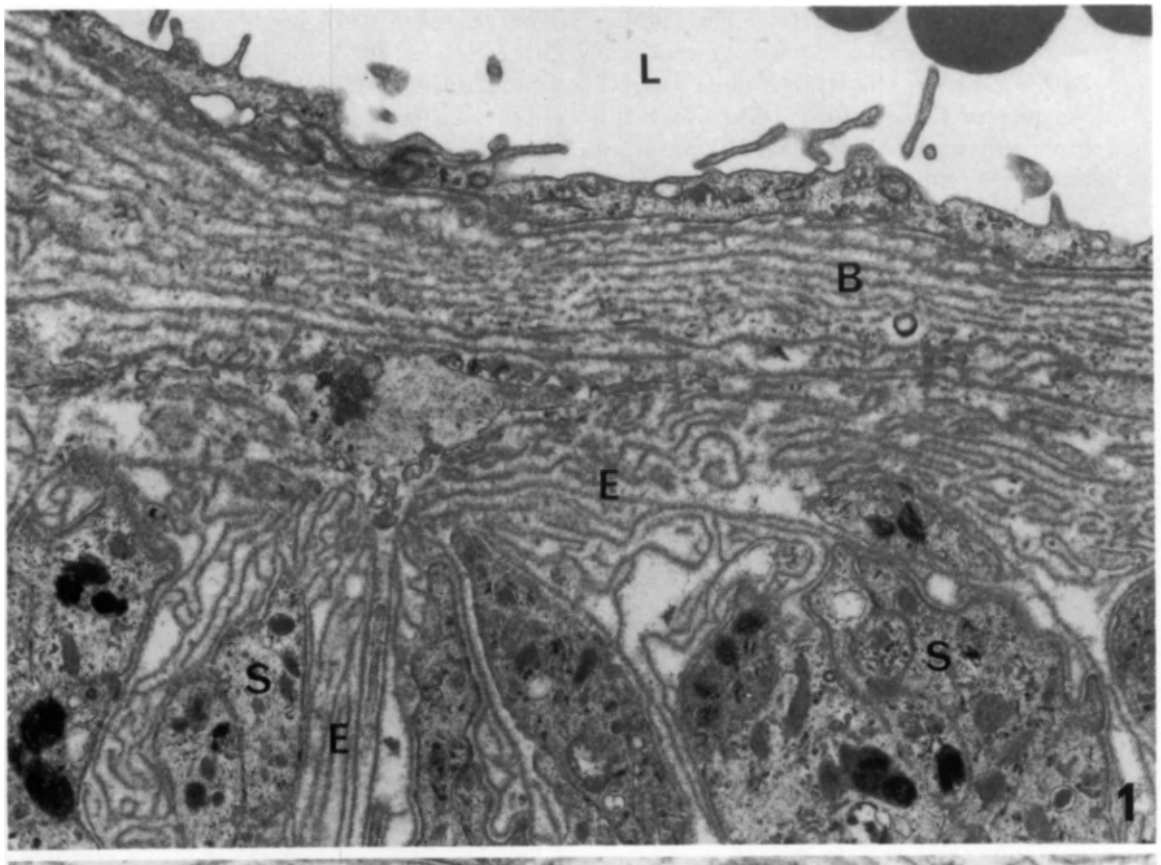
*Macrophages do not have an external lamina and hence neither do melanophages. There was so much redundant external lamina present in this tumour that the absence of external lamina around melanophages could only be appreciated when the melanophages lay well away from the Schwann cells.

Plate 348

Melanotic schwannoma. Same case as *Plates 346* and 347. (*From Robertson and Ghadially, 1983*)

Fig. 1. A singularly large amount of redundant external lamina (E) has been produced by the neoplastic Schwann cells (S) and an extensively reduplicated basal lamina (B) is seen adjacent to a blood vessel. Lumen (L). × 13 000

Fig. 2. Melanotic schwannoma. A melanophage containing compound melanosomes (C) is seen here. Above it lie two cell processes of Schwann cells. The one on the left contains melanosomes in an early stage of development, the one on the right contains well melanized melanosomes. The melanophage is not invested by an external lamina, but redundant folds of lamina produced by neighbouring Schwann cells abut on to the cell membrane in some places. × 13 500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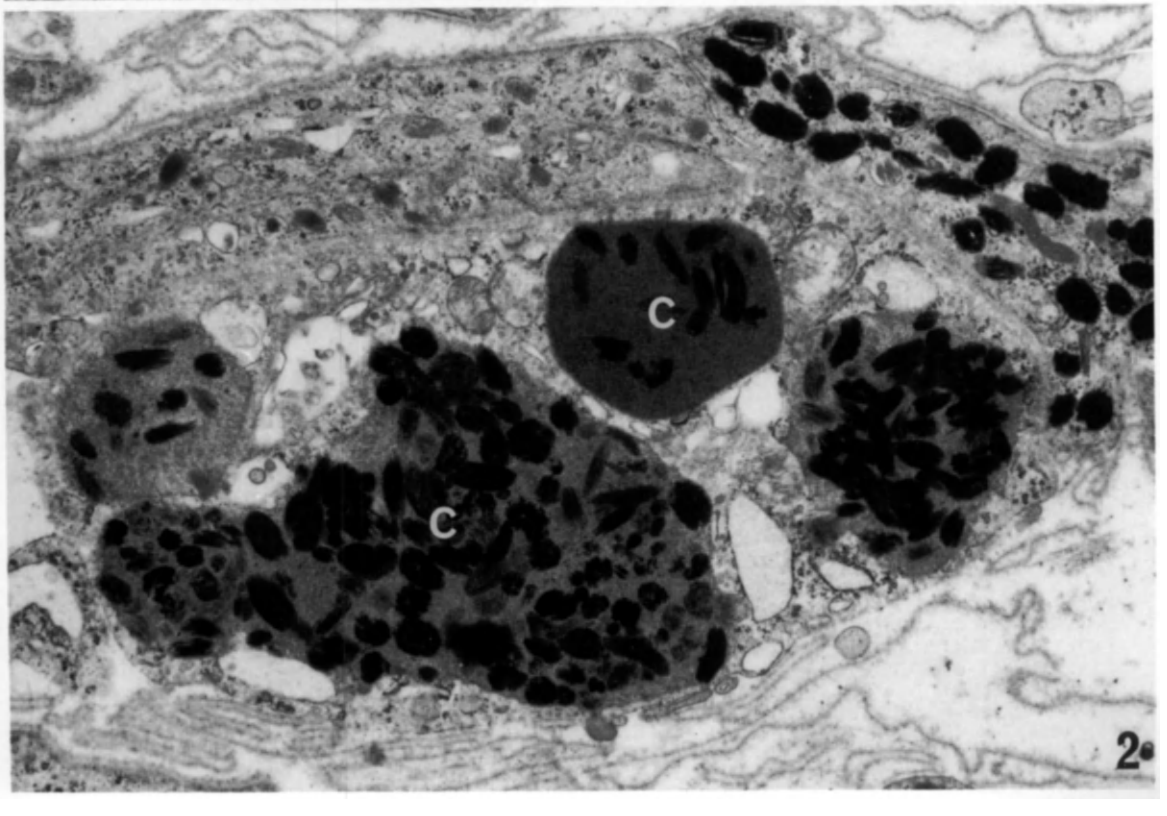
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dispersed in the deep dermis. The cellular blue naevus, as its name implies, is better endowed with compact masses of pigment-producing cells. However, the amount of pigment present in both varieties of blue naevi is quite variable and one can recognize melanotic and hypomelanotic versions of these lesions. Most melanosomes in naevus cells are stage IV melanosomes and granular melanosomes. Stage III melanosomes are rarely encountered and stage II melanosomes are rarer still. As in other melanotic tumours melanophages containing compound melanosomes are found in these tumours.

Before we look at the controversy about the nature of the naevus cells in blue naevi it is worth recalling the principal differences between Schwann cells and melanocytes. They may be summarized by saying that: (1) Schwann cells have a well formed external lamina (composed of a lamina lucida and lamina densa), and so also do the neoplastic Schwann cells of schwannoma and melanotic schwannoma, which often bear an external lamina surrounding each cell or a basal lamina surrounding groups of cells. Neither normal melanocytes nor neoplastic melanocytes of malignant melanoma bear an external lamina. The melanocyte at the dermoepidermal junction does abut a basal lamina but one cannot be certain as to whether this is produced by the melanocyte or adjacent keratinocytes. However, the suspicion remains that the normal melanocyte may not be totally incapable of producing materials found in such laminae and there is at least one report where a lamina is shown (*Fig. 8* in Mazur and Katzenstein, 1980) adjacent to a couple of neoplastic melanocytes in a metastasis from a malignant melanoma. Further such laminae have been found in melanotic tumours of disputable histogenesis (melanocyte *versus* Schwann cells *versus* undifferentiated cell) like desmoplastic melanoma (DiMaio *et al.*, 1982), meningeal melanocytoma invading the spinal cord (Steinberg *et al.*, 1978), soft tissue melanoma (Benson *et al.*, 1985), and melanotic tumour of infancy (Dehner *et al.*, 1979); (2) both cell types have long cell processes but the cell processes of melanocytes do not wrap around extracellular structures (pseudomesaxon formation) as do the cell processes of Schwann cells in a schwannoma; and (3) infolding and coiling of cell membranes to form mesaxons is seen in normal Schwann cells and sometimes also in neoplastic Schwann cells, but not in normal or neoplastic melanocytes.

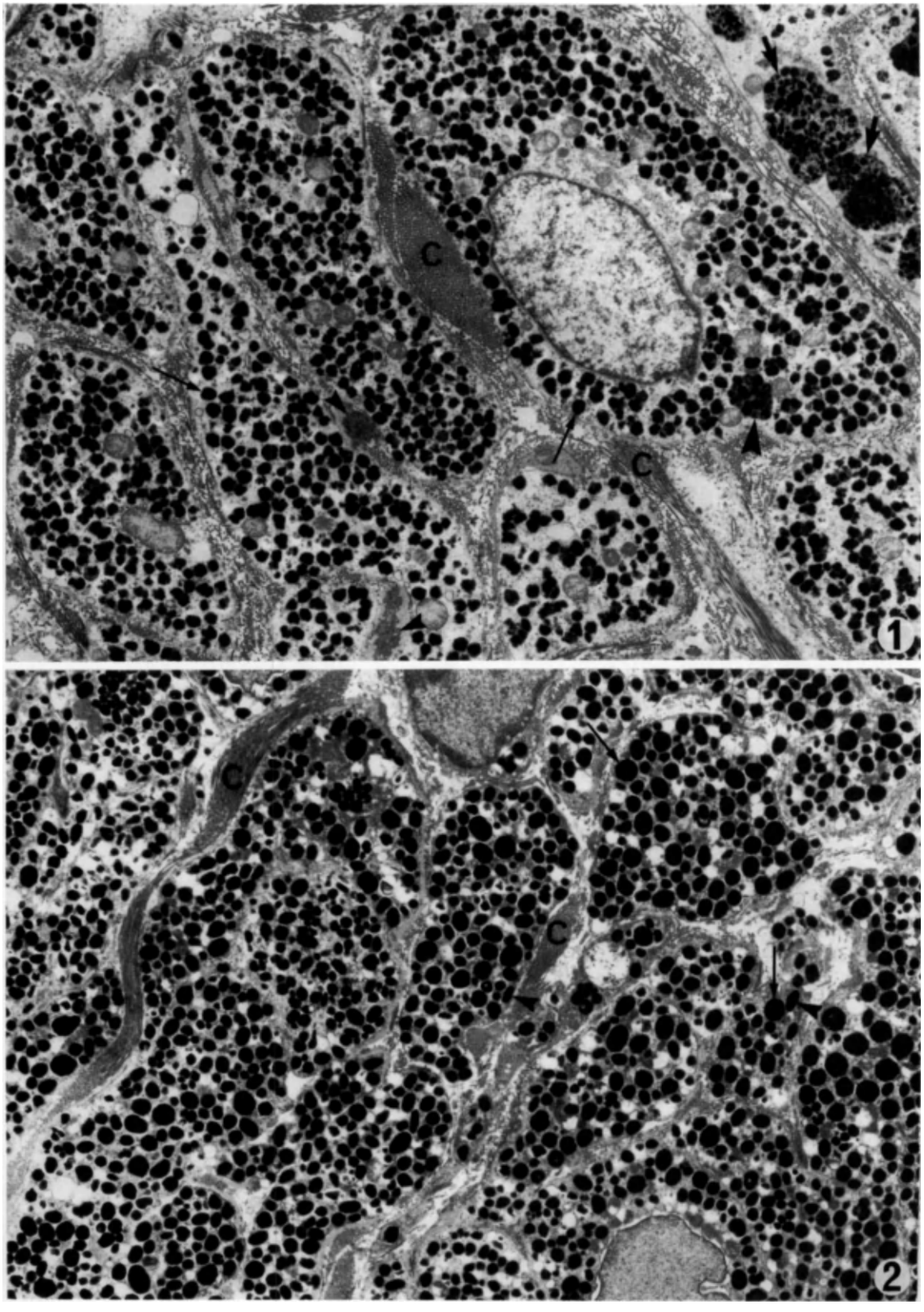
There is no consensus regarding the presence or absence of a basal lamina surrounding a group of cells or an external lamina surrounding individual cells in human cellular blue naevi. A basal or external lamina was not seen by Silverberg *et al.* (1971); Hernandez (1973) and Erlandson (1981) and not mentioned by Mishima (1970) and Allegra (1974) in studies on human cellular blue naevi. On the other hand, Merkow *et al.* (1969) and Kjaerheim *et al.* (1970) claim to have seen an external lamina, but illustrations showing a well developed continuous lamina are not presented. Indeed, the only truly convincing report is by Bhawan *et al.* (1980) who show a continuous indubitable external lamina (composed of well defined lamina lucida and lamina densa) around a cell (their *Fig. 3*) which is clearly a pigment-producing cell containing solitary melanosomes.

Plate 349

(From Ghadially, Ghadially and Lalonde, 1986)

Fig. 1. Human melanotic cellular blue naevus. Numerous naevus cells containing solitary melanosomes (thin arrows) and a melanophage containing compound melanosomes (thick arrows) are seen here. A small compound melanosome (large arrowhead) is present in a naevus cell. Note the collagen (C) and elastic fibres (small arrowheads) in the matrix, and the absence of an external lamina. × 6000

Fig. 2. Hamster melanotic blue naevus. The naevus cells contain numerous rounded (arrows) and elongated (arrowheads) solitary melanosomes. Note the collagenous matrix (C) and the absence of an external lamina. × 4600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Blue naevi, virtually indistinguishable from human blue naevi can be produced by painting the skin of hamsters with chemical carcinogens (Ghadially and Barker, 1960; Illman and Ghadially, 1960; Quevedo *et al.*, 1961, 1968; Ghadially and Illman, 1963, 1966; Oberman and Riviere, 1966; Parish and Searle, 1966).

A comparative study (Ghadially *et al.*, 1986) of 12 human blue naevi and 15 hamster blue naevi failed to reveal an external lamina or basal lamina in human ordinary blue naevi or their equivalent — the hamster small blue naevi (<3 mm in diameter). On very rare occasions, however, small ill-defined patches or minute segments of basal lamina-like material could be discerned. This situation also prevailed in about half the human cellular blue naevi and their equivalent — the hamster larger blue naevi. In the remainder, a small minority of the cells in each tumour showed clear evidence of basal lamina or external lamina formation in that sizeable segments of a slender basal lamina or external lamina were detected. However, in one human tumour and one hamster tumour quite a thick (at times continuous) external lamina was seen adjacent to a few cells (*Plate 350*). It must be stressed that even in these tumours only a few cells in some areas showed evidence of lamina production.

Ultrastructural studies have failed to give an unequivocal answer about the nature of the cells (i.e. whether they are Schwann cells or melanocytes) in blue naevi and their histogenesis. Since cells resembling melanocytes and Schwann cells have been seen in human blue naevi and since Schwann cells and melanocytes both arise from the neural crest, Bhawan *et al.* (1980) state: 'It is therefore tempting to suggest a unifying concept in which neoplastic transformation of a neural crest derived undifferentiated cell could differentiate into predominantly melanocytic, predominantly neural, or a tumour containing both components.'

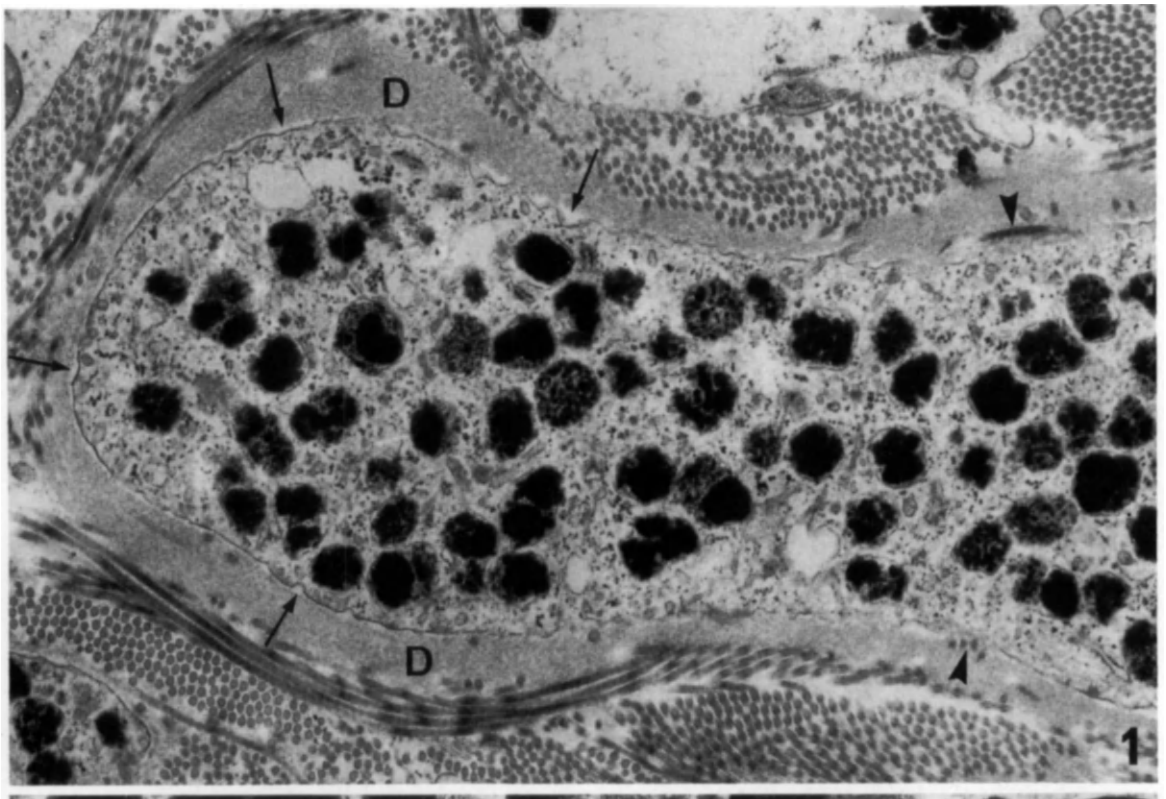
At first sight this seem to be a reasonable compromise between the two conflicting views which have prevailed in the literature regarding the nature of the cells in blue naevi. However, one wonders whether one should argue in this manner for this hypothesis is based on the unproven assumption that neural crest-derived undifferentiated cells occur and persist in the dermis, and one may question the prudence of putting so great a value on the presence of an external lamina or a basal lamina in the absence of any other evidence of schwannocytic differentiation (e.g. formation of mesaxons and pseudomesaxons). Since melanocytes and Schwann cells are closely related, we are not surprised to find that neoplastic transformation of Schwann cells in the melanotic schwannoma leads to the production of melanosomes, why then should one be surprised if the occasional cell (presumably melanocyte) in some blue naevi produces an external lamina? In view of the above, one would say that blue naevi are tumours of melanocytes (probably derived from migrant neural crest cells which failed to reach the epidermis) and that the occasional production of external lamina found in these tumours is an infrequent aberration engendered by the neoplastic state, which reflects the close kinship between the melanocyte and Schwann cell. The occurrence of basal and external laminae in cellular blue naevi and the hamster larger blue naevi, but their virtual absence in ordinary blue naevi and small hamster blue naevi, supports such an idea and suggests that lamina formation is a late event which tends to develop only when a blue naevus has attained a certain degree of size and cellularity.

Plate 350

(From Ghadially, Ghadially and Lalonde, 1986)

Fig. 1. Human melanotic cellular blue naevus. A rather thick external lamina is seen adjacent to a naevus cell. Collagen fibrils (arrowheads) are enmeshed in a medium density band which has the texture of a lamina densa (D). A continuous lamina lucida is not present, but a thin lucent 'line' (arrows) which may represent a poorly developed lamina lucida or a shrinkage artefact is present. Virtually all the melanosomes in this cell are granular melanosomes. × 21 000

Fig. 2. Hamster melanotic blue naevus. Interrupted external laminae of varying thickness are seen adjacent to tumour cells. In some zones the laminae are quite thick (thick arrows), in others relatively thin (thin arrows) or absent (arrowheads). × 27 000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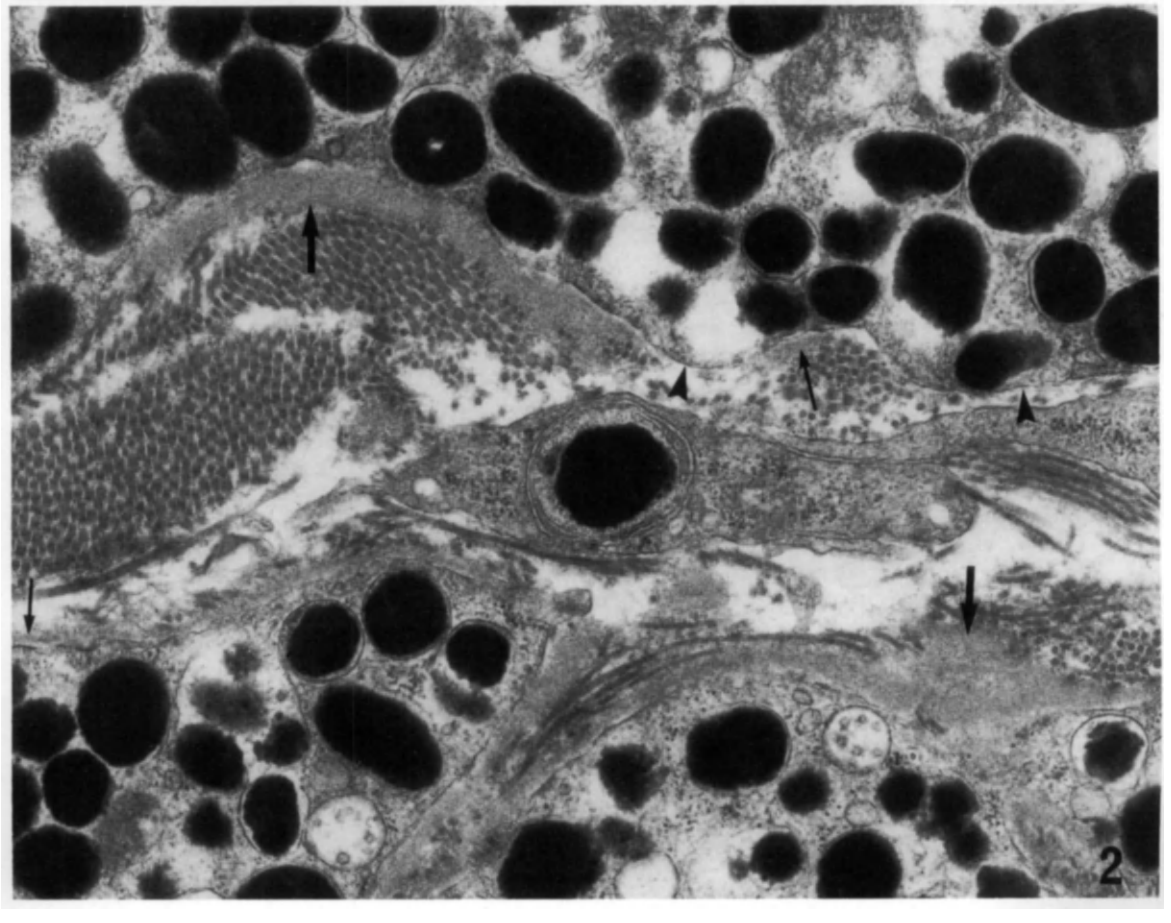
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10

Rod-shaped microtubulated bodies

Introduction

In 1964, Weibel and Palade described a new cytoplasmic component in the vascular endothelial cells of the rat, man and *Amblystoma punctatum*. They called it a 'rod-shaped tubulated body', for ultrastructural studies showed this to be a long single-membrane-bound cylindrical body containing six to 26 microtubules set in an electron-dense matrix. Since then this organelle has been described by many other workers who have used various terms to describe it; these include 'Weibel–Palade bodies', 'rod-bodies', 'tubulated bodies' and 'specific organelle of endothelial cells'. None of these terms is entirely satisfactory. Since the term 'tubulated' referes to the microtubules in the interior of this organelle, it would be more precise to call it a 'rod-shaped microtubulated body'.

It is worth noting that prior to the study by Weibel and Palade (1964) such bodies had been observed in vascular endothelia by various workers on several occasions. Hibbs *et al.* (1958) described dense cylindrical granules in the vasculature of human finger-tips, while micrographs published by Hatt *et al.* (1959) show similar bodies in arterioles and capillaries of human lung. Pease and Paule (1960) saw these bodies in the aortic endothelium of rats and described them as dense granules with compartmentalized internal structure, while Bierring and Kobayasi (1963) described them in the same tissue as small, dense, osmiophilic bodies.

However, it was the classic study of Weibel and Palade (1964) which first clearly described and delineated this body from other cellular structures. Since then many publications dealing with the morphology and distribution of rod-shaped microtubulated bodies in the vascular endothelia of various species have appeared, but the function of this organelle remains unclear although it has been suggested that it may be involved in intravascular clotting, and that it contains factor VIII.

Variations that might occur in pathological states receive only passing mention in a few publications. Since this is a dense body it has at times been mistaken for a lysosome. Attempts to demonstrate acid phosphatase activity in these bodies have been unsuccessful (Lemeunier *et al.*, 1969).

Structure, distribution and variations

Since their recognition by Weibel and Palade (1964), rod-shaped microtubulated bodies have been described in the vascular endothelial cells of many vertebrates. These organelles usually appear scattered randomly in the cytoplasm of the endothelial cells of arteries, veins, capillaries and endocardium (but not lymphatics). Variations in structure and electron density occur, some of which are probably attributable to different techniques of fixation and the staining employed (*Plates 351* and *352*).

Basically this is a single-membrane-bound cylindrical rod-like body which contains a number of microtubules set in an electron-dense matrix. In ultrathin sections, this long organelle is rarely cut along its entire length, so that its true length is difficult to determine. The longest one seen by Weibel and Palade (1964) measured 3.2 μm while that seen in the frog by Steinsiepe and Weibel (1970) measured 2 μm. The diameter of these bodies, as reported by various workers, ranges from 0.1–0.5 μm (human, about 0.15 μm), and that of the microtubules in its substance from 12–27 nm (human, about 20 nm). The matrix varies from moderately to highly electron-dense and appears granular in nature. The microtubules generally pursue a straight parallel course along the long axis of the organelle; sometimes, however, they show a twisted, spiral or whorled configuration. In some studies a central filament has been observed within the microtubules (e.g. Weibel and Palade, 1964; Ghadially and Roy, 1969).

These bodies appear to develop from the Golgi complex (Matsuda and Sugiura, 1970; Sengel and Stoebner, 1970). The first detectable stage of the process seems to be the formation of microtubular elements in enlarged sacs of the Golgi complex (*Plate 352, Figs. 2 and 3*). At this stage the matrix of the body is of low electron density. The body is then probably released into the cytoplasm by a process of pinching off from the Golgi stacks. The possibility that these bodies or their contents may be discharged into the blood stream has often been considered but contact between them and the cell membrane is rarely if ever seen. Although vesicular structures have at times been shown attached to their surface (Steinsiepe and Weibel, 1970), from such static pictures it is difficult to say whether this represents material coming into or leaving these bodies.

Rod-shaped microtubulated bodies have been seen in various species besides humans. These include: (1) rat (Weibel and Palade, 1964; Fuchs and Weibel, 1966; Matthews and Gardner, 1966; Roy and Ghadially, 1967); (2) mouse (Clementi and Palade, 1969); (3) rabbit (Rhodin, 1968; Ghadially and Roy, 1969); (4) dog (Sun and Ghidoni, 1969); (5) pigeon (Raviola and Raviola, 1967); (6) frog (Stehbens, 1965; Steinsiepe and Weibel, 1970); and (7) *Amblystoma punctatum* larvae (Weibel and Palade, 1964).

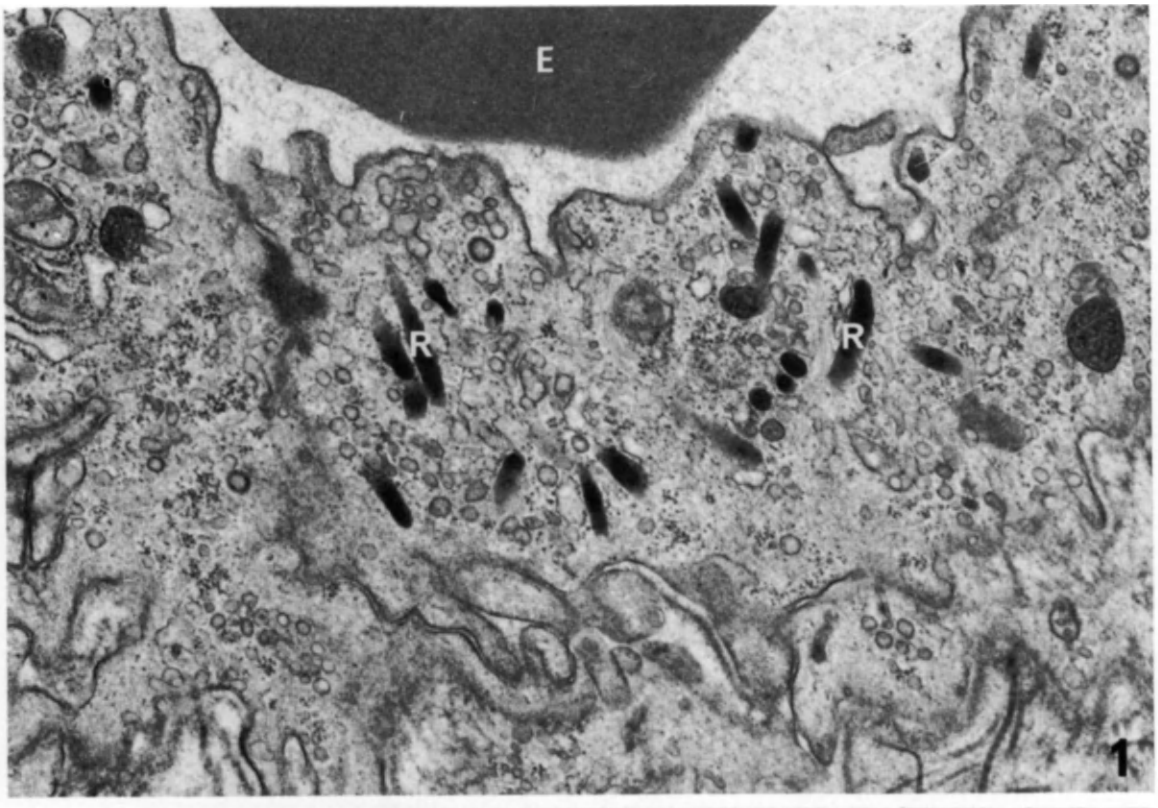
In humans these bodies have been described in blood vessels* of: (1) umbilical cord (said to occur more frequently in the vein than the artery) (Dubois *et al.*, 1965; Parry and Abramovich, 1972); (2) carotid body and paraganglia (Grimley and Glenner, 1966, 1968); (3) kidney (Jacobsen *et al.*, 1966); (4) skin (White and Clawson, 1967); (5) lymph node (*Plate 351, Fig. 1*); (6) synovial

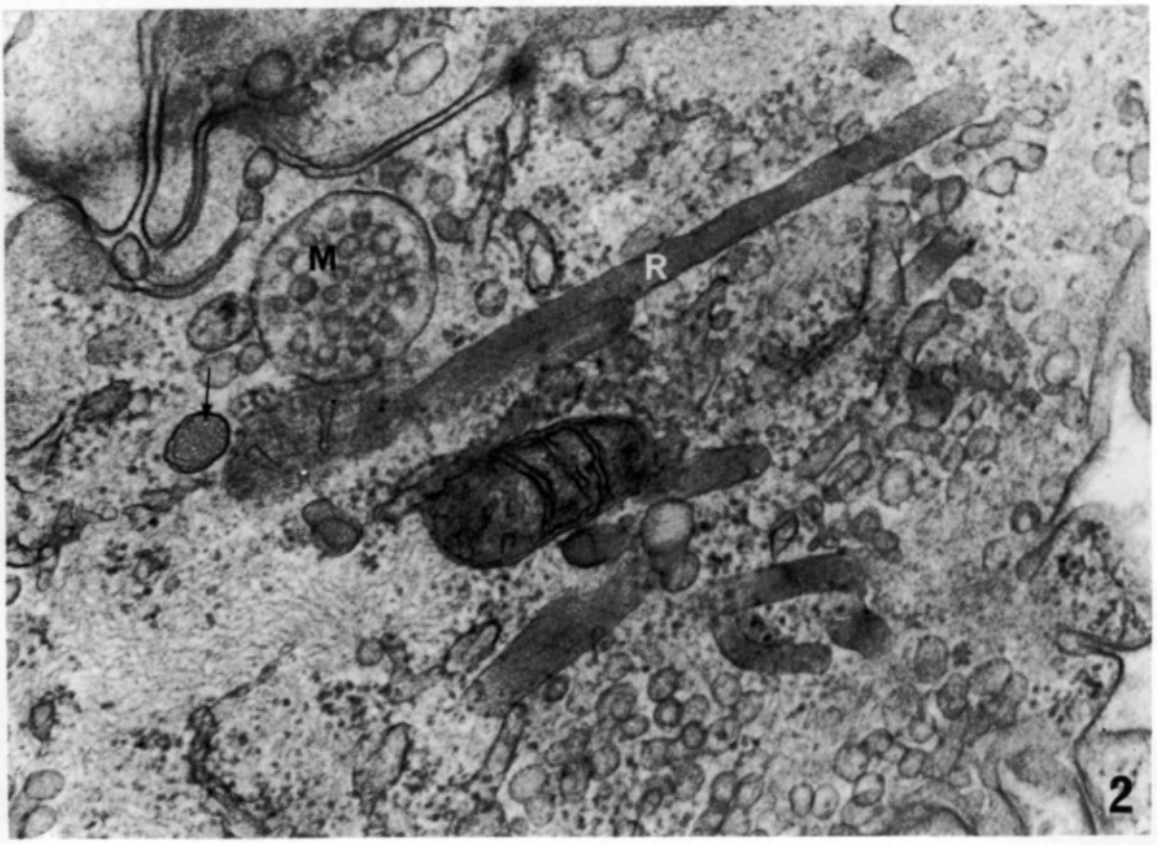
*This list is by no means complete. In fact rod-shaped microtubulated bodies are found in blood vessels of virtually every tissue and organ, normal and pathological (including tumours).

Plate 351

Fig. 1. Blood vessel from a human lymph node, showing lumen containing an erythrocyte (E) and vascular endothelial cells containing markedly electron-dense rod-shaped microtubulated bodies (R). × 28 000

Fig. 2. Vascular endothelial cell from subsynovial tissue of an osteoarthritic joint. The length of these organelles is difficult to appreciate in sectioned material, but here one of the rod-shaped microtubulated bodies (R) has been sectioned quite a distance along its length. Another, cut transversely, shows microtubules (arrow) in its interior. Note also the multivesicular body (M) which is frequently found in vascular endothelial cells. × 60 000 (*From Ghadially and Roy, 1969*)


E
R
R
1


M
R
2

membrane in normal subjects and in cases of traumatic arthritis, rheumatoid arthritis and osteoarthritis (Roy and Ghadially, 1967; Ghadially and Roy, 1969) (*Plate 351, Fig. 2*); (7) eye (Vegge and Ringvold, 1969; Matsuda and Sugiura, 1970; Matsuda, 1978) (*Plate 352*); (8) senile cutaneous angiomas (Stehbens and Ludatscher, 1968); (9) glomus tumours (Venkatachalam and Greally, 1969); (10) various vasoformative tumours (*see* pages 832–835) such as haemangio-endothelioma, haemangioblastoma and angiosarcoma (for reference *see* Ghadially, 1985); and (11) parathyroid adenomas (Elliot and Arhelger, 1966).

Rod-shaped microtubulated bodies are seen (for references *see* Kumar *et al.*, 1985) in greater numbers: (1) in human brain tumours than in normal brain; (2) in rheumatoid synovium than in normal synovium; and (3) in chick chorioallantoic membrane treated with low molecular weight angiogenesis factor (isolated from Walker sarcoma or human rheumatoid joint) than in normal untreated chorioallantoic membrane. However, serotonin which is capable of stimulating growth and division of endothelial cells does not produce an increase in the number of rod-shaped microtubulated bodies. Therefore Kumar *et al.* (1985) suggest that serotonin merely speeds up the normal growth and differentiation of new capillaries while angiogenesis factor actually induces the formation of new capillaries from existing mature capillaries. This involves dissolution of existing basal lamina and migration of endothelial cells which occurs well before endothelial cell proliferation.

Quantitative studies on the distribution of these bodies in various parts of the vascular system in the rat and frog (Fuchs and Weibel, 1966; Steinsiepe and Weibel, 1970) show that these are ubiquitous bodies found in vessels of all calibres and also in the endocardium. In the rat, the cytoplasmic volume occupied by these bodies in endothelial cells is said to be directly proportional to the size of the vessel, but in the frog this is said to depend more on the distance from the heart than on the vessel size. In the frog the volume density of these organelles in endothelial cell cytoplasm is reported to be: thoracic aorta, 8 per cent; abdominal aorta, 6.6 per cent; capillaries, 0.3 per cent; vena abdominalis, 6.7 per cent; vena cava, 3.7 per cent; endocardium, 0.5 per cent.

Such a distribution correlates neither with hydrostatic pressure nor with oxygen or carbon dioxide tension of the blood. It has been suggested that these bodies are involved in blood coagulation because these granules are thought to resemble α-granules of thrombocytes (Weibel, 1964) and probably contain platelet factor III (Siegel and Lüscher, 1967). Some support for this idea may also be found in the work of Shimamoto and Ishioka (1963), who showed that after administration of adrenalin the aortic wall of the rabbit produces a coagulation-enhancing substance, and Burri and Weibel (1968), who found that in segments of rat aorta incubated *in vitro* for short periods with adrenalin there is a quantitative loss of up to 40 per cent of these bodies from the endothelium.

Wagner *et al.* (1982) have shown by immunoelectron microscopy that rod-shaped microtubulated bodies contain factor VIII antigen (von Willebrand factor) but not tubulin, fibronectin or vimentin. Factor VIII is known to affect platelet binding to arterial subendothelium *in vivo* (Sakariassen *et al.*, 1979) and to interact with fibronectin and fibrinogen in mediating platelet binding to solid substrata *in vitro* (Lahav and Hynes, 1981).

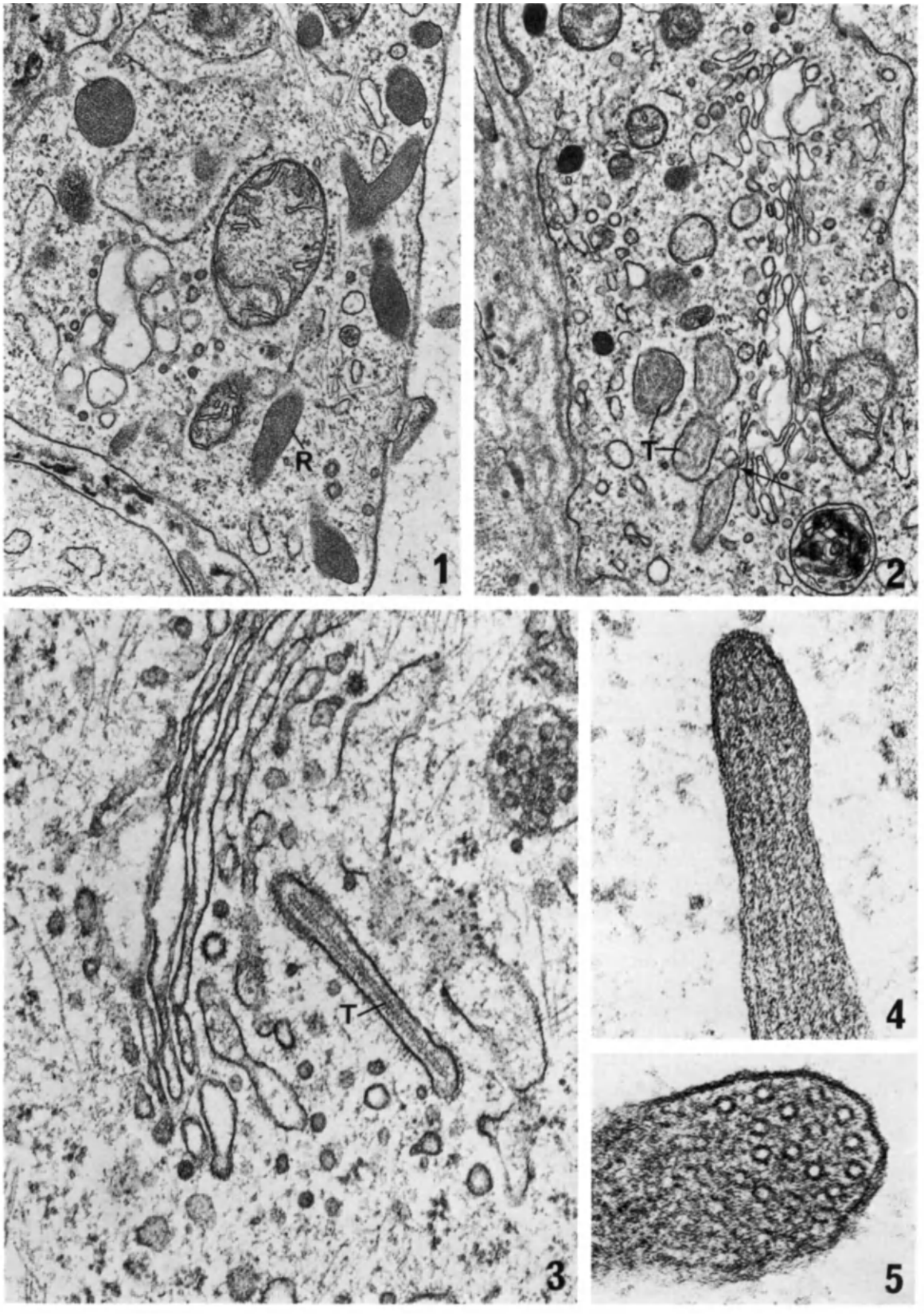
Plate 352

Rod-shaped microtubulated bodies from vascular endothelial cells of the iris from a case of Behçet's disease. (*From Matsuda and Sugiura, 1970*)

Fig. 1. A number of rod-shaped microtubulated bodies (R) are seen in a vascular endothelial cell. × 33 000

Figs. 2 and 3. A possible way in which these bodies are formed is depicted here. Near the Golgi complex are seen structures acceptable as enlarged Golgi sacs containing microtubules (T). One of these appears to be in the process of being pinched off from the Golgi stacks (arrow). × 42 000; × 60 000

Figs. 4 and 5. Longitudinal and transverse sections of rod-shaped microtubulated bodies, showing the microtubules in their interior. × 147 000; × 168 000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Rod-shaped microtubulated body in vasoformative tumours

As mentioned earlier (pages 828–831), the rod-shaped microtubulated body is found in the endothelial cells lining blood vascular spaces (endocardium, arteries, veins and capillaries) but not in those lining lymphatics. Since its recognition by Weibel and Palade (1964), the rod-shaped microtubulated body has been widely accepted as the specific organelle* of the endothelial cells of the blood vessels. Neoplastic endothelial cells retain the ability to produce these specific organelles (i.e. rod-shaped microtubulated bodies) to a greater or lesser degree, and hence they are a useful marker in identifying tumours of these cells (*Plates 353–354*). The various ways in which rod-shaped microtubulated bodies† help in diagnosis of vasoformative tumours may be summarized by saying that they assist in: (1) detecting small and slit-like vascular lumina; (2) distinguishing vascular lumina from others; (3) distinguishing endothelial cells from others; (4) diagnosis of angiosarcoma; and (5) distinguishing a haemangiomatous tumour from a lymphangiomatous tumour. Some comments and amplification of these points will now be presented and we shall also look at some examples where rod-shaped microtubulated bodies have assisted in deducing the histogenesis of some tumours.

A problem with vasoformative tumours is that the characteristic vascular lumina are not always well formed and hence not easily identifiable with the light microscope (item 1). Electron microscopy can help by demonstrating small or slit-like vascular lumina lined by endothelial cells (identified as such by the presence of rod-shaped microtubulated bodies) which cannot be seen or appreciated as such with the light microscope (*Plate 353*). Further, even if some lumina are detected at light microscopy, one may be left wondering whether they are vascular lumina or the intercellular lumina of other tumours like adenocarcinoma or mesothelioma (item 2). Here again electron microscopy may help by identifying endothelial cells by the presence of rod-shaped microtubulated bodies.

Yet another problem is deciding about the nature of cells present in vasoformative tumours (item 3) and certain well vascularized tumours. The value of rod-shaped microtubulated bodies lies in the fact that they permit us confidently to distinguish neoplastic endothelial cells from other cells (e.g. pericytes and smooth muscle cells) in vasoformative tumours and also distinguish non-neoplastic endothelial cells in well vascularized tumours. Thus, for example, Carstens (1981) reports two cases where the finding of rod-shaped microtubulated bodies was helpful. In one case it showed that the tumour was a haemangioma and not a haemangiopericytoma and in another case it helped to establish that the tumour was a haemangioblastoma and not an angiomatous meningioma.

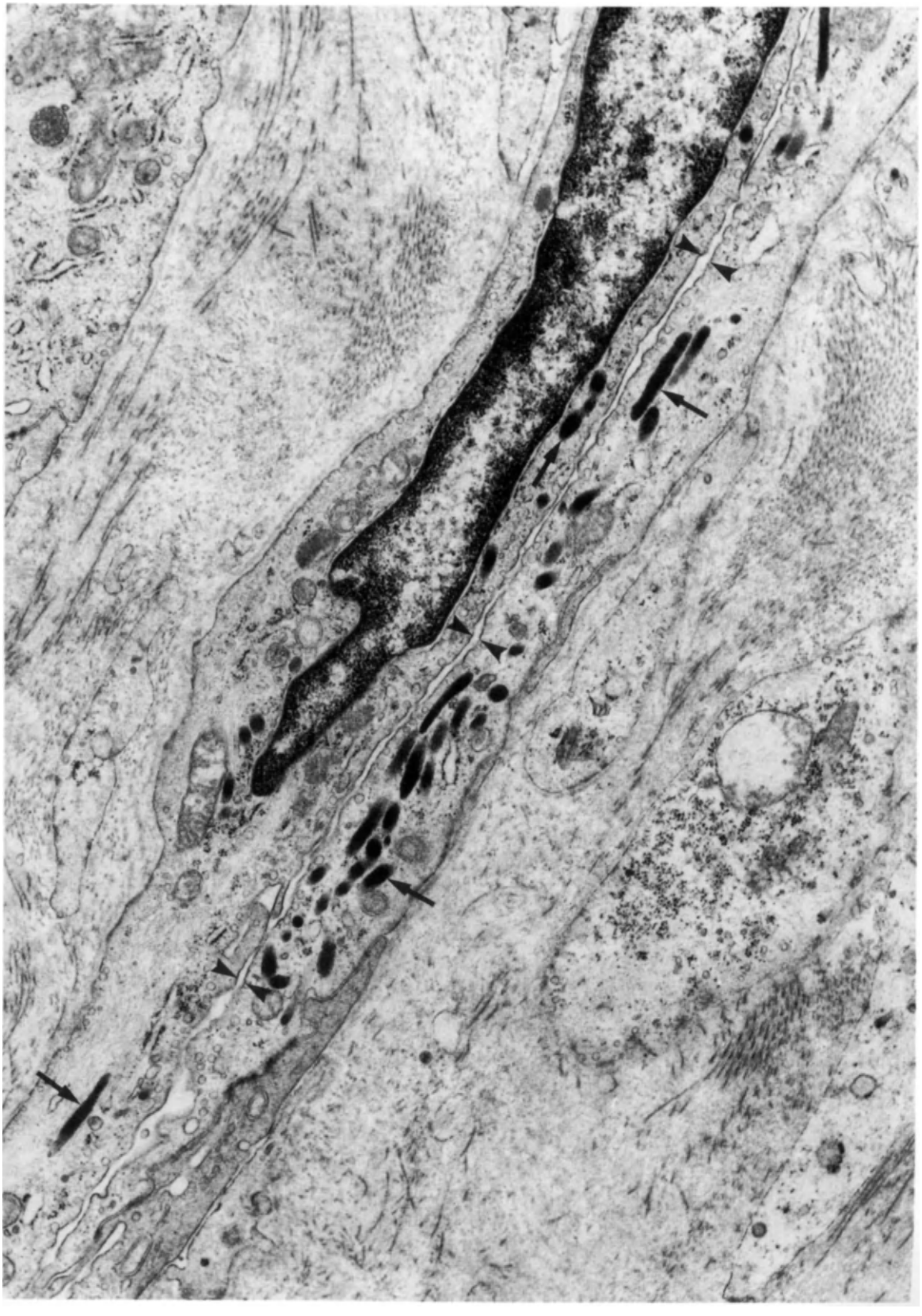
Rod-shaped microtubulated bodies are prominent in benign endothelial neoplasms but not in in their malignant counterpart (i.e. angiosarcoma) (item 4). It would appear that neoplastic endothelial cells of angiosarcoma often lose their ability to form confidently indentifiable rod-shaped microtubulated bodies. Nevertheless the diagnosis of several difficult to diagnose

*The paper by Zelickson (1966) is often quoted by those who erroneously imagine that rod-shaped microtubulated bodies are not specific for endothelial cells and that they are also found in pericytes. True, Zelickson makes such a claim for pericytes of human normal skin capillaries, but the only so-called 'rod-shaped microtubulated bodies' that the author shows in a pericyte (his *Fig. 1*) are a couple of nondescript rounded electron-dense bodies which could equally well be lysosomes. This unconfirmed claim cannot be seriously entertained.

†Several features besides rod-shaped microtubulated bodies have to be taken into consideration in the diagnosis of vasoformative tumours. It is not possible to deal with all these here or discuss fully the histogenesis and diagnosis of vasoformative tumours (for this *see* Ghadially, 1985). Only the role of rod-shaped microtubulated bodies in diagnosis and evaluation of the histogenesis of some controversial lesions is presented here.

Plate 353

Benign haemangioendothelioma. Seen here is a slit-like vascular lumen (between arrowheads), which can be confidently identified as such because it is bounded by endothelial cells which in turn can be identified as such by the presence of rod-shaped microtubulated bodies (arrows). × 19 000



(with the light microscope) angiosarcomas has been established by demonstrating rod-shaped microtubulated bodies in tumour cells (e.g. Carstens, 1981).

Rod-shaped microtubulated bodies are at times helpful in distinguishing haemangiomatous from lymphangiomatous neoplasms (item 5). Thus, for example, the distinction between haemangioma and lymphangioma is relatively easy because the former contains many rod-shaped microtubulated bodies (more than normal endothelial cells), the latter none. However, the distinction between haemangiosarcoma and lymphangiosarcoma can be difficult or impossible. If rod-shaped microtubulated bodies are found then the tumour is a haemangiosarcoma; if they are not found, it could still be a haemangiosarcoma because: (1) failure to find rod-shaped microtubulated bodies does not necessarily mean that they are absent; and (2) failure to find rod-shaped microtubulated bodies may be no more than a reflection of anaplasia or poor differentiation associated with neoplasia.

Besides the diagnostic value of this organelle, there is also the usefulness of rod-shaped microtubulated bodies in deciding whether a cell type in a controversial neoplasm is of endothelial differentiation. Thus, for example there has been much dispute about the nature of the cells in sclerosing haemangioma and its histogenesis. Carstens and Schrodt (1974) studied nine cases and were able to demonstrate rod-shaped microtubulated bodies in cells that lined identifiable lumina as well as in cells that formed organoid structures, and were thus able to support Gross and Wolbach's (1943) hypothesis that this group of lesions is of endothelial derivation.

Light and electron microscopy show that haemangioblastoma (a tumour of the central nervous system, mainly cerebellum) is composed of three major cell types: endothelial cells, pericytes and stromal cells. There has been much controversy about the nature of the stromal cells. Two studies (Carstens, 1981; Ho, 1984) have now demonstrated rod-shaped microtubulated bodies in these cells so their endothelial nature cannot be seriously doubted.

Similarly, there has been much dispute about the nature of the cells and the histogenesis of Kaposi's sarcoma. Leu and Odermatt (1985) have found rod-shaped microtubulated bodies by electron microscopy and factor VIII by immunohistology, not only in cells lining vascular channels but also in the spindle cells in the stroma and hence conclude that this is a vasoformative tumour.

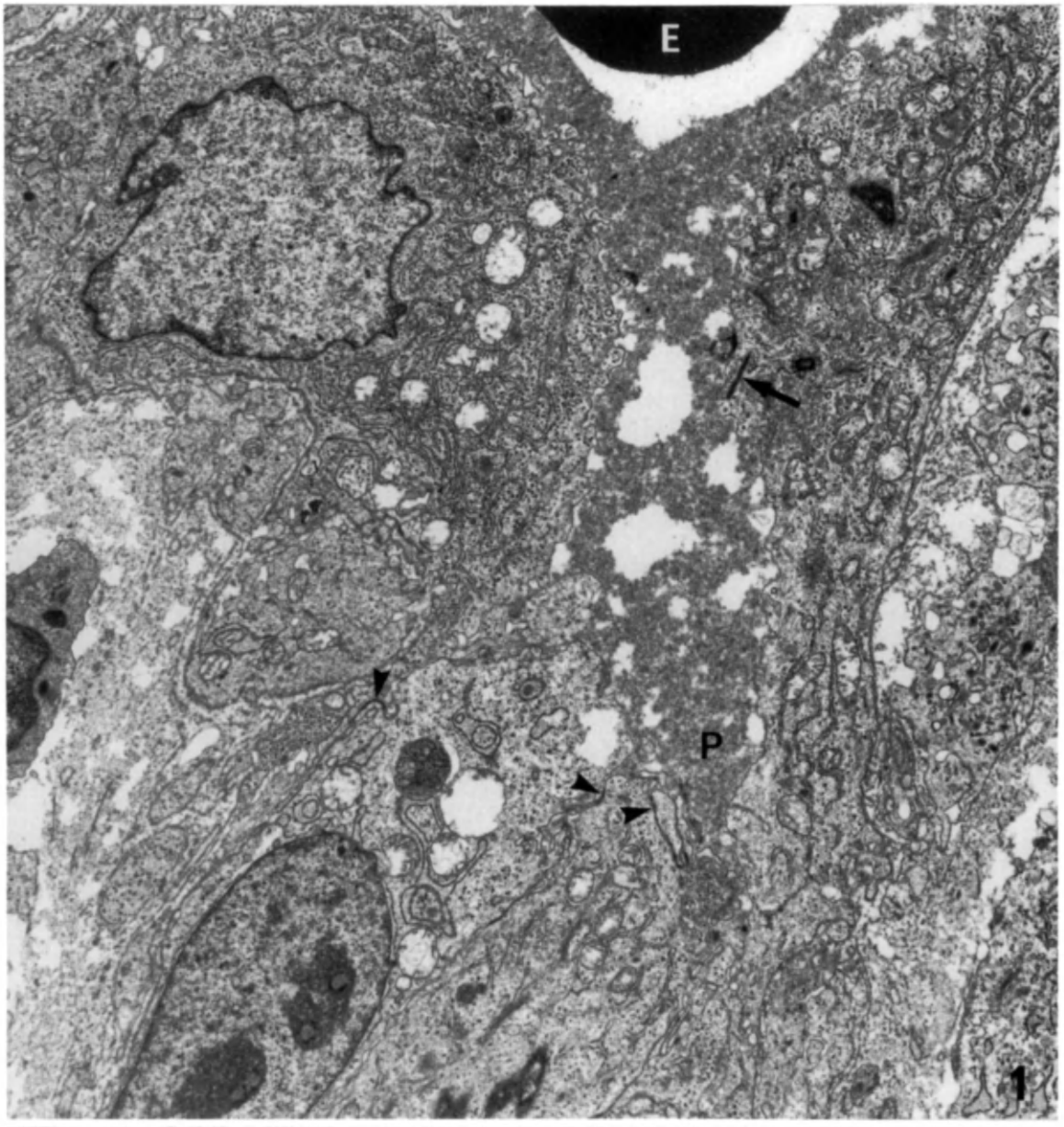
Finally a word of warning. Blood vessels are found in all tumours, and so are rod-shaped microtubulated bodies in their endothelial cells. Therefore, the mere finding of rod-shaped microtubulated bodies in a tumour means nothing, they must be demonstrated in indubitable tumour cells before reaching a diagnosis of vasoformative tumour.

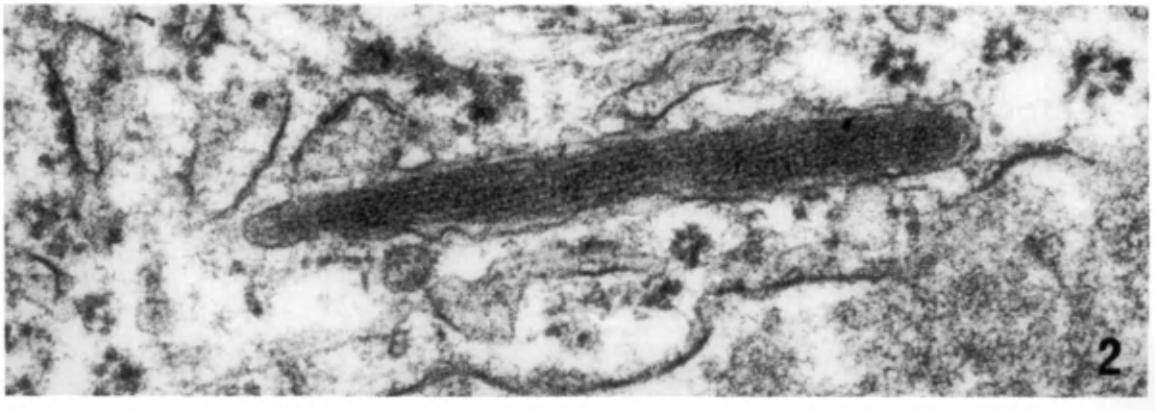
Plate 354

Poorly differentiated angiosarcoma. (*From a block of tissue supplied by Dr I. Dardick and Dr E. Liepa*)

Fig. 1. Malignant endothelial cells united by tight junctions (arrowheads) are seen lying adjacent to a space occupied by some particulate material (P) and an erythrocyte (E). One of the neoplastic cells contains a rod-shaped microtubulated body (arrow) which is shown at a higher magnification in *Fig. 2.* × 7200

Fig. 2. Higher-power view of the rod-shaped microtubulated body shown in *Fig. 1.* The interior of the body shows the characteristic striated appearance. × 87000


E
P
1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11

Intracytoplasmic filaments

Introduction

Intracytoplasmic filaments of one type or another are a universal component of the cytoplasmic matrix. However, in routine electron microscopic preparations most cell types are seen to contain only rare filaments or a few filaments scattered at random in the cytoplasm. These, as a rule, attract little attention; it is only when such filaments are abundant that curiosity as to their nature and significance is aroused. Intracytoplasmic filaments may be classified primarily as myofilaments and other intracytoplasmic filaments.

Myofilaments comprise a special class of intracytoplasmic filaments. Two types of filaments, thick (about 15 nm)* and thin (about 6 nm), are recognized in striated muscle cells. The former contain myosin, the latter actin and some tropomyosin and troponin. In striated muscle the two types of filaments are set side by side. They slide past one another during contraction. In the usual preparations of vertebrate smooth muscle, most of the filaments are very fine (6 nm) and only occasional thicker filaments (15 nm) are seen. However there is now much evidence that the thicker myosin filaments are present in substantial numbers in smooth muscle also, but special methods are required to preserve and demonstrate them. It has also become apparent that actin and/or myosin (not necessarily as filaments) occur not only in muscle cells but in most other cells, even red blood cells.

The term 'other intracytoplasmic filaments' was at one time used to describe intracytoplasmic filaments other than myofilaments. Since these filaments have a diameter of about 10 nm (8–11 nm) they are now described as '10 nm filaments', 'intermediate filaments' or 'intermediate-sized filaments' (i.e. intermediate in size when compared with the thin actin filaments and the thick myosin filaments).

Intermediate filaments varying in orientation and abundance are seen in a variety of cell types. There is evidence that an increase in intermediate filaments occurs with age, at least in some cell types (e.g. chondrocytes of articular cartilage and neurons) and examples may also be found where such an increase is associated with pathological processess including tumours (*Plate 355*). It has long been suspected that these intermediate filaments are proteinaceous in nature. Although it is not possible on ultrastructural appearances alone to distinguish

unequivocally between intermediate filaments occurring even in such diverse cell types as chondrocytes and neurons, it was argued that there must be chemical differences.

This has now proven to be so; for biochemical and immunofluorescent techniques have clearly established intermediate filaments as a distinct filamentous system composed of chemically heterogeneous subunits, on the basis of which five major classes of filaments may be identified. These include: (1) tonofilaments† (also called prekeratin filaments or cytokeratin filaments) found in epithelial cells; (2) vimentin filaments found in mesenchymal cells but they have also been described in cultured cells of epithelial, myogenic and neurogenic origin; (3) desmin filaments (also called 'skeletin filaments') found in muscle cells (smooth, skeletal and cardiac); (4) neurofilaments found in neurons; and (5) glial filaments found in astrocytes.

Myofilaments and intermediate filaments are long thread-like structures. There is no real evidence that they branch or bifurcate. Whether filaments (myofilaments, intermediate filaments or in fact any other type of filament within or outside the cell) branch or not is almost impossible to ascertain by just looking at electron micrographs of sectioned material. Factors such as crossing and overlaying of filaments within the section thickness, filaments contacting each other at acute angles and weaving in and out of the section thickness at varying angles so that their full course is not visualized can create appearances which may be erroneously interpreted as evidence of branching. (For a critique and references on this point *see* Franke *et al.*, 1979.) No wonder then that most authors sidestep this issue and make no comments about such matters in their papers. But those oblivious to such problems or unaware of the magnitude of the problem speak blithely about branching filaments. For example, Carr (1972) in his paper on filaments (which he calls 'microfibrils'‡) in macrophages speaks about 'microfibrils which often branched'. The illustrations presented to support this contention unequivocally show overlaying of filaments but nothing that would make one suspect that branching filaments are present (*see also* footnote on page 940 where the myth about branching microtubules is dealt with).

*A review of the early literature shows that in the past the diameters of most filaments were underestimated and indeed these low figures are quoted in Fawcett (1966) and in the 1st edition of this book (Ghadially, 1975). For example myosin filaments were said to be 10 nm in diameter. It seems likely that preparative procedures were at least in part responsible for this. One may speculate that the introduction of glutaraldehyde fixation which better preserves proteins and double staining (which too accentuates the diameter of filaments) are factors responsible for differences in measurements found in the old and the new literature.

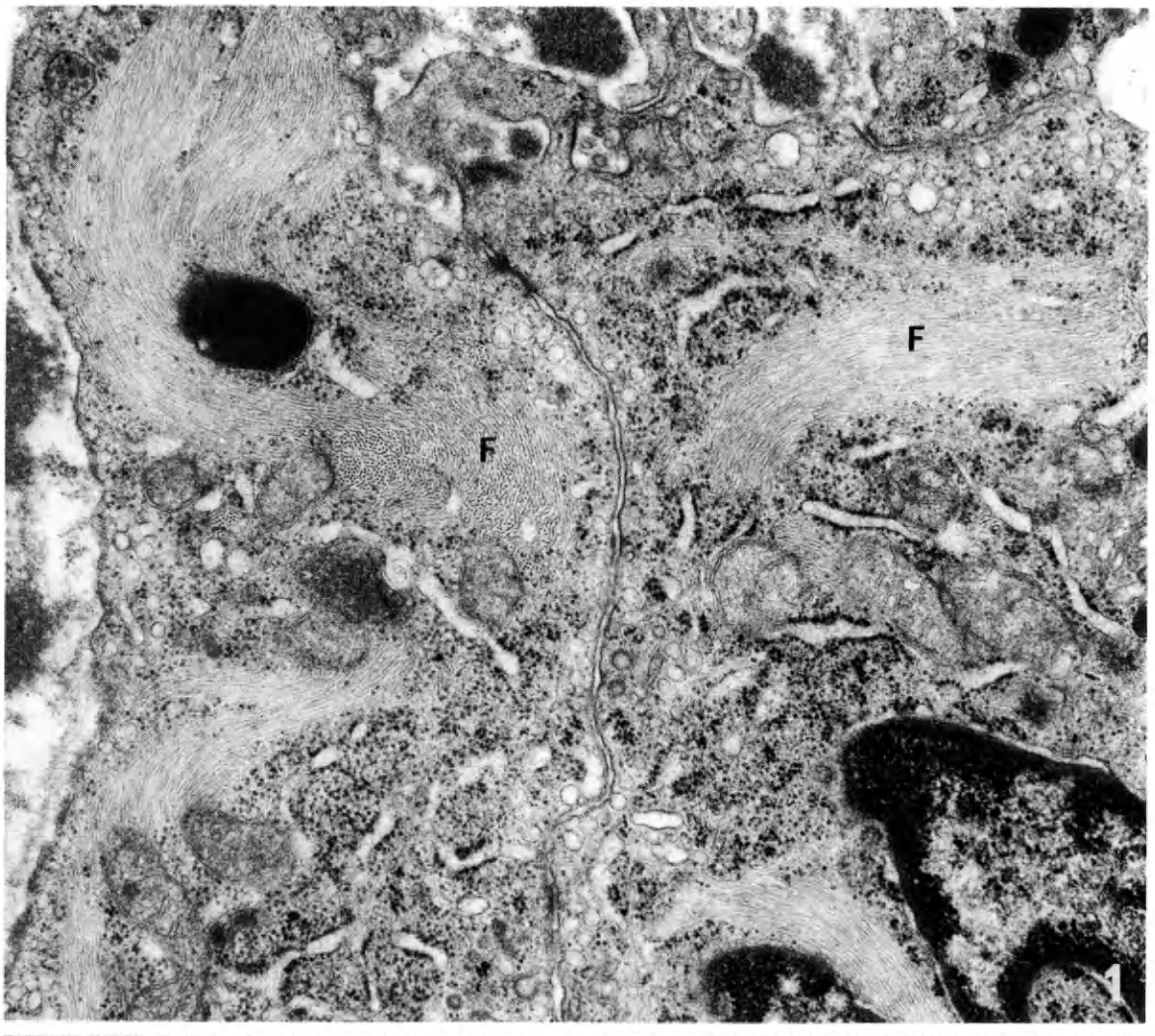
†Tonofilaments should not be called 'keratin filaments' or 'keratin-like filaments'. For a discussion on this point *see* page 884.

‡Time hallowed histological nomenclature defines a fibril as a 'bundle or aggregate of filaments'. A filament is an entity on its own, it is not a 'small fibril' or 'microfibril'. (*see* pages 1215–1221 for details).

Plate 355

Fig. 1. Cardiac myxoma. The cells of the tumour contain several aggregates of intermediate (vimentin) filaments (F). × 32 000

Fig. 2. Cardiac myxoma. High-power view of intermediate filaments. × 58 000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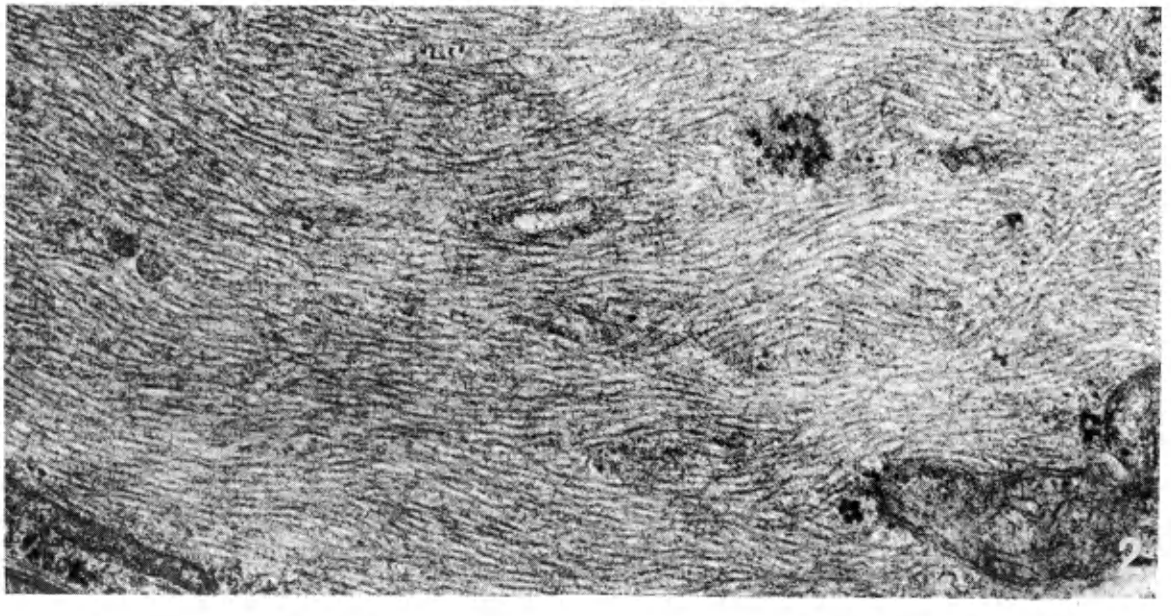
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Myofilaments in striated muscle

It has already been noted that two types of myofilaments*—thick and thin (myosin and actin)—occur in striated muscle (page 839). Such filaments are arranged in a highly ordered fashion to form contractile elements called myofibrils. Numerous myofibrils occur in the cytoplasmic matrix (sarcoplasm) of a skeletal muscle fibre, which is in fact a long cylindrical cell which, in addition to myofibrils, contains various organelles such as numerous nuclei, mitochondria and endoplasmic reticulum, and also inclusions such as glycogen and lipid. Under polarized light the myofibrils and myofibres display alternating bright anisotropic bands (A-bands) and dark isotropic bands (I-bands). In stained preparations viewed with ordinary light, the densities are reversed. Other features that can be shown in suitable preparations by light microscopy but are more clearly demonstrable by electron microscopy (*Plate 356*) include†: (1) a dark line (Z-line or Zwischenscheibe) which traverses the middle of the I-band; (2) a pale zone (H-band or Hensen's stripe) which bisects the A-band; and (3) a narrow dark line (M-line or Mittelscheibe) which traverses the centre of the H-band. The portion of a myofibril between two consecutive Z-lines is called a sarcomere. This is the contractile unit of striated muscle. At the Z-line the thin actin filaments from two neighbouring sarcomeres are believed to be attached laterally to one another. In cross-section the Z-line shows a lattice pattern (for references and details, *see* page 850). The relative lengths of the I- and A-bands are dependent upon the state of contraction or relaxation of the myofibril. The A-band remains quite constant in length, but the I-band is longest when the muscle is stretched, medium sized when it is relaxed, and shortest when the muscle is contracted. The thin actin filaments in the I-band extend into the A-band which contains the thick myosin filaments. Cross-sections through the A-band of vertebrate skeletal and cardiac muscle show that the thick filaments form a hexagonal array or lattice.

The thin filaments are interposed between the thick filaments at the trigonal points in the hexagonal array so that each thick filament is seen surrounded by six thin filaments, the ratio of thin to thick filaments being 2:1. The longer, thicker myosin filaments of invertebrate striated muscle are capable of interacting with a greater number of thin filaments. In muscle from various species, thin to thick filament ratios varying from 3:1 to 6:1 have been found.

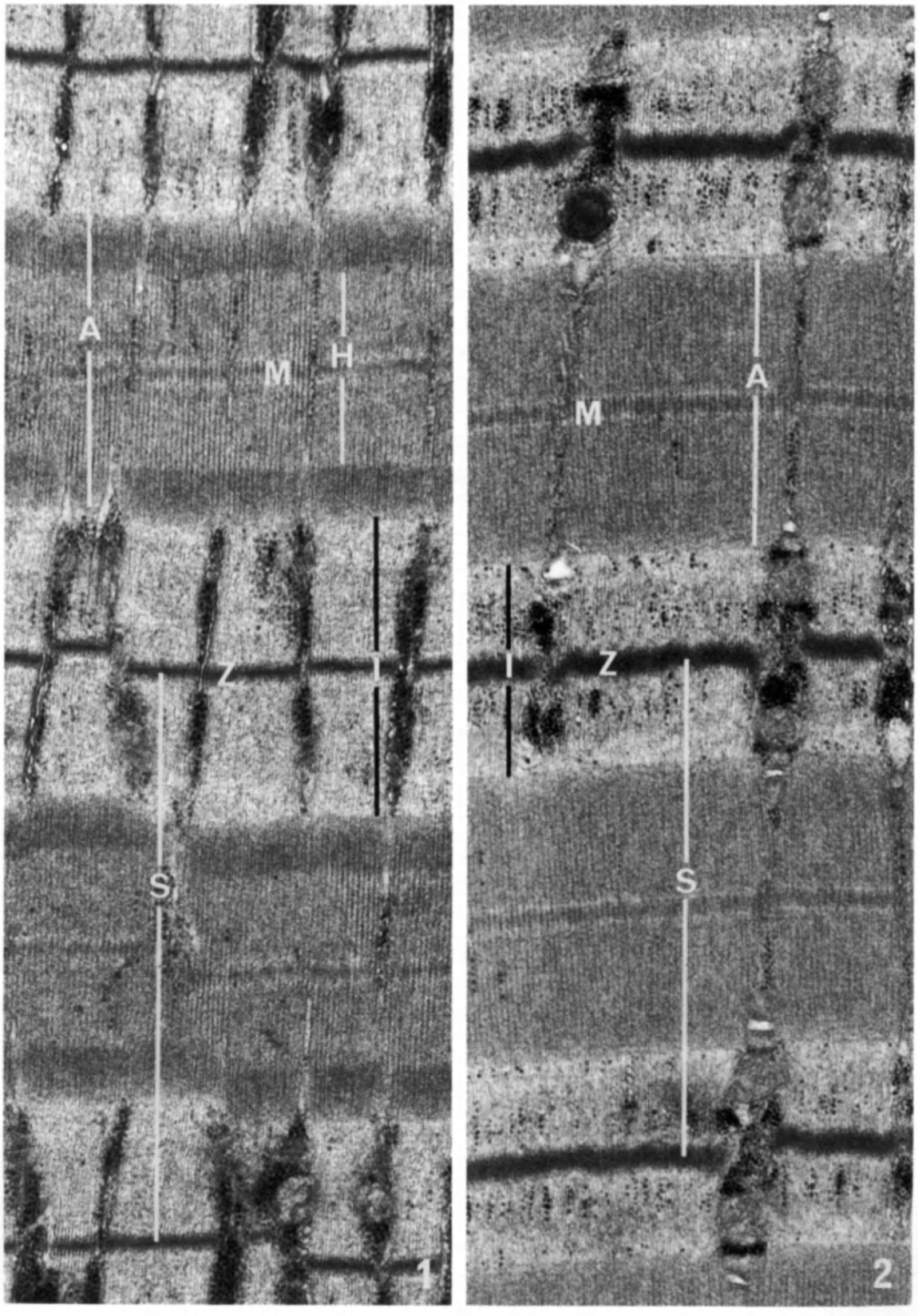
During contraction the thin filaments slide over the thick filaments and extend deeper into the A-band. This draws the two Z-lines closer together and reduces the length of the sarcomere with resultant shortening of the fibre. In relaxed muscle the thin filaments extending into the A-band from opposite ends do not meet at the M-line, but leave a gap which determines the width of the H-band. The H-band is thus the region of the A-band from which thin filaments are absent at any given moment. This explains why the H-band is wide in stretched muscle, of a medium size in relaxed muscle, and very narrow or entirely absent when the muscle is in the contracted state (*Plate 356*). (For references to the literature on myofibrils and structure of striated muscle, *see* Spiro and Hagopian, 1967; Mair and Tomé, 1972.)

*Intermediate filaments (desmin) also occur, but they are difficult to demonstrate in normal adult striated muscle cells (*see* page 890 for more details and also *Plate 380*).

†It is beyond the scope of this text to describe certain elusive entities called 'N-lines' (at least seven of them are said to exist) which are usually invisible but are rendered visible by chance and entities called 'gap filaments'; neither of which have a role in the sliding filament theory of muscle contraction (*see* Locker, 1984 and Locker and Wild, 1984 for information on these entities).

Plate 356

Figs. 1 and 2. Stretched and contracted skeletal muscle, respectively, are shown. The principal features are identified in these electron micrographs as follows: sarcomere (S), A-band (A), I-band (I), H-band (H), M-line (M) and Z-line (Z). Note the wide H-band and I-band in the stretched myofibrils shown in *Fig. 1*, and the absence of an H-band and a narrow I-band in the contracted myofibrils shown in *Fig. 2*. Although the two muscle specimens illustrated here are similar in appearance, one is from a rabbit (*Fig. 1*) and other from a man (*Fig. 2*). × 30 000; × 30 000


A
M
H
Z
I
S
1
A
M
Z
I
S
2

The description of the structure of striated muscle given above and illustrated in *Plate 357* stems from the classic studies of Huxley (1957, 1960) and Huxley and Hanson (1954, 1960) who gave us the now famous, sliding filament theory of muscle contraction. However, what is illustrated in the diagrams and what one actually sees in the usual 60–90 nm thick sections through routinely processed muscle (especially human biopsy material) are somewhat different, so some explanations are in order.

As mentioned before, the widely accepted view is that in vertebrate (including human) striated muscle each thick myosin filament is surrounded by six thin actin filaments occupying the trigonal position—that is equidistant from and shared by three thick myosin filaments, and that the ratio of thin to thick filaments is 2:1.

However, the fact of the matter is that this perfect pattern of arrangement of thick and thin filaments is often not demonstrable and there seem to be too few or too many thin filaments around the thick filaments, which are not all that regularly or equidistantly spaced between the thick filaments. Some people believe that such deviations from the ideal model are artefactual, while others believe (Nistal *et al.*, 1977) that at least in some instances such deviations reflect real differences in thick filament-thin filament arrangements and ratios (thin to thick filament ratio said to be as high as 3:1 or 4:1).

Newstead (personal communication) suggests (and I concur) that too many thin filaments in the A-band may be explained by assuming a state of spasm or hypercontraction which drives the actin filaments across the middle of the sarcomere. The I-bands would then virtually disappear and all the filaments would huddle up in the A-band. Similarly, too few filaments could result from some of the thin filaments moving out of the A-band and crumpling up in the I-band. Such changes could easily occur during excising, chopping up and fixing muscle.

In routine longitudinal sections (60–90 nm thick) it is really not possible to demonstrate the arrangement of thin filaments in the A-band. What one usually sees is a grey area between the thick filaments in which one can now and again discern short lengths of thin filaments. This is because more than one layer of filaments is included in the section thickness. If superimposition of filaments is to be avoided the section has to be no more than about 20–25 nm thick, and of course it has to be parallel to the long axis of the sarcomere. Glycerol extraction and subsequent washing (a procedure which removes 30–40 per cent of soluble muscle protein) enhances contrast and hence more clearly demonstrates the filaments and also the cross bridges that radiate (at 40 nm intervals) from each myosin filament towards the neighbouring actin filaments. The bridges are, in fact, the heads of the myosin molecules* plus a short part of their rod-like tail portion. Sliding of filaments is produced by repetitive cycles of attachment and detachment of the heads to the actin filament. During contraction flexing of the heads drives the actin filaments deeper into the A-band with resultant shortening of the sarcomere.

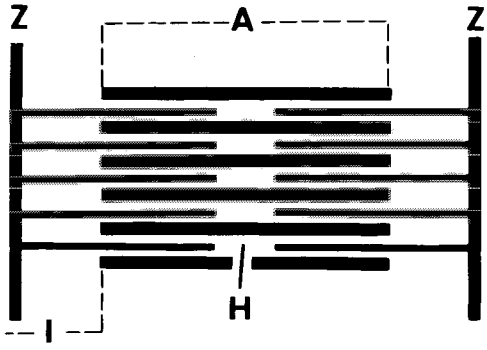
*Space does not permit inclusion of a discourse on actin and myosin molecules. This is of no great consequence because this information is available in several standard histology texts.

Plate 357

Figs. 1–4. Diagrams of striated muscle in the resting state. Longitudinal sections (*Figs. 1 and 2*) through striated muscle showing sarcomeres bounded by Z-lines (Z). One (*Fig. 1*) or two (*Fig. 2*) thin filaments may be seen between each pair of thick filaments in the A-band (A). The H-band (H) contains thick filaments but no thin filaments. In the I-band (bisected by the Z-line) only thin filaments are seen. The transverse sections (*Figs. 3 and 4*) through the A-band (A) show that the thick filaments form a hexagonal lattice, and that each thick filament is surrounded by six thin filaments which are situated at the trigonal positions of the lattice so that each is shared by three equidistant thick filaments. The parallel dashed lines explain how a section through certain planes can show either one or two thin filaments between the thick filaments as depicted in *Figs. 1 and 2* respectively.

Fig. 5. Biopsy of human skeletal muscle. Transverse section through an A-band shows that virtually all the thick filaments are surrounded by six thin filaments (demonstrated by circles). × 170 000

Fig. 6. Biopsy of human skeletal muscle. Different case from *Fig. 5*. Transverse section through an A-band shows that some of the thick filaments are surrounded by about nine or ten thin filaments (demonstrated by circles). × 140 000



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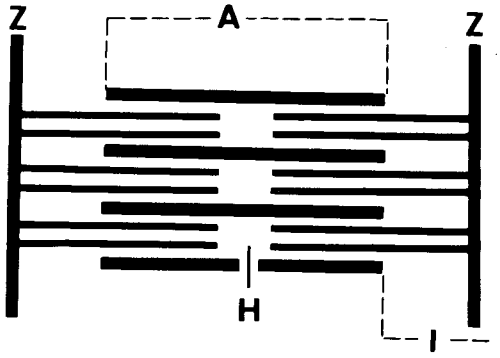

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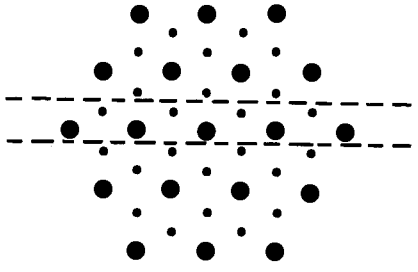

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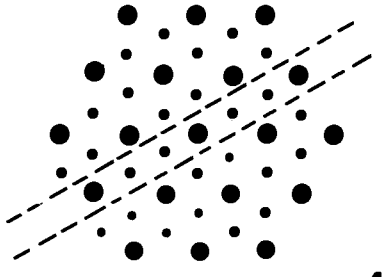

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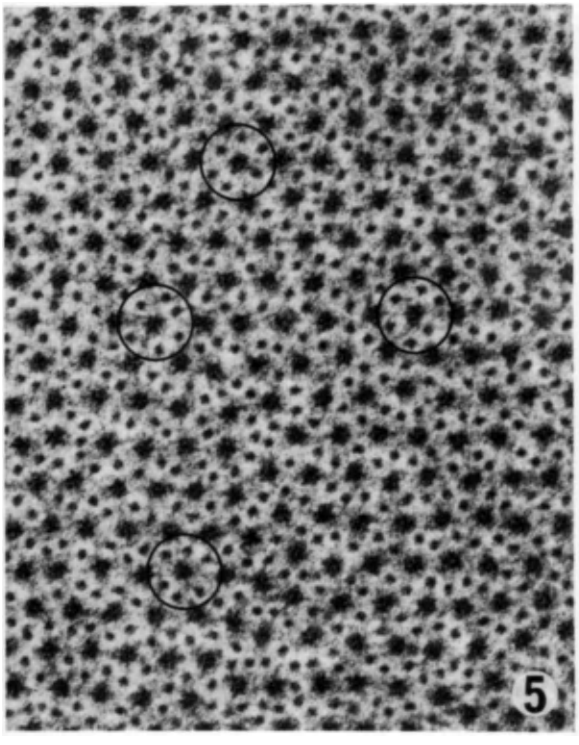
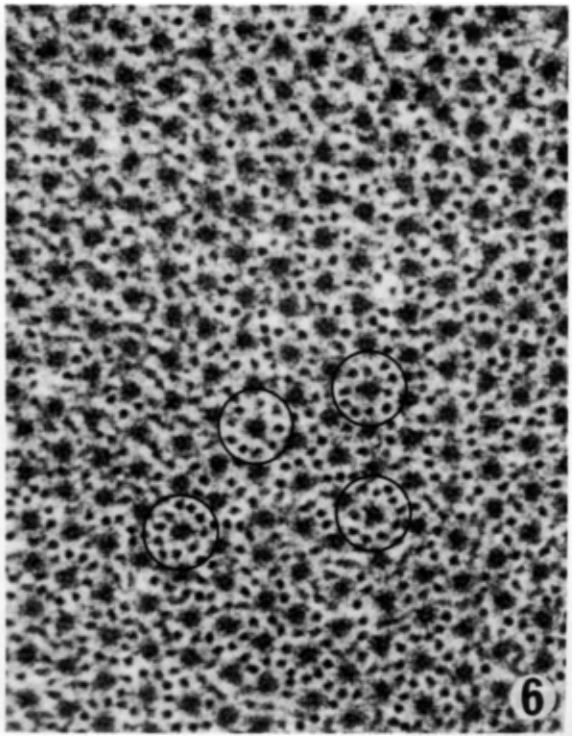




Ring fibres

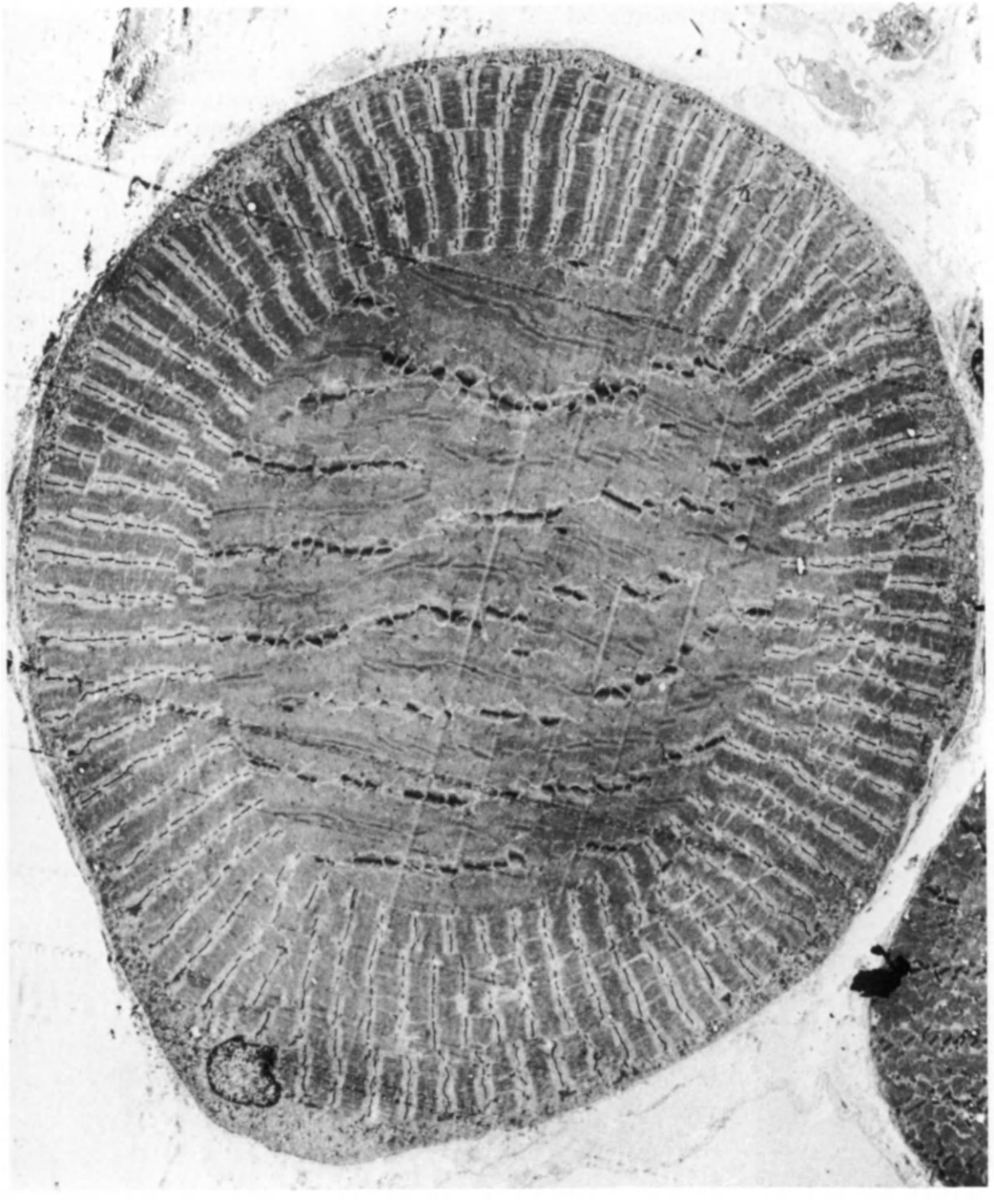
The term 'ring fibre' (striated annuli, ringbinden) designates a muscle fibre with an aberrant set of circumferentially placed myofibrils which follow a spiral or circular course, forming a collar around the usual central longitudinal core of myofibrils. Thus a cross-section through the ring fibre shows longitudinally sectioned myofibrils encircling transversely or obliquely cut myofibrils in the centre (*Plate 358*). In longitudinal sections through a ring fibre the collar of encircling myofibrils is cut transversely or obliquely while the main central mass of myofilaments is cut longitudinally. No membrane separates the two sets of myofibrils. The long axes of the muscle nuclei are often parallel to the aberrant collar of myofibrils rather than to the central, longitudinally orientated myofibrils.

Ring fibres have been noted in: (1) amphibian muscle (Bataillon, 1891; Weiss and James, 1955; Jonecko, 1962); (2) muscle from amputation stumps (Apatenko, 1964); (3) apparently normal muscle, especially from the external ocular muscle of elderly subjects (Rubinstein, 1960; Bethlem and van Wijngaarden, 1963); (4) muscle from various conditions such as myasthenia gravis, dermatomyositis, periarteritis nodosa, scleroderma, sarcoidosis and disorders affecting the lower motor neuron (for references, *see* Schotland *et al.*, 1966); and (5) muscular dystrophies, particularly myotonic dystrophy (Engel, 1962; Lapresle *et al.*, 1964, 1966; Schotland *et al.*, 1966; Lapresle and Fardeau, 1968; Schroder and Adams, 1968; Hayward and Mair, 1970). Ring fibres have also been noted in the myocardium (Le Menn and Emeriau, 1970) in cases of obstructive cardiomyopathy, being particularly frequent in a case of aortic stenosis.

The function and significance of ring fibres are not well understood. The suggestion that they might be a fixation artefact (Adams *et al.*, 1962) is not tenable in the light of experimental studies on this subject. Ring fibres can be produced experimentally by interrupting the continuity of muscle between its origin and insertion by cutting either the muscle fibres or its tendon (Rumyantseva, 1957; Morris, 1959; Bethlem and van Wijngaarden, 1963). In such instances the number of ring fibres increases with the passage of time. There is evidence that in man their number increases with advancing age. These findings taken in conjunction with their frequent occurrence in diseased human muscle have led Schotland *et al.* (1966) to suggest that 'ring fibres are reactive structures developing in response to pathologic alterations within the muscle fibre'.

Plate 358

Cross-section through a ring fibre showing a band of longitudinally sectioned myofibrils encircling obliquely cut myofibrils in the centre. From a case of Duchenne muscular dystrophy. × 2100 (*From Oteruelo, 1972*)



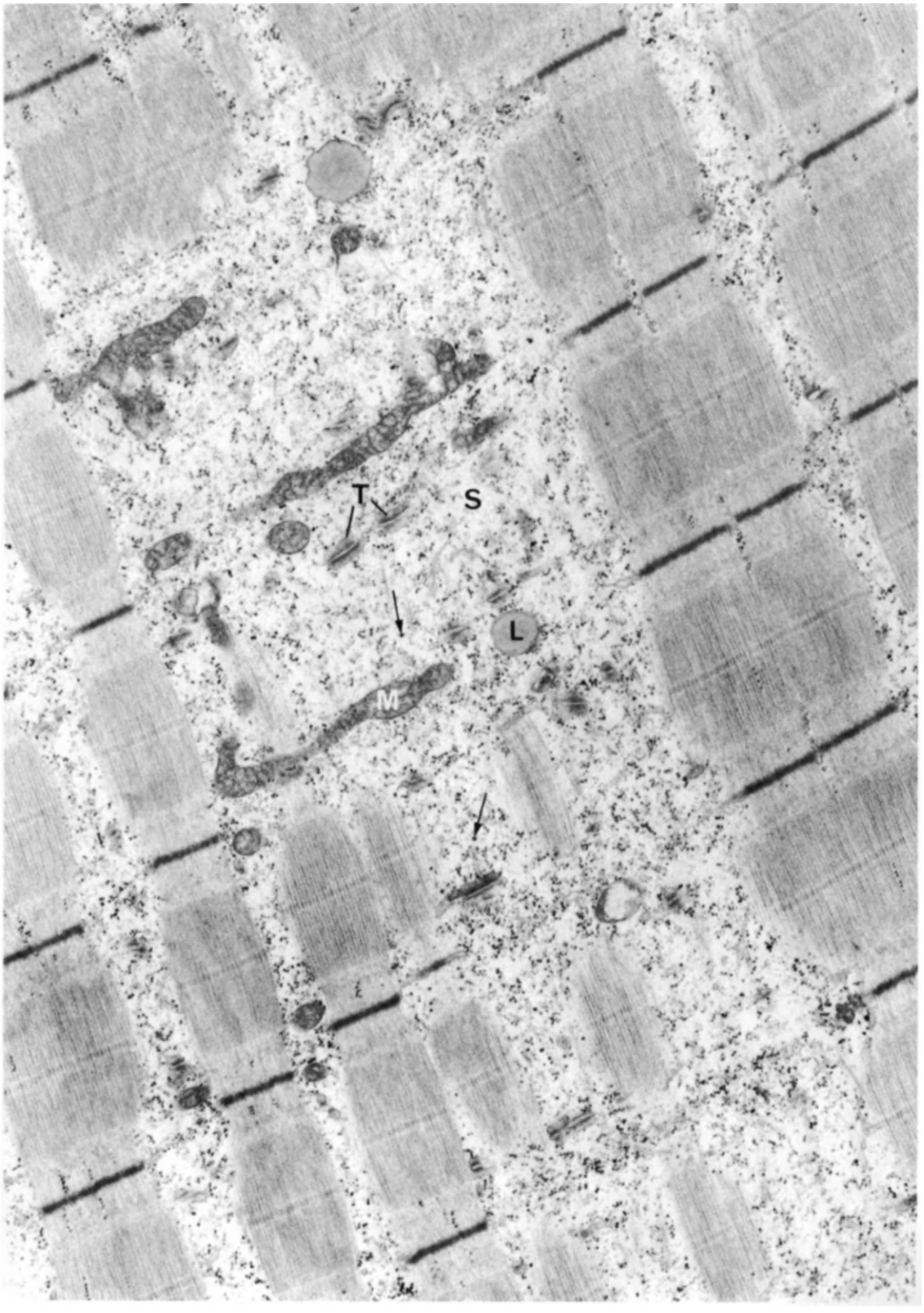
Myofibrillary degeneration

The term 'myofibrillary degeneration' is used to describe changes affecting myofilaments and Z-lines in myofibres undergoing degeneration and atrophy from various causes. Degeneration and atrophy of myofibres has been observed in a variety of pathological states affecting muscle. These include denervation, deprivation of blood supply, metabolic insufficiency, cachexia, mechanical compression, drug action and many other diseases, including muscular dystrophy and myositis (Stenger *et al.*, 1962; Pellegrino and Franzini, 1963; Gonatas *et al.*, 1965; Zacks, 1970; Mair and Tomé, 1972; Dastur *et al.*, 1979).

The degenerating myofibres and myofibrils show a variety of ultrastructural alterations but none of these is specific or pathognomonic for any particular muscular disease or disorder. Depending upon the severity and stage reached by the degenerative process, various morphological changes are seen. Thus, in early or mild lesions there is a decrease in the width of the myofibrils due to a loss of peripheral myofilaments but the normal sarcomere structure is preserved. More marked changes include the destruction and loss of myofibrils over one or more sarcomere lengths (*Plate 359*), and various changes in the Z-line (which are discussed on page 850). As a consequence of such a loss of myofibrils, there is a relative abundance of intervening sarcoplasm, and various structures such as mitochondria, triads and glycogen particles, which persist for a time, appear more prominent. The loss of myofibrils leads to a shrinkage of the myofibre so that its surface becomes scalloped or markedly irregular. Dissociation of the folded cell membrane from the external lamina is also sometimes seen. The undulating configuration of the surface of the fibre produces sarcoplasmic projections or masses called sarcoplasmic pads. These contain occasional nuclei, many mitochondria, glycogen, lipid and disorientated disintegrating filaments. When a substantial loss of myofilaments has occurred the size and shape of the myofibre are grossly altered. Progressive changes along this line lead ultimately to necrosis of the muscle fibre.

Plate 359

The loss of myofilaments from the periphery of myofibrils and the disappearance of entire sarcomeres are evident in this degenerating myofibre from an atrophic deltoid muscle of man. Monoparticulate glycogen (arrows), lipid (L), triads (T) and transversely orientated mitochondria (M) (instead of the normal longitudinally disposed ones) are seen in the sarcoplasm (S) from which the myofilaments have disappeared. × 20 000 (*Ghadially and Ailsby, unpublished electron micrograph*)


T
S
L
M

Morphological alterations of the Z-line

The Z-line in normal skeletal muscle varies between 50–100 nm in thickness. It is wider in red than in white fibres (Shafiq *et al.*, 1966; Tice and Engel, 1967) and in glutaraldehyde-fixed material compared with osmium-fixed material (Engel and MacDonald, 1970).

The structure of the Z-line has been studied by many workers (for references, *see* Landon, 1970), most of whom have found that in osmium-fixed material transverse sections through this structure present the appearance of a square lattice composed of 5 nm filaments with a spacing of 22 nm. The similarity between the crystalline lattice of the Z-line and tropomyosin B crystals (Fawcett, 1968) led to the suggestion that tropomyosin probably occurs in the Z-line and not just in the I-band as suggested by immunofluorescence studies. However, current belief is that tropomyosin does not occur in the Z-line but that it contains vinculin, α-actinin and zeugmatin (Maher *et al.*, 1985; Darnell *et al.*, 1986).

Alterations in Z-line morphology have been seen in various diseases of muscle and also at times in normal or apparently normal muscle. One such change is streaming of the Z-line (*Plate 360*). In such instances the Z-line develops a zig-zag appearance and the Z-band material extends into the I- and A-bands. Such a change may affect one or more sarcomeres in a single myofibril or affect many adjacent myofibrils. A more advanced stage of streaming leads to a disintegration of the Z-lines which then present as dispersed dense masses of Z-line material.

Light microscopists are familiar with the appearances called 'target' and 'targetoid' fibres, seen in diseased muscle (*see below*). Here, sarcomeres of several adjacent myofibrils are replaced by patchy electron-dense material, presumably derived from disintegrating Z-lines and myofibrils. It is thought that this change is also probably related to Z-line streaming and disintegration (Mair and Tomé, 1972). The target fibre derives its name from the light microscopic appearance of such fibres seen in sectioned material. Three concentric zones are seen: (1) a central pale-staining zone lacking striations, but showing fine wavy filaments; (2) an intermediate zone which stains faintly but in which cross-striations can be detected by polarized light; and (3) a peripheral zone which is more or less normal looking but may show histochemical abnormalities. In targetoid fibres the intermediate zone is lacking. Engel (1961) reported the occurrence of target fibres in denervated muscle and thought that this was a sign of denervation. However, since then, such fibres have been found in a variety of other pathological states by numerous workers (Shafiq *et al.*, 1967; Schotland, 1969; Tomonaga and Sluga, 1969, 1970; Mair and Tomé, 1972).

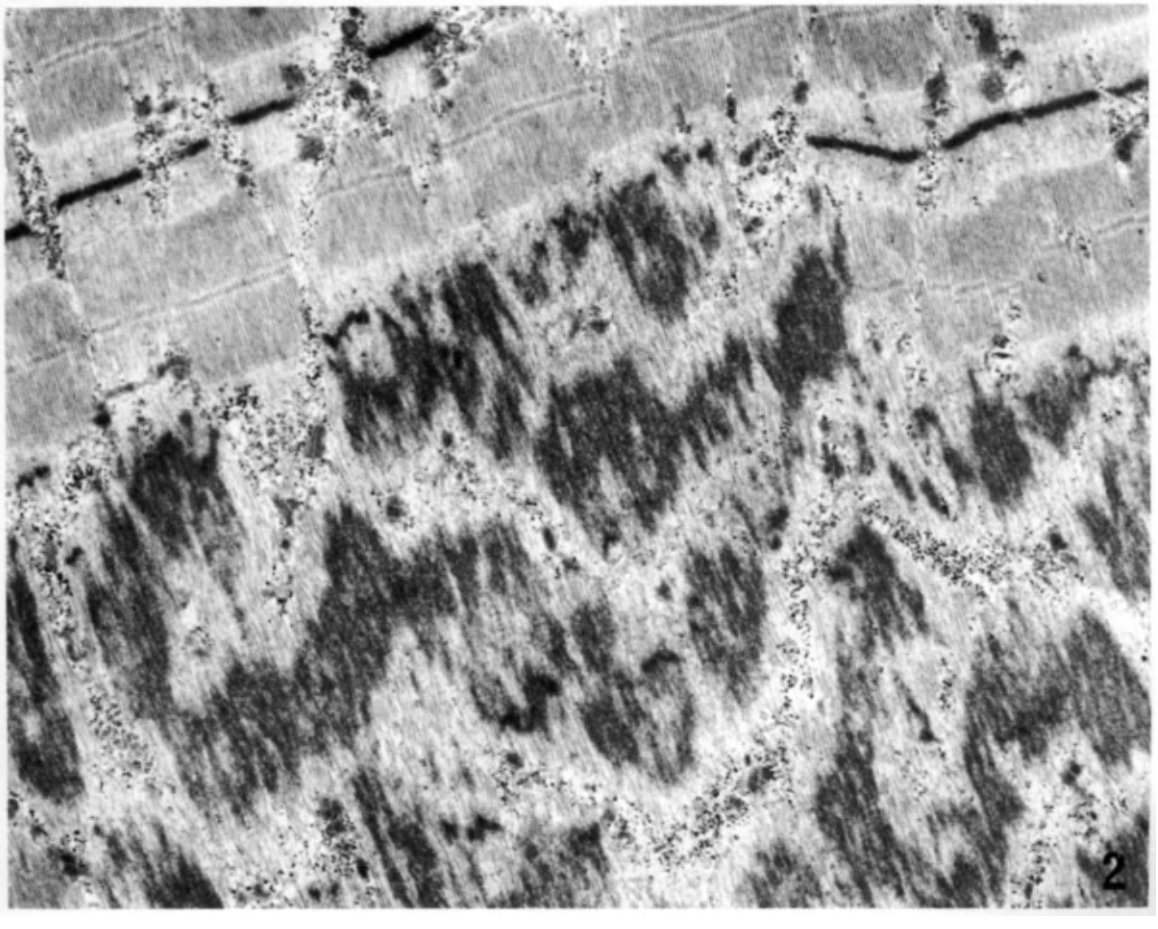
Plate 360

From the same specimen as *Plate 359*. (*Ghadially and Ailsby, unpublished electron micrographs*)

Fig. 1. Streaming of three Z-lines (arrows) of adjacent sarcomeres is seen here. × 25 000

Fig. 2. Several adjacent myofibrils, showing a more advanced streaming leading to a disintegration of the Z-lines and of the sarcomeres. × 16 500


1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However, the most interesting change affecting the Z-line is the formation of dense rods (*Plate 361, Fig. 1* and *Plate 364*). Such rods may be solitary or multiple and small or large (up to 5 μm long and 1 μm in diameter). These structures were first described by Shy *et al.* (1963) in a familial myopathy characterized by non-progressive weakness. In histological preparations, rod-like or thread-like structures were found; hence they named the condition 'nemaline myopathy' (Gr. *nema* = thread). The origin of these rods from the Z-line was noted by Price *et al.* (1965) and it was suggested that they might contain tropomyosin B. Since then, however, rod formation has been reported in a variety of other myopathies and muscle diseases, including denervation and polymyositis (for references, *see* Mair and Tomé, 1972), and in the appparently normal heart of aged cats and dogs (Fawcett, 1968; Munnell and Getty, 1968).

According to Engel and MacDonald (1970), the rods are characterized by their origin from Z-lines and their continuity with the I-filaments. They also display a periodic pattern of lines parallel and perpendicular to the long axis of the rod. In fact, the ultrastructure of the rods and their lattice pattern are identical to that of Z-lines with which they are sometimes continuous (Mair and Tomé, 1972).

Yet another anomaly of the Z-lines seen in diseased muscle is a structure called the cytoplasmic body (MacDonald and Engel, 1969). It has a density and granularity similar to the Z-line and is hence believed to be derived from it. However, unlike the rods, it does not show a periodic structure, for it is composed of randomly orientated filaments; those in the centre are compacted and appear electron dense, while those at the periphery form a lighter halo.

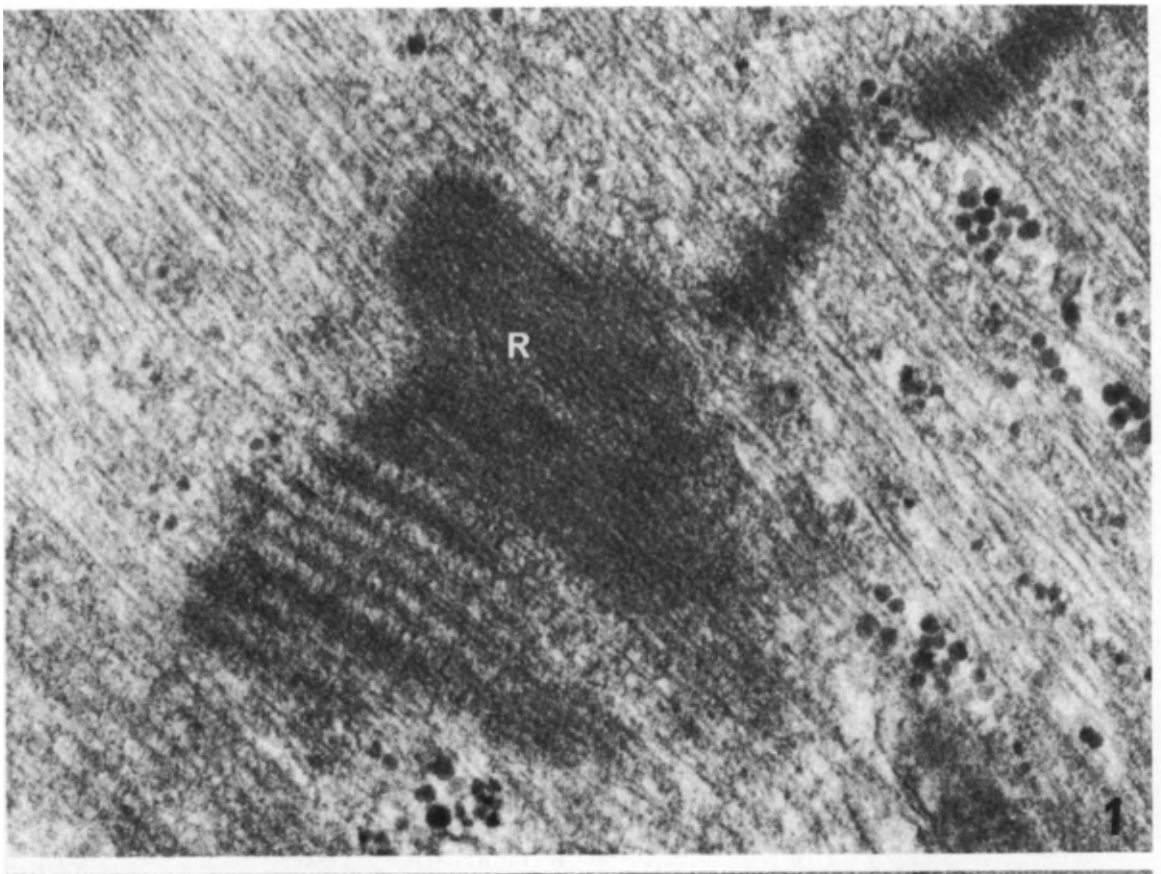
Various other anomalies of the Z-lines have also been described. These include fragmentation and disappearance, widening and doubling of the Z-lines (*Plate 361, Fig. 2*). Doubling of Z-lines has been reported in diseased muscle but is also seen in normal muscle fibres at the myotendinous junction and in extra-ocular muscles (Grabow and Chou, 1968; Engel and MacDonald, 1970; Mair and Tomé, 1972).

Of the numerous alterations of Z-line morphology, three have received more study than others. These include Z-line streaming, rod formation and cytoplasmic bodies. It is clear that these are related anomalies of structure which, in their earlier stages of formation, resemble each other so closely as to be indistinguishable (Engel and MacDonald, 1970). Furthermore, it is clear that while there might be a tendency for one or other variety of Z-line anomaly to be prominent in a given muscular disorder, it is also evident that on the whole such changes are common to a variety of different pathological states affecting muscle.

Plate 361

Fig. 1. The rod (R) in this illustration is clearly seen to be derived from a Z-line. Continuity between the thin filaments of the I-band and the filaments in the rod is evident, as is the periodic parallel arrangement of the filaments in the rod. Below and to the left of the rod is another, less compact, rod-like formation. From human atrophic deltoid muscle; same case as *Plate 360*. × 70 000 (*Ghadially and Ailsby, unpublished electron micrograph*)

Fig. 2. Doubling of Z-lines in a dystrophic human muscle. × 35 000 (*Ghadially and Ailsby, unpublished electron micrograph*)


R
1


2

Myofilaments in rhabdomyoma and rhabdomyosarcoma

Myofibrils composed of thick and thin filaments are a highly characteristic feature of striated muscle and its tumours (*Plates 362–364*). The rare, benign rhabdomyoma usually poses no diagnostic problems, nor does it undergo malignant transformation. Ultrastructurally, the rhabdomyoma contains cells with rudimentary sarcomeres which show most or all the bands found in normal skeletal muscle. The Z-lines are hypertrophied, forming structures that resemble the rods seen in nemaline myopathy and other conditions (page 852). Quite large glycogen deposits are seen in some cells and occasional cells contain mitochondria with crystalline inclusions (Cornog and Gonatas, 1967; Tandler *et al.*, 1970).

With the light microscope one cannot visualize myofilaments or distinguish thick and thin filaments, but one can do this with the electron microscope. Hence, it follows that with the electron microscope one can at times unequivocally establish the diagnosis of rhabdomyosarcoma where the diagnosis was in doubt by light microscopy. Similarly, there are occasions where the diagnosis of rhabdomyosarcoma can be quite effectively refuted by electron microscopy, but experience shows that in a substantial number of cases (probably one-third to one-half) the electron microscope also fails to produce an unequivocal answer. This is because the tumour is just not differentiated enough or the rare well-differentiated cell with thick and thin filaments does not happen to be included in the minute amount of material that can be examined with the electron microscope.

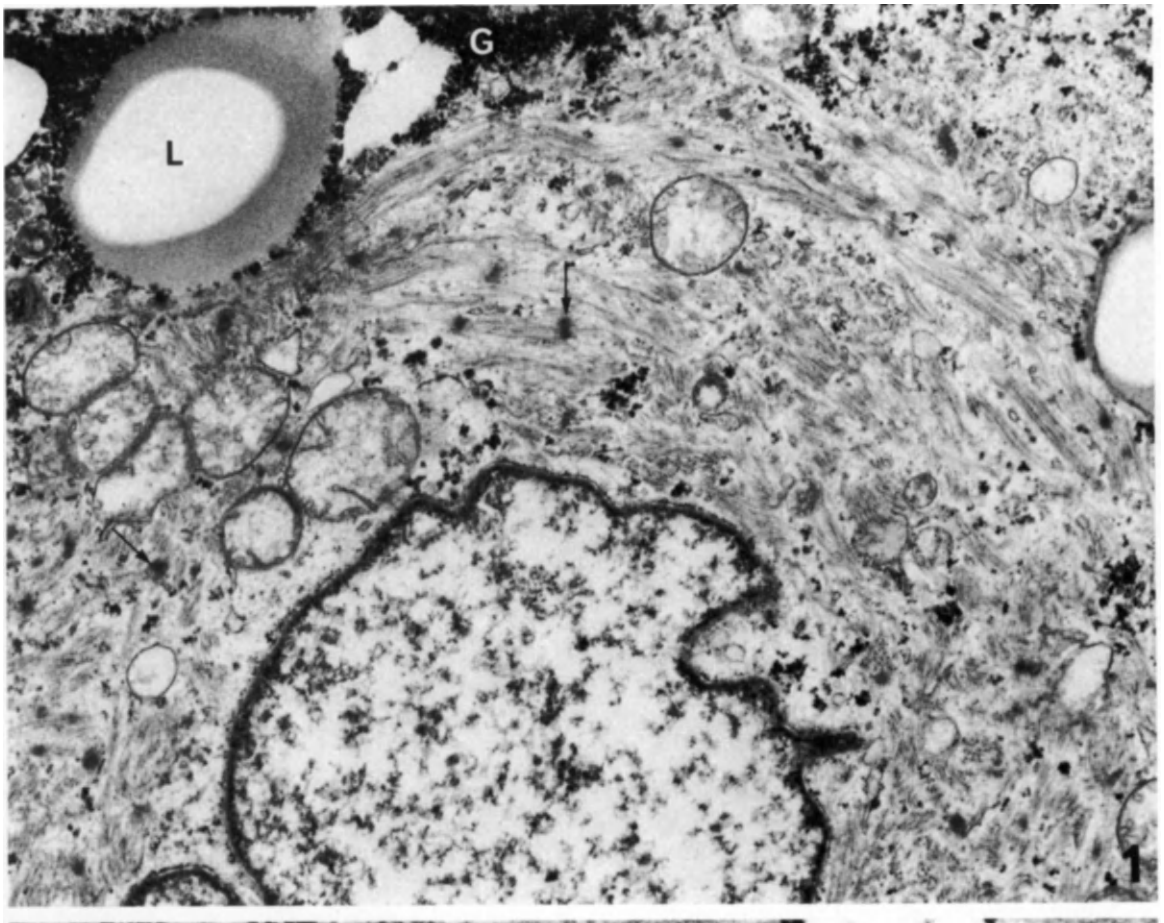
These difficulties and the conceptual dilemma as to what is the minimum requirement for labelling a tumour a rhabdomyosarcoma is reflected in the numerous papers on the ultrastructure of rhabdomyosarcoma. Some authors have been quite happy to make the diagnosis of rhabdomyosarcoma on the basis of filaments alone or filaments with densities which could be construed as altered Z-lines but could also equally well be focal densities of the type seen in smooth muscle; however, others have insisted (quite rightly) on much stricter criteria. A detailed list of references to this literature is not necessary because two excellent papers giving many references are available. The first is the paper by Morales *et al.* (1972) who set down clearly the diagnostic criteria for rhabdomyosarcoma and the second is the paper by Bundtzen and Norback (1982) who review 56 reports on the ultrastructure of rhabdomyosarcoma in humans. They tabulate the ultrastructural features and give a logical and lucid analysis of diagnostic problems. Morales *et al.* (1972) describe the ultrastructure of '15 histologically malignant tumours in which a light microscope diagnosis of rhabdomyosarcoma had been rendered previously or was strongly suspected' and they also review past published cases in a most thorough and critical fashion, which is in accord with my own thinking about this matter.

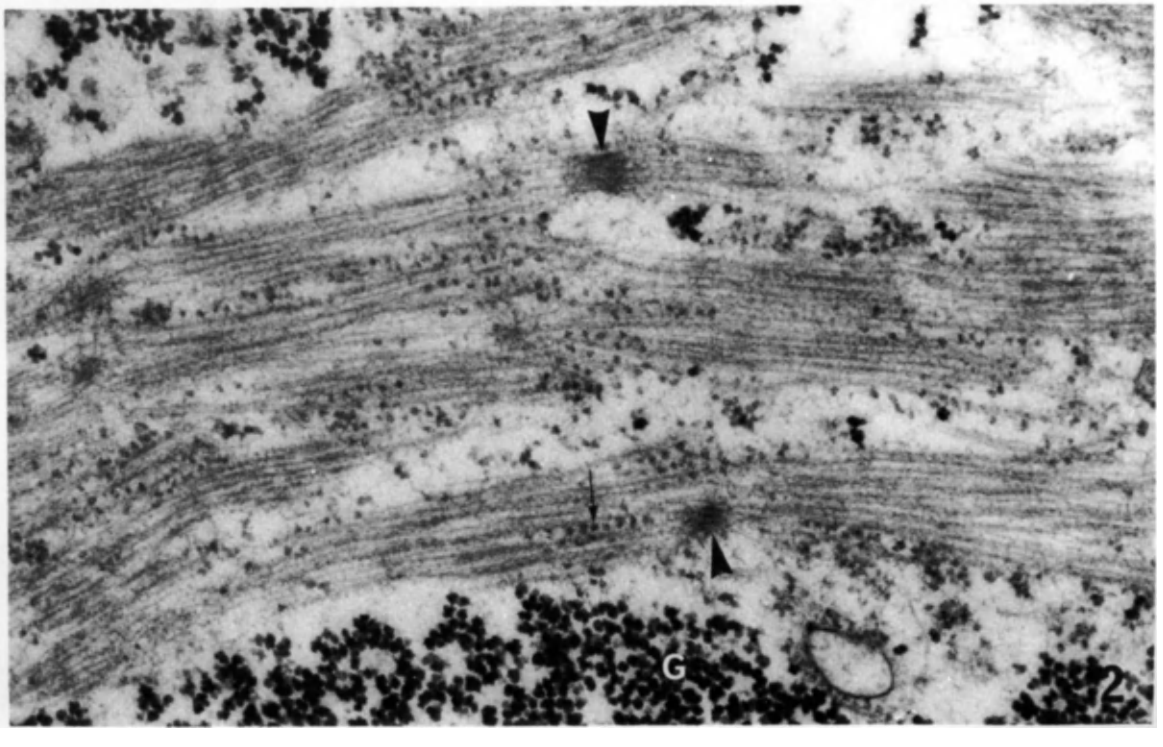
They divide their tumours into two groups: Group A (six cases) where thin (6–8 nm) and thick (12–15 nm) filaments were present and at times I-bands and Z-lines were also seen. Group B (nine cases) lacked these features, but thin filaments (6–8 nm in diameter) were found distributed irregularly in the cytoplasm or arranged in bundles along the long axis of the cell.

Plate 362

Fig. 1. Rhabdomyosarcoma of the orbit. Neoplastic cell showing fibrils with attenuated Z-lines (arrows), glycogen (G) and lipid (L). × 16 500

Fig. 2. Rhabdomyosarcoma of the orbit (different case from that shown in *Fig. 1*). Tumour cell containing straight fibrils composed of thick and thin filaments. Note also the attenuated Z-lines (arrowheads) and ribosomes (arrow) in Indian file arrangement. The larger, more electron-dense glycogen particles (G) are easily distinguished from the ribosomes. × 58 000 (*From Ghadially, 1980*) (*From a block of tissue supplied by Dr A. H. Cameron*)


G
L
1


G
2

On this basis they considered the diagnosis of rhabdomyosarcoma as firmly established in the six cases in Group A but not in the nine cases in Group B. They accept the possibility that some of the tumours in Group B where only thin filaments were seen could have been rhabdomyosarcomas. In three of the tumours in Group B ultrastructural study clearly demonstrated that they were not rhabdomyosarcomas (two leiomyosarcomas, one osteogenic sarcoma).

Plate 362, Fig. 2 shows a cell from an embryonal rhabdomyosarcoma of the orbit. The appearance seen here is diagnostic of rhabdomyosarcoma, because slender fibrils composed of quite straight, thick and thin filaments are clearly evident. A bonus here is that there are also some densities which are acceptable as attenuated Z-lines, but this would not be absolutely necessary for diagnosing rhabdomyosarcoma. Also reassuring is the finding of ribosomes arranged in what is known as an 'Indian file' along some of the myofilaments, for such an appearance is thought to indicate that synthesis of new filaments is occurring. It is worth noting that this appearance has been seen in developing or regenerating striated muscle and rhabdomyomas and rhabdomyosarcomas.

Thick and thin filaments can be readily identified in transverse sections through the A-band (the I-band contains thin filaments only) or normal muscle. Each myosin filament is surrounded by six actin filaments occupying the trigonal position—that is, equidistant from and shared by three myosin filaments. In rhabdomyosarcoma such a precise geometric arrangement can hardly be expected to occur, nor is the orientation of filaments likely to be so perfect that all of them will be transversely cut and, hence, well visualized. However, careful search does often reveal thick and thin filaments (*Plate 363, Fig. 1*) mimicking the appearance seen in transverse sections of normal striated muscle. And this, too, is an appearance which can be confidently accepted as diagnostic of rhabdomyosarcoma.

Thus, on the basis of the appearances noted above one could assert that this is a rhabdomyosarcoma, but at this stage one should pause to consider whether one is indeed looking at: (1) a neoplastic rhabdomyoblast containing myofilaments in a rhabdomyosarcoma; or (2) an 'accidently included' degenerating muscle fibre* in an infiltrating tumour; or (3) some quite different tumour in which rhabdomyoblasts occur.

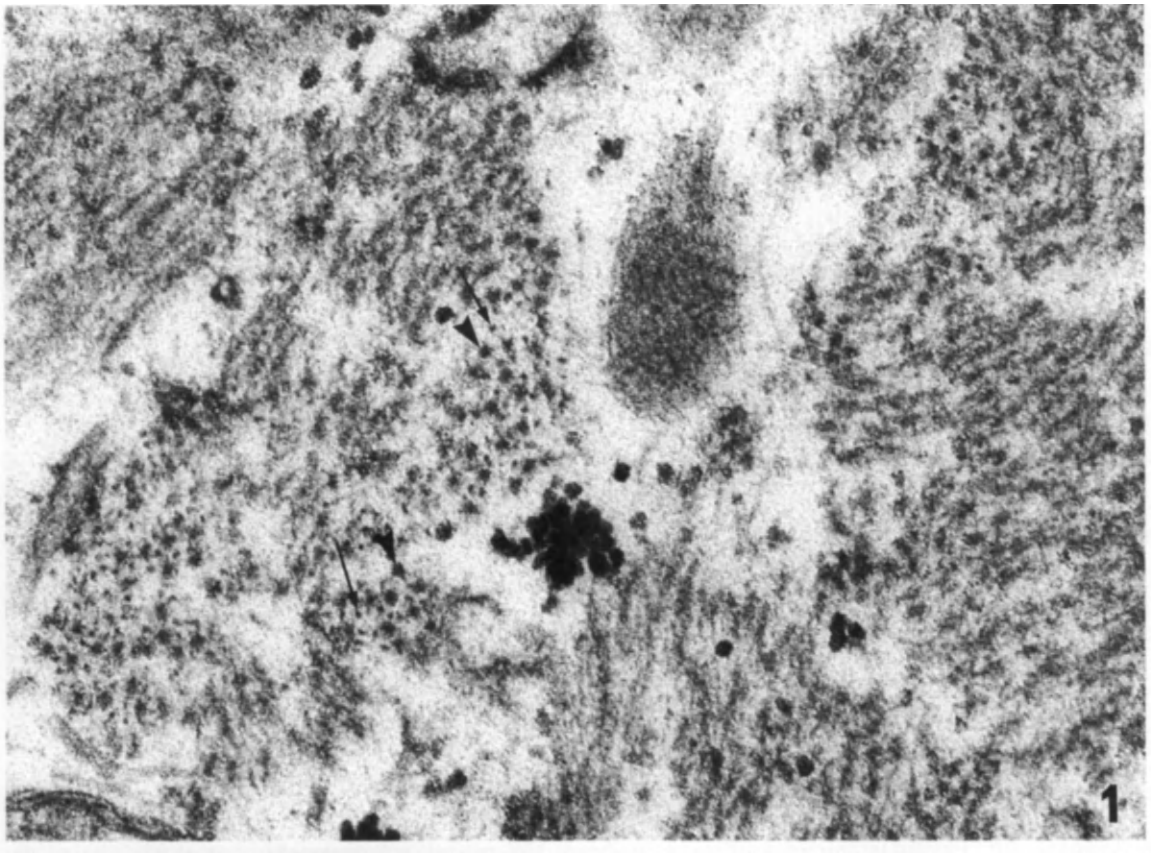
The problem of 'accidently included' muscle fibres arises when the biopsy is taken from a tumour in or adjacent to a muscle mass. Examples of degenerating muscle fibres may be found in any tumour (usually in the peripheral parts of the tumour) which infiltrates and destroys muscular tissues. Such degenerating or necrotic muscle fibres do not contain the kind of nuclei one sees in malignant tumour cells, they contain normal-looking nuclei, or nuclei showing marked chromatin margination, pyknosis, karyolysis or karyorrhexis. The myofibrils may or may not be well preserved but when adequately preserved they often show a degree of 'perfection' or 'order' one associates with the normal and not the neoplastic state (a 'too good to be true' appearance). Attention to such details often but not invariably helps one to decide whether one is looking at an included muscle fibre or a rhabdomyoblast in a rhabdomyosarcoma.

*An excellent illustration of this is presented by Penney *et al.* (1978) who show (their *Figure 14*) a muscle fibre 'decomposed or degraded by one or more tumour cells' in a squamous cell carcinoma of the oral cavity.

Plate 363

Fig. 1. Rhabdomyosarcoma (same case as *Plate 362, Fig. 2*). Note obliquely and transversely cut thick (arrowheads) and thin (arrows) filaments. Compared to normal striated muscle, there are fewer thin filaments and an orderly arrangement is lacking. × 116 000 (*From Ghadially, 1980*) (*From a block of tissue supplied by Dr A. H. Cameron*)

Fig. 2. Rhabdomyosarcoma (same case as *Plate 362, Fig. 1*). A damaged or degenerating cell showing a tangled mass of filaments and entrapped glycogen. × 18 000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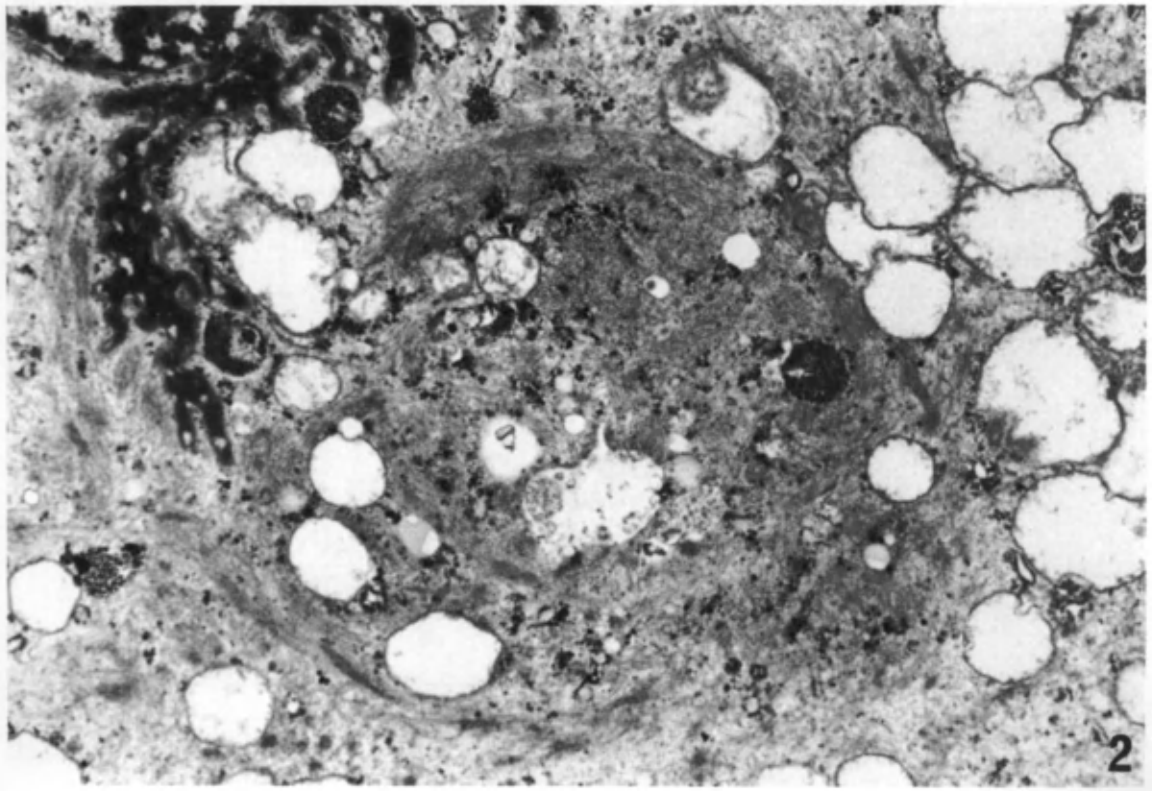
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It is of course well known that several tumours besides rhabdomyosarcomas contain neoplastic rhabdomyoblasts. These include tumours such as: (1) malignant mixed mesenchymal tumour of uterus (Stembridge *et al.*, 1964); (2) some tumours of thymus (Henry, 1972); (3) some medulloblastomas (called 'medullomyoblastoma' by some and regarded as a teratoma by others) (Misugi and Liss, 1970; Smith and Davidson, 1984); (4) Wilm's tumour which may at times contain a few or numerous rhabdomyoblasts (Tremblay, 1971; Sens *et al.*, 1984); and (5) hepatoblastoma.

I have seen a hepatoblastoma which contained many rhabdomyoblasts and was hence mistaken for a rhabdomyosarcoma. Clinically, the tumour was thought to be a neuroblastoma. The tentative histological diagnosis made on a small piece removed for frozen section was: 'portion of a rhabdomyosarcoma—upper abdomen'. This diagnosis was supported by electron microscopy. However, when the tumour was removed (right hemi-hepatectomy) histological studies showed that this was a hepatoblastoma of mixed epithelial and mesenchymal type and that the rhabdomyomatous part was but a modest portion of this tumour. This shows how sampling problems can lead to an erroneous diagnosis.

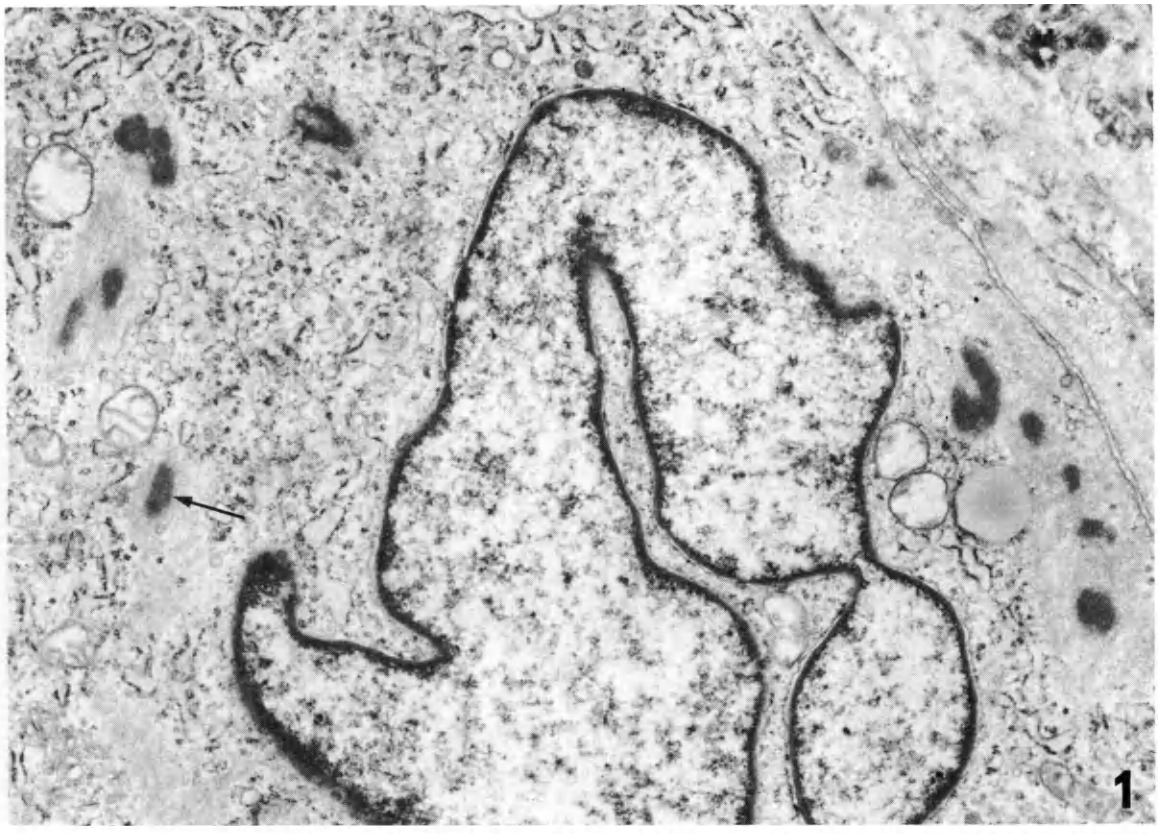
It will be apparent from the above that only when the problem of: (1) included muscle fibres; and (2) the occurrence of rhabdomyoblasts in other varieties of tumours have been excluded, can one confidently diagnose rhabdomyosarcoma on the basis of appearances such as those shown in *Plates 362* and *363, Fig. 1*. Besides these truly diagnostic appearances mentioned above, there are some other features which although of limited value on their own, constitute good supportive evidence that the tumour is likely to be a rhabdomyosarcoma. These features include: (1) collections of filaments and small or large dense structures which may be of irregular or rod-like shape (*Plate 364*) (similar to those seen in various myopathies, particularly nemaline myopathy. These are thought to be derived from Z-line material); and (2) masses of tangled filaments reminiscent of those found in damaged muscle (*Plate 363, Fig. 2*).

Various other features found in rhabdomyosarcoma worth a passing mention include: (1) markedly irregular nuclei (although at times the nuclei are remarkably smooth and oval), prominent nucleoli and an abundance of polyribosomes lying free in the cytoplasm as expected in a malignant tumour; (2) modest amounts of rough endoplasmic reticulum; (3) pleomorphic, often swollen mitochondria; (4) a fair to quite a substantial amount of glycogen in the cytoplasm (usually the monoparticulate form, but a few rosettes are sometimes seen) and rarely also in the nucleus; (5) a few lipid droplets; (6) interrupted external lamina or external lamina-like material; (7) infrequent sightings of subplasmalemmal densities and paired subplasmalemmal densities (i.e. desmosome-like structures); and (8) very rare sightings of annulate lamellae (*see Plate 248* in Chapter 6).

Plate 364

Rhabdomyosarcoma (same case as *Plate 362, Fig. 1*)

Figs. 1 and 2. The filaments in these tumour cells are not well orientated to form characteristic fibrils, but rod-like (*Fig. 1*) and irregular masses (*Fig. 2*) of Z-line material (arrows) are evident. × 16 500; × 49 000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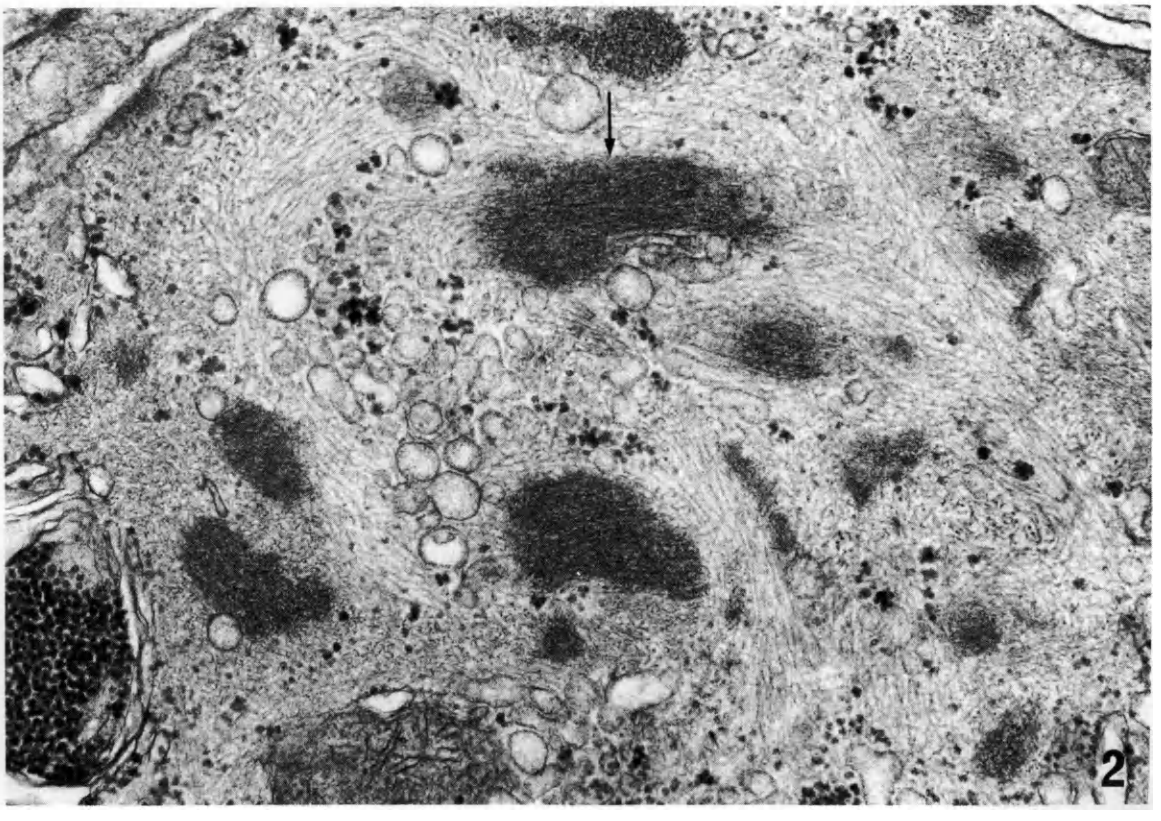
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Myofilaments in smooth muscle

In routinely prepared tissues fixed in osmium or glutaraldehyde the cytoplasm of smooth muscle cells has a somewhat dense homogeneous appearance because the very fine filaments present here are difficult to resolve except at quite high magnifications. Nevertheless, these myofilaments are morphologically distinguishable from many other intracytoplasmic filaments (e.g. tonofilaments in keratinocytes and intermediate (vimentin) filaments in chondrocytes) because they show an orderly parallel arrangement and focal densities along their course (*Plate 365*). In addition to the presence of such filaments, the typical smooth muscle cell as found in the wall of the gut or uterus is characterized by its spindle shape, a nucleus which is folded when the muscle is contracted, a relative scarcity of endoplasmic reticulum and Golgi complex and an abundance of micropinocytotic vesicles which are frequently referred to in this instance as plasmalemmal vesicles.

At one time it was thought that smooth muscle contained* only thin actin filaments, or at the most only a few thick myosin filaments. The arrangement of filaments in vertebrate smooth muscle has been the subject of intense study and the existence or non-existence of organized myosin filaments has been debated extensively.

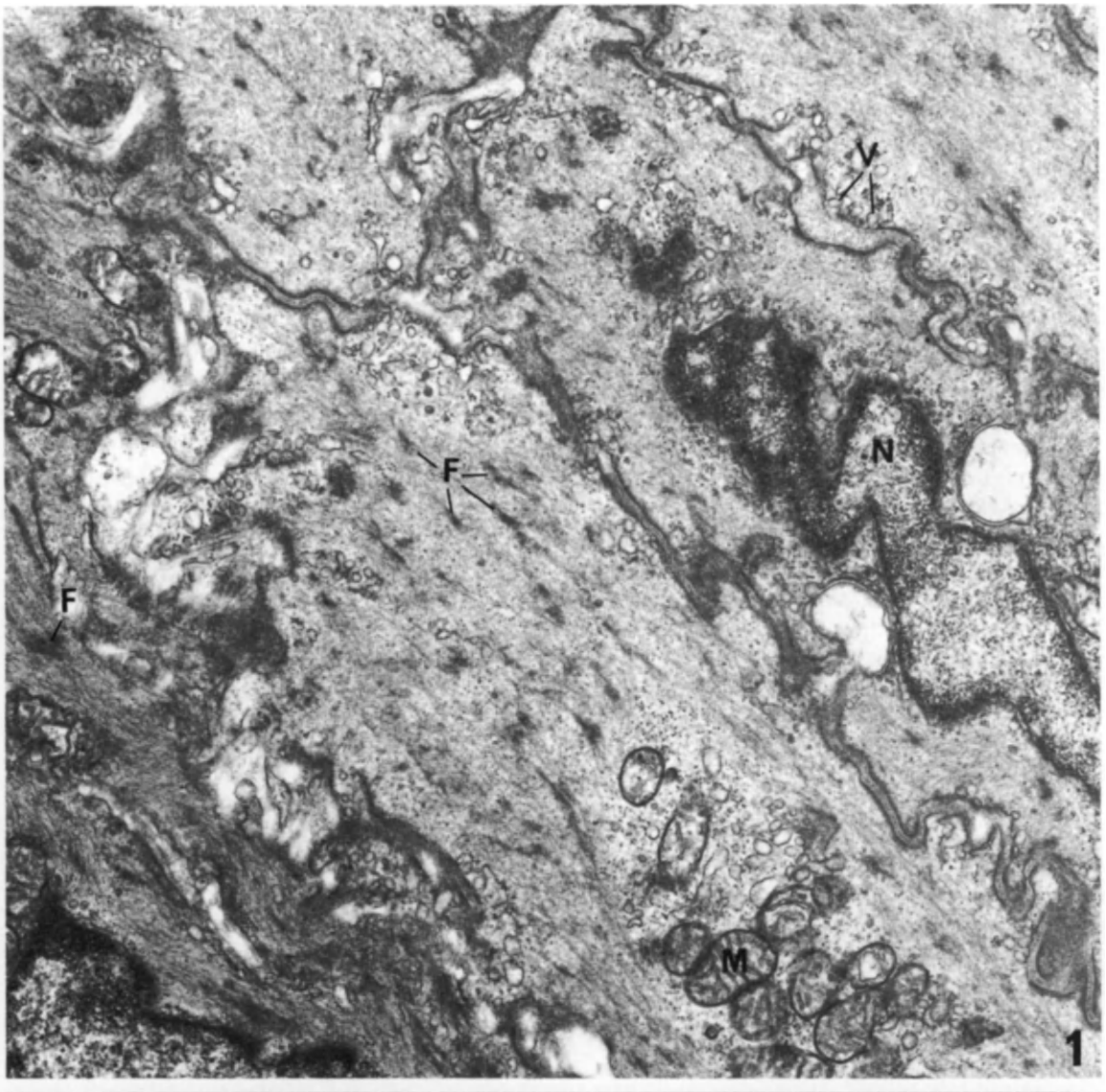
Early studies revealed two sizes of filaments in some invertebrate smooth muscle but not in vertebrate smooth muscle (Hanson and Lowy, 1960, 1964a, b). Similarly early x-ray diffraction studies also failed to detect myosin filaments (Elliot, 1964, 1967). Later studies, however, have shown reflections attributable to myosin filaments in the taenia coli of the guinea-pig (Lowy *et al.*, 1970), and various authors have now also demonstrated the presence of thick filaments in preparations containing isolated filaments and in ultrathin sections of vertebrate smooth muscle (for references, *see* Newstead, 1971; Garamvolgyi *et al.*, 1971). In some instances quite an impressive number of thick filaments surrounded by thin filaments have been demonstrated. The difficulty of demonstrating thick filaments in smooth muscle led Kelly and Rice (1968) to suggest that thick filaments were formed when the muscle contracted, but others contended that this might be due to poor preservation of smooth muscle by conventional methods of tissue fixation. Recent studies tend to support the latter concept.

*Intermediate filaments containing desmin are also found in smooth muscle. This is dealt with on page 890.

Plate 365

Fig. 1. Smooth muscle cells from the wall of human small intestine, showing myofilaments with focal densities (F) along their course, micropinocytotic vesicles (V), folded nucleus (N) and numerous mitochondria (M), but the Golgi complex and endoplasmic reticulum are not clearly evident. × 21 000

Fig. 2. Muscularis mucosae from the same specimen as *Fig. 1.* Note the abundance of micropinocytotic vesicles (arrows). × 28 000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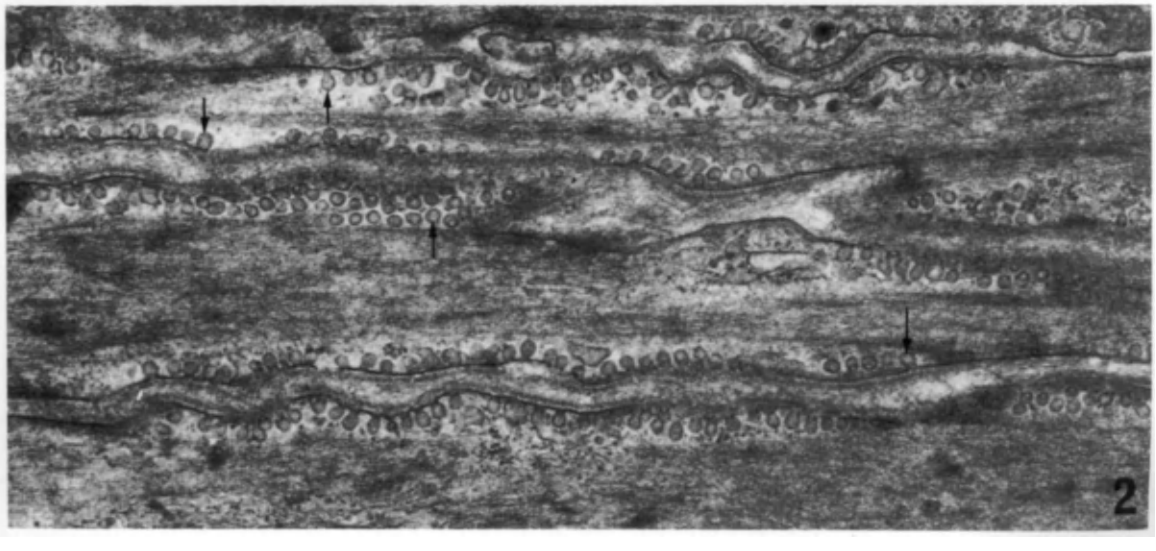
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Myofilaments in leiomyoma and leiomyosarcoma

There are occasions when it is difficult to distinguish leiomyosarcoma from other tumours with the light microscope. In such instances electron microscopic examination of tumour tissue may help to establish the diagnosis. Conversely, it can at times effectively refute the diagnosis of leiomyosarcoma made with the light microscope.

The unequivocal establishment of the histogenesis of a neoplasm with the electron microscope often rests upon the demonstration of some specific organelle or feature which occurs in a particular cell line and its tumour but in no other. Such examples include beta cell granules in insulinoma and the characteristic myofibrils composed of thick and thin filaments which are found in rhabdomyosarcomas (pages 854–859).

There is, alas, no such single specific trait which distinguishes the smooth muscle cell from all others*. However, this cell can usually be distinguished by a combination of features, which include: (1) a nucleus which is often folded (concertina fashion) or notched or shows many invaginations; (2) a cytoplasm which is packed with thin actin filaments with focal densities† along their course; (3) a few mitochondria and sparse elements of endoplasmic reticulum and Golgi complex situated adjacent to the poles of the nucleus; (4) abundant micropinocytotic vesicles; (5) subplasmalemmal densities into which some of the myofilaments appear to be anchored: and (6) a thin but usually distinct external lamina‡.

The cells of leiomyomas bear a close resemblance to normal smooth muscle cells (Meyer *et al.*, 1968; Welsh and Meyer, 1969; Ferenczy *et al.*, 1971). Virtually all the features of normal smooth muscle, such as folded nucleus, filaments with densities, subplasmalemmal densities and an external lamina, can be detected in these tumours (*Plate 366*). However, some slight variations may also be noted, the most common ones being a relative paucity of micropinocytotic vesicles and an external lamina that is not quite so well developed and continuous as in the normal state.

*In this connection it is worth noting that filaments with focal densities are found in myoepithelial cells (*Plate 370*) and myofibroblasts (*Plate 373*). Such cells occur in tumours, and tumours of such cells are also known to occur. Because of this, and other considerations (for details *see* Ghadially, 1980) the mere finding of cells containing filaments and focal densities does not on its own establish the diagnosis of leiomyosarcoma.

†In smooth muscle the spindle-shaped or oval densities lie parallel to the filaments but the Z-lines of striated muscle lie at right angles to the filaments. Unfortunately, this distinction is at times not maintained in tumours. Thus, for example in slender fibrils of rhabdomyosarcoma, the Z-line may form a square or oval mass, while in smooth muscle tumours regimentation of focal densities may mimic a Z-line.

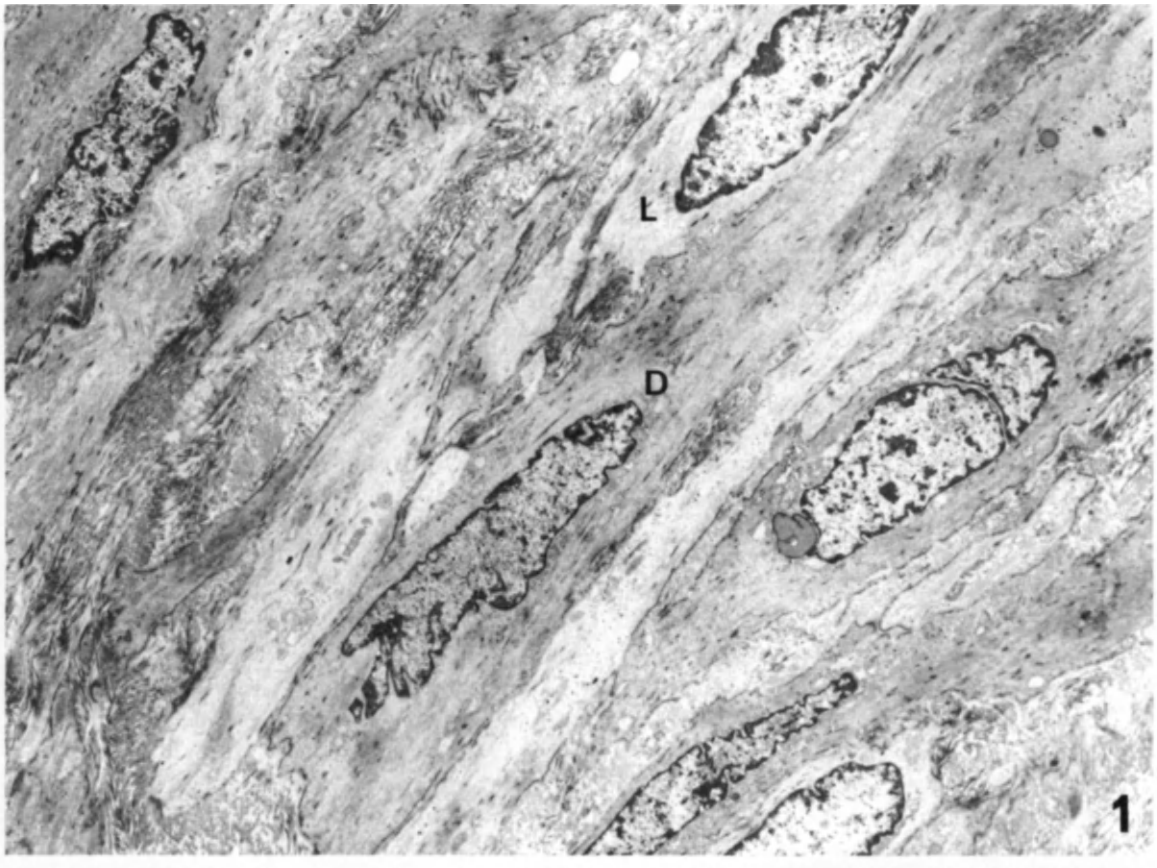
‡This is often referred to as a 'basal lamina' or, worse still, 'basement membrane'. The basal lamina lies at the base of various epithelia. A similar lamina invests cells such as smooth muscle cells and Schwann cells. There is nothing 'basal' about this lamina. It should, therefore, be called an 'external lamina'. In electron microscopy the term 'membrane' is restricted to describe only those structures where the characteristic trilaminar appearance is evident, e.g. plasma membrane and cytomembranes such as mitochondrial membranes and lysosomal membrane. For this reason and also because of the fact that the basement membrane of the light microscopist includes not only the basal lamina of the electron microscopist, but also some underlying collagen fibrils, it would be erroneous to call the 'basal lamina' 'basement membrane'. It is, of course, quite legitimate to continue using the term 'basement membrane' in light microscopy.

Plate 366

Leiomyoma of uterus.

Fig. 1. Light (L) and dark (D) neoplastic smooth muscle cells are seen set in a collagenous stroma. (For details about the light cell-dark cell phenomenon *see* page 954) × 4000

Fig. 2. Neoplastic smooth muscle cell showing the characteristic folded nucleus. The actin filaments in smooth muscle cells and their tumours are so fine that they are difficult to resolve. As Fawcett (1966) points out 'the cytoplasm looks surprisingly homogeneous even at moderately high magnifications'. The focal densities (arrows) are, however, easily seen. × 16000 (*From Ghadially, 1985*)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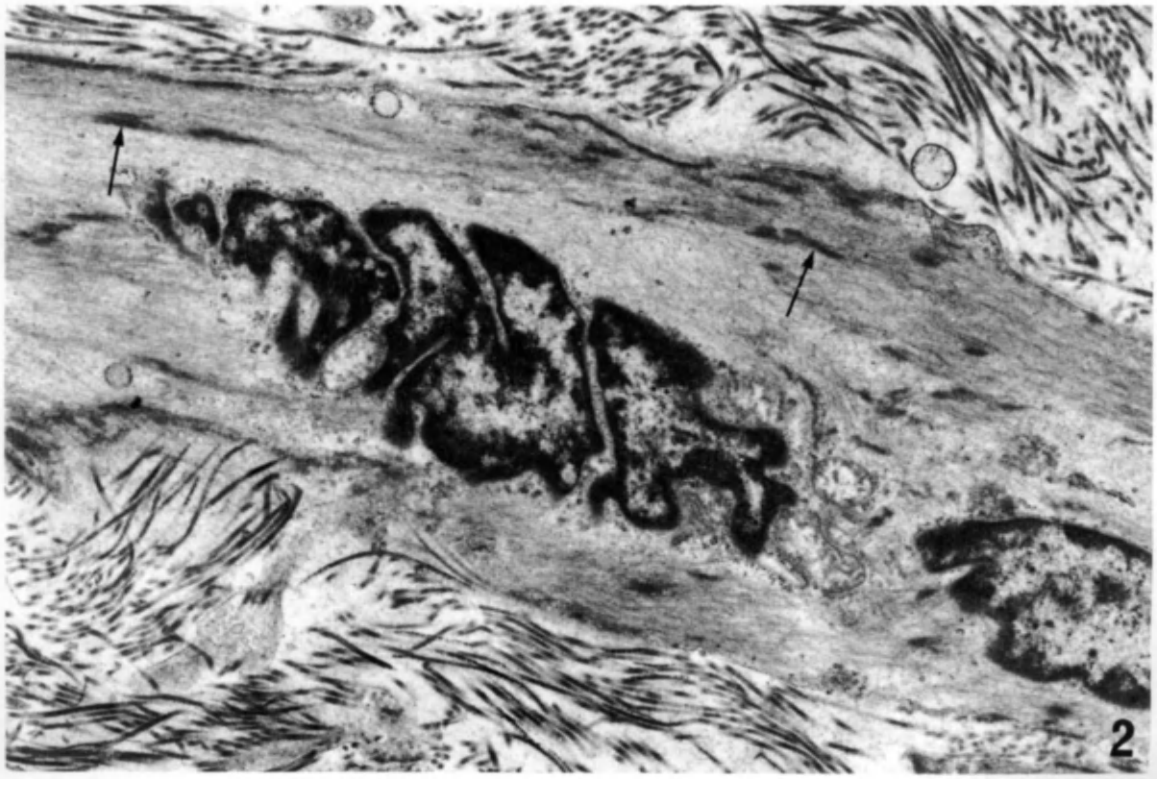
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Ultrastructural observations on leiomyosarcomas from various sites have been reported (Ferenczy *et al.*, 1971; Morales *et al.*, 1975; Prichett *et al.*, 1975; Johnson *et al.*, 1978; Nevalainen and Linna, 1978; Roth *et al.*, 1978; Seifert, 1978; Henrichs *et al.*, 1979; Sanerkin, 1979; Anderson *et al.*, 1980; Seo *et al.*, 1980; Murao, 1982; Rushton *et al.*, 1983), but several of these are essentially case reports or light microscopic studies where the ultrastructural features are not well documented or illustrated.

Ultrastructural differences between leiomyoma and various grades of leiomyosarcoma (*Plates 366* and *367*) depend on the degree of differentiation. In better differentiated examples, thin filaments are plentiful, and so are focal densities which can at times be quite large. In markedly anaplastic versions of leiomyosarcoma, filaments may be sparse and even when a fair number are present, one has to look around for focal densities to convince oneself that one has myofilaments and not just 'other intracytoplasmic filaments'.

However, parallel arrays of thin filaments and associated fusiform densities are not always observed in tumours considered to be obvious or indubitable leiomyosarcomas of the GI tract by light microscopy (Mackay and Osborne, 1978; Erlandson, 1981; Henderson and Papadimitriou, 1982). This situation at times prevails also in leiomyosarcomas from other sites. I studied a tumour of the abdominal wall, diagnosed by several histopathologists as a well differentiated leiomyosarcoma, where no bundles of thin filaments (with or without focal densities) were demonstrable in almost all of the cells, but after much searching rare cells containing a few small parallel arrays of thin filaments were seen. However, no focal densities were detected. Whether one should call such tumours leiomyosarcoma or anaplastic sarcoma is debatable. Be that as it may, I believe that for the ultrastructural diagnosis of leiomyosarcoma, an irreducible minimum requirement is the demonstration of bundles of thin filaments with focal densities along their course.

Irregularity of nuclear form is of limited value in distinguishing leiomyoma from leiomyosarcoma since this is a normal feature of this cell type (i.e. smooth muscle cell) but bizarre grossly enlarged nuclei and nucleoli are of some value. Other features in leiomyosarcoma may be summarized by the following points: (1) the filaments show a much greater degree of disorientation than in leiomyoma; (2) quite large areas of the cell may be devoid of filaments and such areas are occupied by various other organelles such as polyribosomes, atypical mitochondria, rough endoplasmic reticulum* and Golgi complex; (3) paucity of pinocytotic vesicles; (4) paucity of subplasmalemmal densities; (5) aberrations, discontinuities or virtual absence of the external lamina; and (6) far fewer collagen fibrils in the stroma compared with leiomyoma.

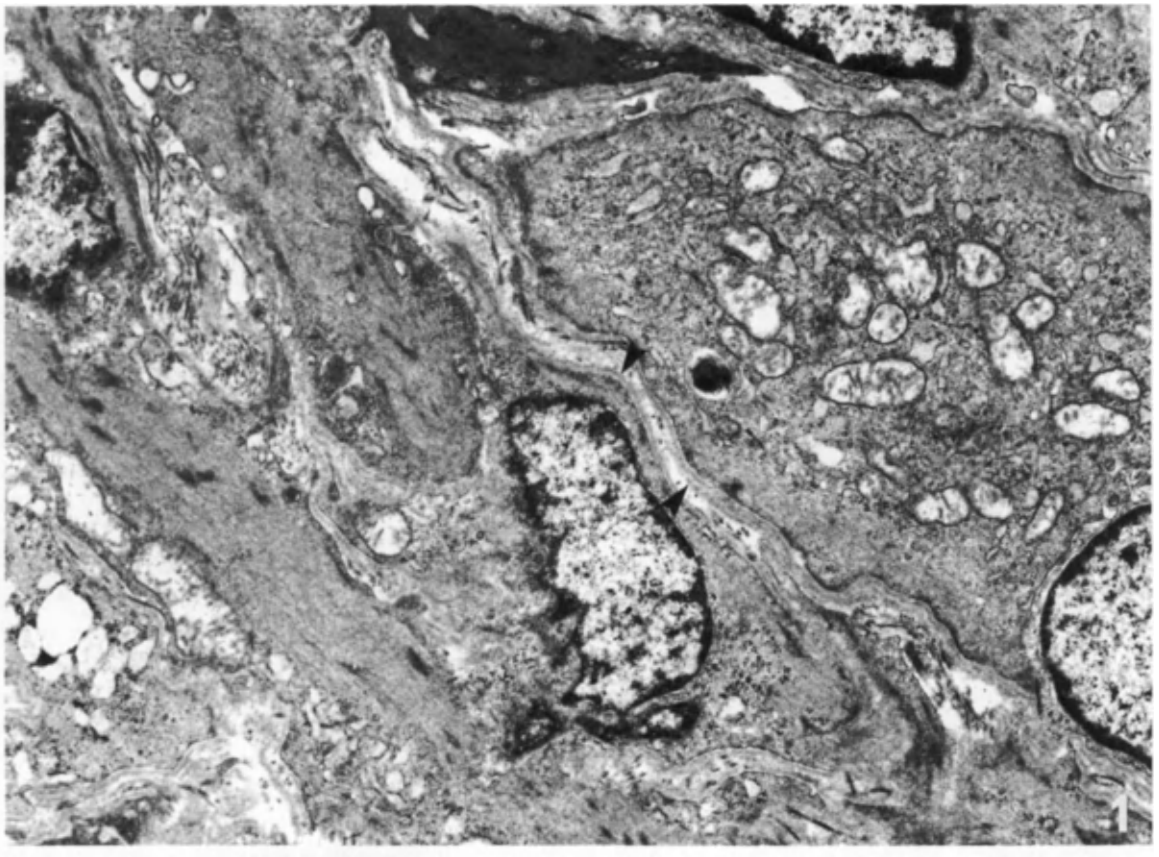
The recognition of malignancy in smooth muscle tumours can be quite difficult by light microscopy. Correlation of light and electron microscopic findings may be of some value here, for Morales *et al.* (1975) have found that while the presence of well-developed and abundant structures such as myofilaments, focal densities, subplasmalemmal densities and pinocytotic vesicles is no assurance that a tumour is benign, the converse situation—that is to say, a paucity or atypia of one or more of these features—is highly likely to be an indicator of malignancy, even in cases where the tumour appears benign by light microscopic examination.

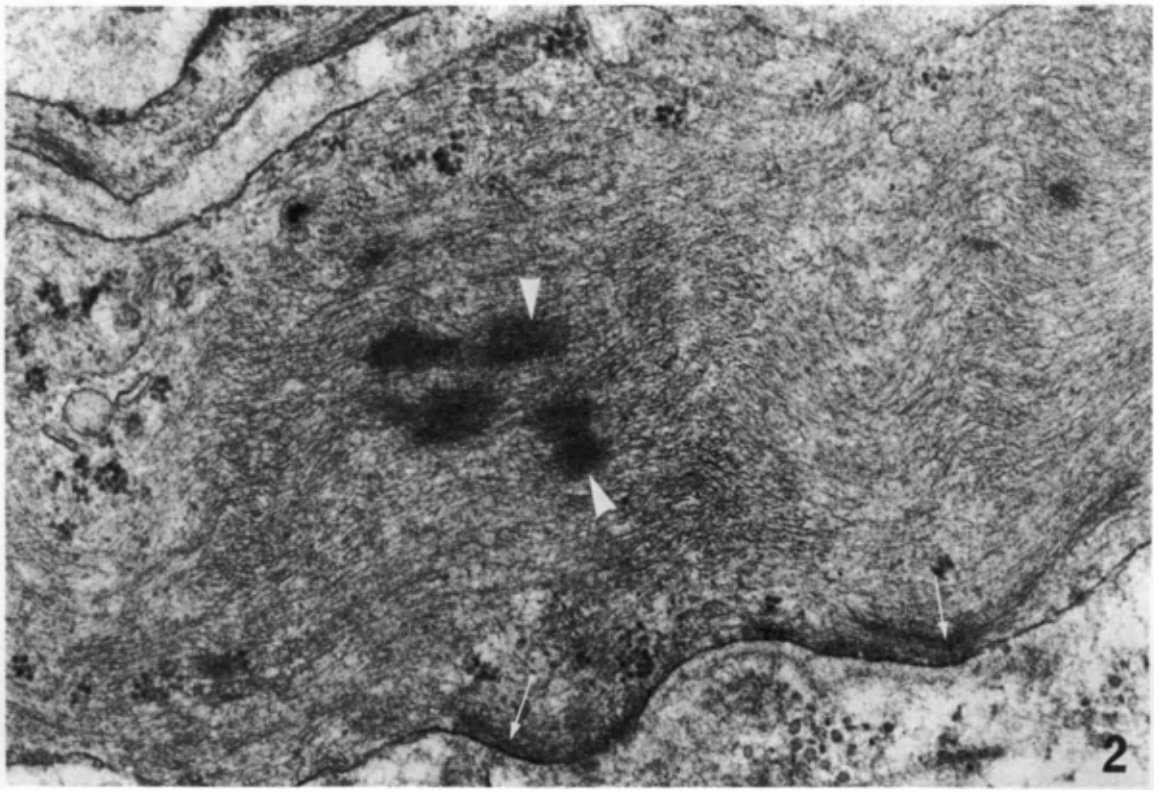
*A cell containing a large amount of rough endoplasmic reticulum and tracts of filaments with focal densities at the periphery of the cell is a myofibroblast. Occasionally, a few such cells are seen in leiomyosarcoma. Also seen are cells intermediate in morphology between a myofibroblast and a smooth muscle cell. This phenomenon is not unexpected. It adds weight to the idea that one of the ways in which a myofibroblast develops is by modulation of a smooth muscle cell (*see* page 876).

Plate 367

Fig. 1. Leiomyosarcoma. The cell in the top right-hand corner contains numerous mitochondria and some rough endoplasmic reticulum; the other cells are better-endowed with filaments and focal densities. An external lamina (arrowheads) invests the tumour cells. × 16 000 (*From Ghadially, 1980*)

Fig. 2. Leiomyosarcoma (same case as *Fig. 1*). Note filaments with quite large focal densities (arrowheads) along their course and subplasmalemmal densities (arrows). × 45 000 (*From Ghadially, 1980*)





Myofilaments in cells other than muscle

As noted in the previous section, the presence of filaments with focal densities along their course is a characteristic feature of typical smooth muscle cells found in sites such as the uterus or gut. However, the presence of such filaments is by no means the hallmark of a smooth muscle cell, for some other cells of substantially different morphology and function also contain filaments which show focal densities along their course. Furthermore, there are cells which are suspected of pronounced contractile activity where an orderly arrangement of filaments ranging in diameter from 3–17 nm (depending to some extent on preparative procedures) have been found. At least in some instances, correlated histochemical studies show that myosin is associated with such filaments (for more details and references, *see* Newstead, 1971).

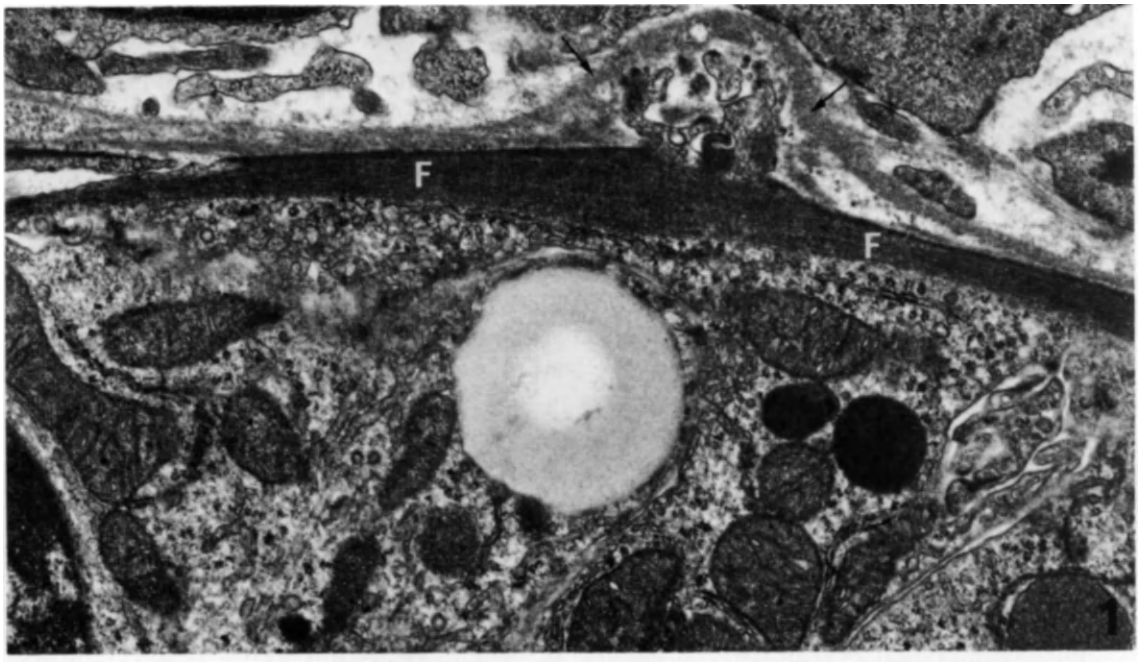
An example of this is seen in the epithelial cells of renal tubules where tracts of filaments occur orientated circumferentially around the tubules (*Plate 368, Fig. 1*). The presence of myosin has been demonstrated here by histochemical methods, and the contractile nature of these filaments is also evidenced by the folding of the basal lamina seen in electron micrographs of this region (Newstead, 1971). Furthermore, studies on glycerol-extracted rat kidney tubule cells show that both actin and myosin are present and it is suggested (Rostgaard *et al.*, 1972) that this provides strong evidence that a two-filament contractile system, based on the interaction of actin and myosin, exists here.

Similarly, filamentous elements have been described in the endothelial cells of many small vessels (Hama, 1961; Dubois *et al.*, 1966; Florey, 1966; Majno *et al.*, 1970; Chen and Weiss, 1972) and in some examples (particularly in arterial endothelia) a band of filaments (called a 'myoid zone' or 'myoid band') is seen on the basal aspect of the endothelial cells (Parry and Abramovich, 1972).

Plate 368

Fig. 1. Basal portion of a proximal tubule epithelial cell from a rat kidney, showing a circumferentially orientated tract of filaments. (F). It would appear that in this specimen (fixed by perfusion with glutaraldehyde) an agonal contraction of the filamentous band occurred, producing a fold in the basal lamina (arrows). Within the pocket so created lies some extruded cellular material. × 19 000 (*Newstead, unpublished electron micrograph*)

Fig. 2. A cell from Wharton's jelly, packed with fine filaments which are difficult to resolve. Some of the focal densities are randomly distributed (arrows) while others are aligned to form irregular dense bands (B). From a collapsed human umbilical cord. × 24 000 (*From Parry, 1970*)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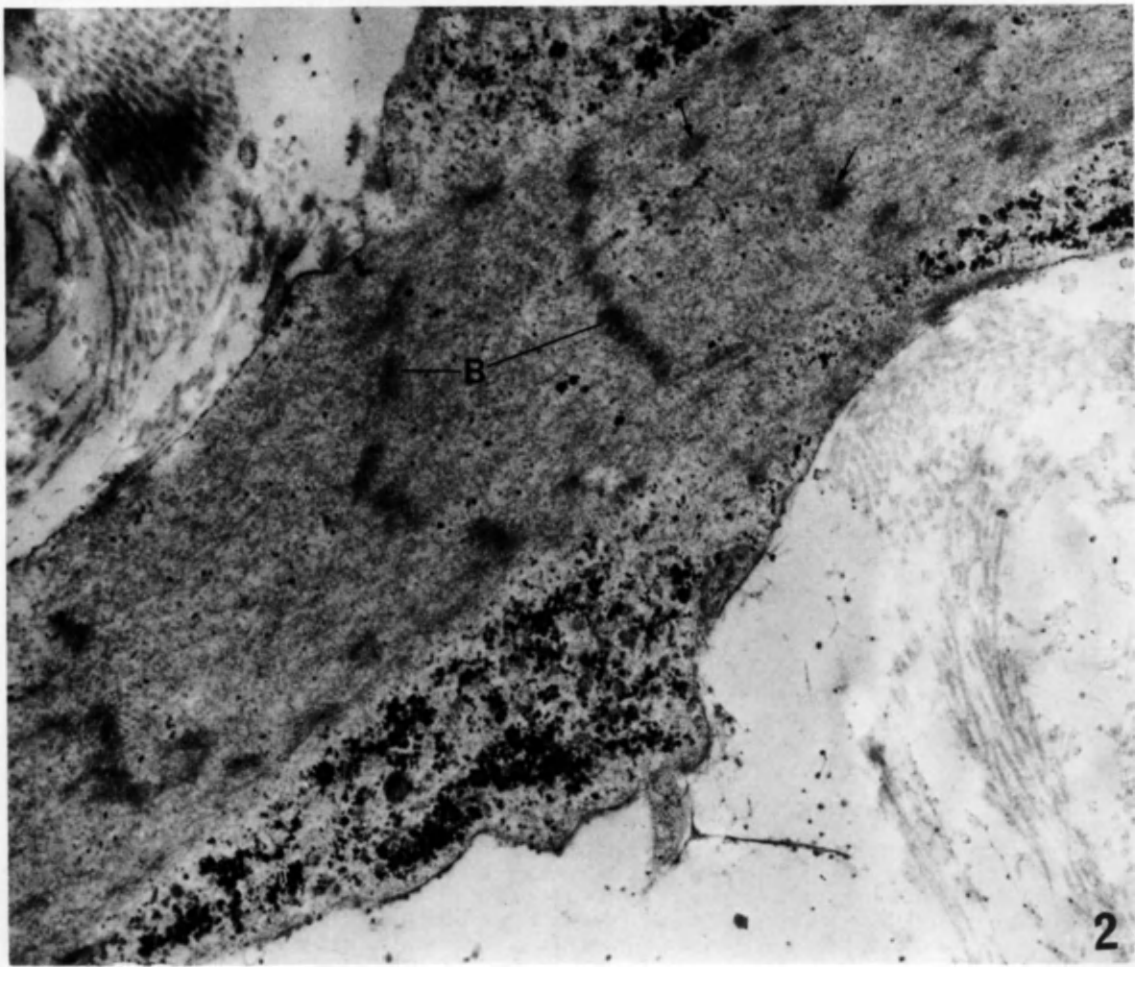
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Further in the *Arteriolae rectae* of the rat kidney thin filaments with focal densities along their course have been clearly demonstrated in the endothelial cells (Newstead and Munkacsi, 1969). It had been shown that some endothelia have the power of independent contraction (Majno *et al.*, 1970) and evidence has also been presented that actomyosin occurs in endothelial cells (Becker and Murphy, 1969). Filaments containing actin have been demonstrated also in lymphatic endothelial cells (Lauweryns *et al.*, 1975).

It is worth noting that in the above-mentioned examples a myoid band was found at the base of endothelial or epithelial cells (i.e. a basal or abluminal myoid band), but in the endothelial cells of the small cerebral arteries and arterioles from hypertensive rats a myoid band was seen by Giacomelli *et al.* (1970) at the apex of the endothelial cells (i.e. an apical or adluminal band). One wonders whether increased intraluminal pressure led to the development of the adluminal myoid band. Support for this concept comes also from our studies (Ghadially *et al.*, 1986) on an intraocular lacrimal gland choristoma where an adluminal myoid band was seen (*Plate 369*) in the epithelial cells lining cysts which had presumably developed by distension of ductal elements in this tumour or tumour-like lesion derived from lacrimal glandular tissue sequestrated in the globe during development.

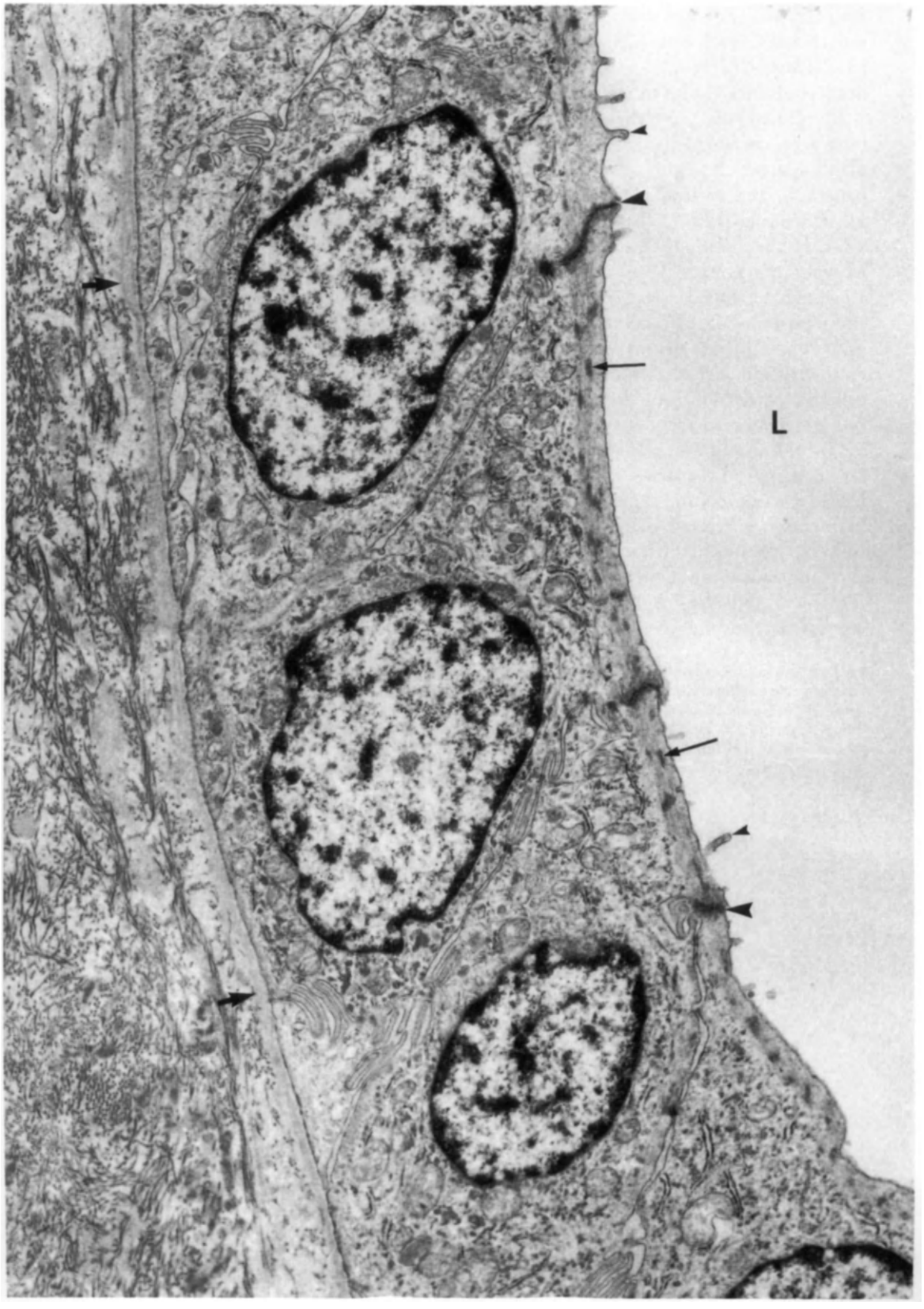
However, some epithelial and endothelial cells are not the only non-muscle cells which contain actin filaments. Indeed submembranous bundles of thin filments* (5–7 nm) have now been described in many cells types. These range from amoeba to several vertebrate cells. Furthermore, it has been demonstrated that the major component of these filaments is similar to muscle actin (Spooner *et al.*, 1971; Wessells *et al.*,1972; Lazarides and Weber, 1974; Goldman *et al.*, 1975, 1979).

Such sparse actin filaments occur also in fibroblasts but focal densities as seen on the actin filaments in smooth muscle are not seen in fibroblasts. However, electron microscopy has revealed cells of an intermediate morphology called 'myofibroblasts' which show some of the

*The thin actin filaments are a part of the cytoskeleton. The cytoskeleton is a ramifying network composed of intermediate filaments, microtubules and actin filaments. Most of the actin filaments are located under the cell membrane.

Plate 369

Choristoma of lacrimal gland. Wall of cyst lined by a single layer of cuboidal epithelial cells united by terminal bars (large arrowheads). Note the lumen (L) of the cyst, the basal lamina (thick arrows), the sparse microvilli (small arrowheads) and the adluminal myoid band composed of thin filaments with focal densities (thin arrows) along their course. × 12500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features of fibroblasts but contain tracts of thin filaments with focal densities like smooth muscle cells. Such cells (i.e. myofibroblasts) have been seen in several sites such as Wharton's jelly (*Plate 368, Fig. 2*), granulation tissue, atherosclerotic plaques and certain tumours. These matters are dealt with in the next section (page 872).

Myofibroblasts were discovered by electron microscopists, but the myoepithelial cell has long been known to light microscopists. Myoepithelial cells have been observed in the prostate gland, Harderian gland, apocrine glands, exocrine sweat glands and mammary glands (but not pancreas), and various pathological states affecting breast tissues such as sclerosing adenosis, fibroadenoma (*Plate 370*) and carcinoma (Richardson, 1949; Hurley and Shelly, 1954; Silver, 1954; Hibbs, 1958, 1962; Hollman, 1959; Munger and Brusilow, 1961; Munger *et al.*, 1961; Rowlatt and Franks, 1964; Murad and Scarpelli, 1967; Murad and Haam, 1968; Carter *et al.*, 1969; Schafer and Bässler, 1969; Tannenbaum *et al.*, 1969; Harris and Ahmed, 1977). Such cells and their numerous branching processes form a basket-like structure around the gland acinus,a shape quite different from the spindle-shaped classic smooth muscle cell. These cells lie on the epithelial side of the basement membrane. They are of ectodermal origin, in contrast to muscle cells which derive from the mesenchyme. On rare occasions a desmosomal junction may be noted between adjacent myoepithelial cells.

Like the smooth muscle cell, the myoepithelial cell is contractile, and electron microscopy has revealed typical filaments with focal densities, both in the cell processes and in the cell body. However, the epithelial character of these cells is evidenced by the presence of cytokeratin filaments (i.e. tonofilaments) in them, but vimentin filaments characteristic of mesenchymal cells and desmin filaments characteristic of muscle cells are not detectable by immunofluorescence and biochemical methods in the cells (Franke *et al.*, 1980).

(Gabbiani and Majno, 1972; Hueston *et al.*, 1976); (16) intrinsic fibrosis of muscle and in stenosing tenosynovitis (Madden, 1973; Madden *et al.*, 1975); (17) pseudomalignant myositis

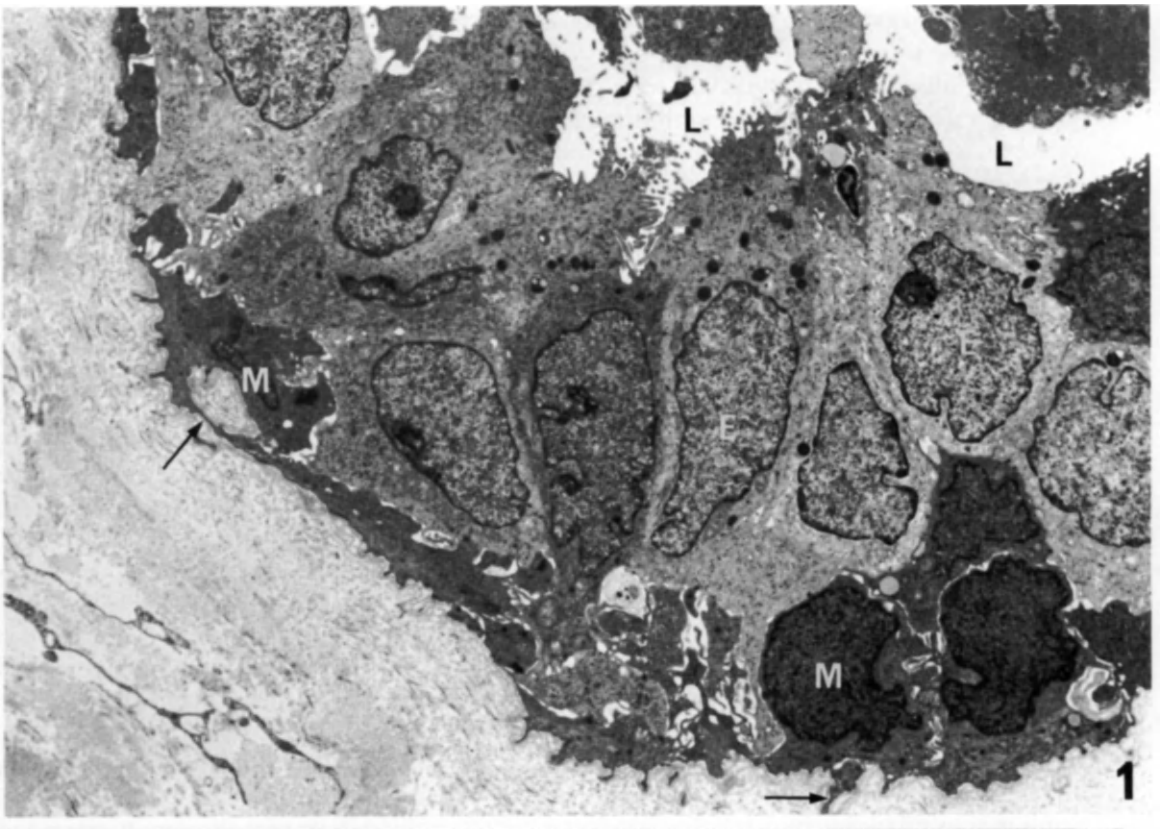
*Thin filaments are called 'microfilaments' by some workers, while others use the term 'microfilaments' in a collective sense for any or all intracytoplasmic filaments, particularly if they are not certain as to the nature of the filament. I have come across several papers where it is impossible to know what the author is talking about when he talks about 'microfilaments'. In view of this I have avoided the term 'microfilaments'. If general agreement could be reached, it would be both legitimate and desirable to call the thin actin filaments 'microfilaments' and the thick myosin filaments 'macrofilaments'. And, of course, the term 'intermediate filaments' would fit nicely in this scheme (*see* footnote on page 910).

Plate 370

Fibroadenoma of breast.

Fig. 1. Low-power view of a neoplastic ductule or alveolus showing a lumen (L) surrounded by epithelial cells (E). At the base of the epithelial cells lie myoepithelial cells (M) and their cell processes (arrows). × 3800

Fig. 2. Higher-power view showing filaments with focal densities (arrows) in a myoepithelial cell. Note also the cell processes (arrowheads) and a reduplicated basal lamina (B). × 12000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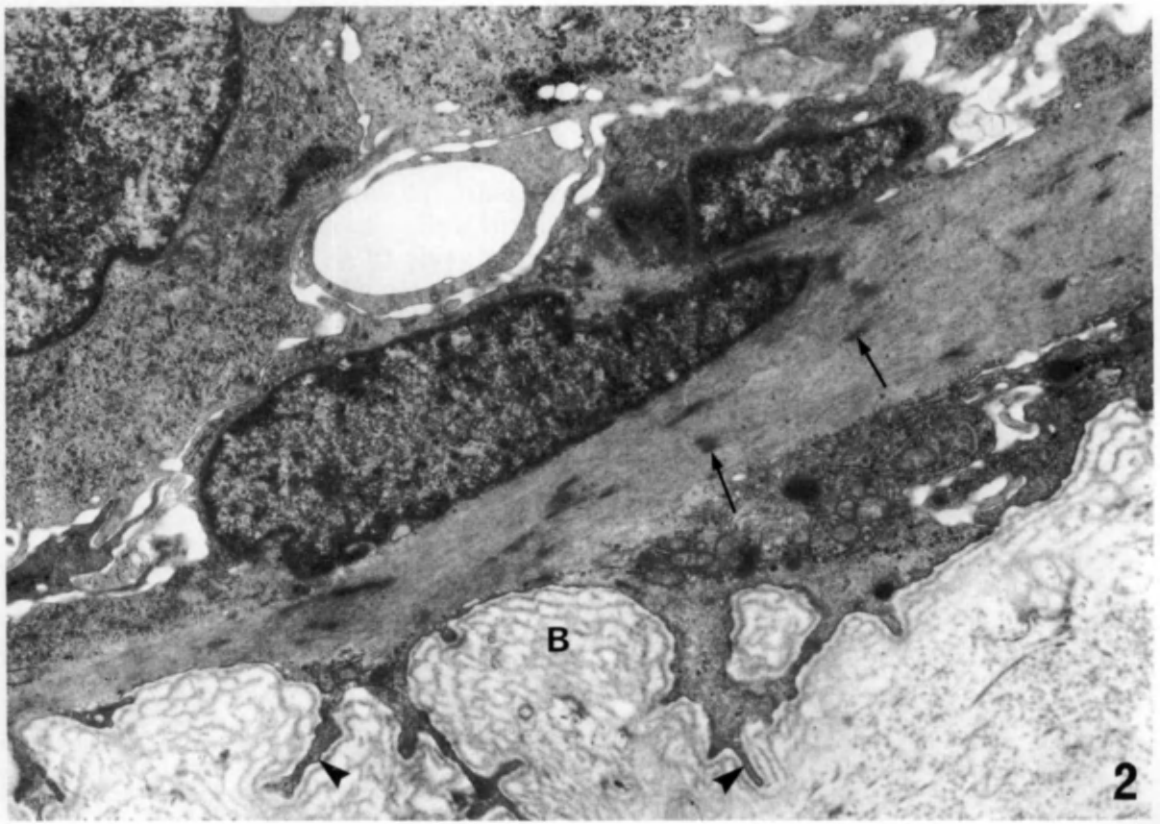
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Myofibroblasts and myofibroblastoma

The term 'myofibroblasts' was coined by Majno *et al.* (1971) to define cells which exhibit some of the ultrastructural features of both smooth muscle cells and fibroblasts (*Plates 371–375*). Such cells bear a resemblance to smooth muscle cells in that they contain tracts of filaments with focal densities along their course and densities along the cell membrane (called 'subplasmalemmal densities', 'attachment sites' or 'dense plaques') to which some of these filaments are attached, but there are also morphological and functional differences between such cells and smooth muscle cells. Examples of morphological differences include a relative infrequency of pinocytotic vesicles and an abundance of elements of the Golgi complex and rough endoplasmic reticulum. It is the latter two features which make them akin to fibroblasts. Fibroblasts lack an external lamina but such a lamina ensheaths smooth muscle cells. Myofibroblasts can at times have a fairly well developed external lamina, but more often than not this is interrupted, occurs only in patches as a 'basal-lamina-like material', or is absent. In the *in vivo* situation, junctions or junction-like structures are very rarely seen between fibroblasts, but they are more frequently seen between smooth muscle cells. In some instances such structures* are seen between myofibroblasts, but in other instances they are not.

Thus the term 'myofibroblasts' accurately reflects their morphological status. On the other hand, on the basis of functional characteristics these cells have been called 'multifunctional mesenchymal cells' (Wissler, 1967; Ghadially, 1975) because not only are they capable of synthesizing actin filaments and the precursors of collagen and elastic fibres, proteoglycans and cell coat (i.e. external lamina), but also of trapping lipids and lipoproteins (e.g. in atheroma).

Myofibroblasts have been seen in the many varied circumstances listed below. For example, myofibroblasts have been seen in some normal tissues such as: (1) intestinal villi† (Güldner *et al.*, 1972); (2) placental villi (Feller *et al.*, 1985); (3) alveoli of lung‡ (Kapanci *et al.*, 1974); (4) seminiferous tubules in human testis (Böck *et al.*, 1972); (5) testicular capsule of rat (Gorgas and Böck, 1974); (6) theca externa of ovarian follicles (O'Shea, 1970); (7) adrenal capsule (Bressler, 1973); (8) renal capsule of mammals (Kobayashi, 1978); (9) Wharton's jelly of umbilical cord (Parry, 1970); (10) Achilles tendon of rabbit (Ippolito *et al.*, 1977); (11) mesenteric lymph nodes of guinea pigs (Milanesi *et al.*, 1984); and (12) human fetal spleen (Fukuda, 1981).

Myofibroblasts have been seen in: inflammed tissues, injured tissues, repair tissues, scars, fibroblastic tumours or tumour-like lesions (fibromatosis) and tumours or lesions containing fibroblasts and histiocytes. Such examples include: (13) granulation tissue (Gabbiani *et al.*, 1971, 1976; Hirschel *et al.*, 1971; Majno *et al.*, 1971; Ryan *et al.*, 1974); (14) avascular fibrous tissue (Ryan *et al.*, 1973); (15) palmar nodules of Dupuytren's contracture (but not in the fascia itself)

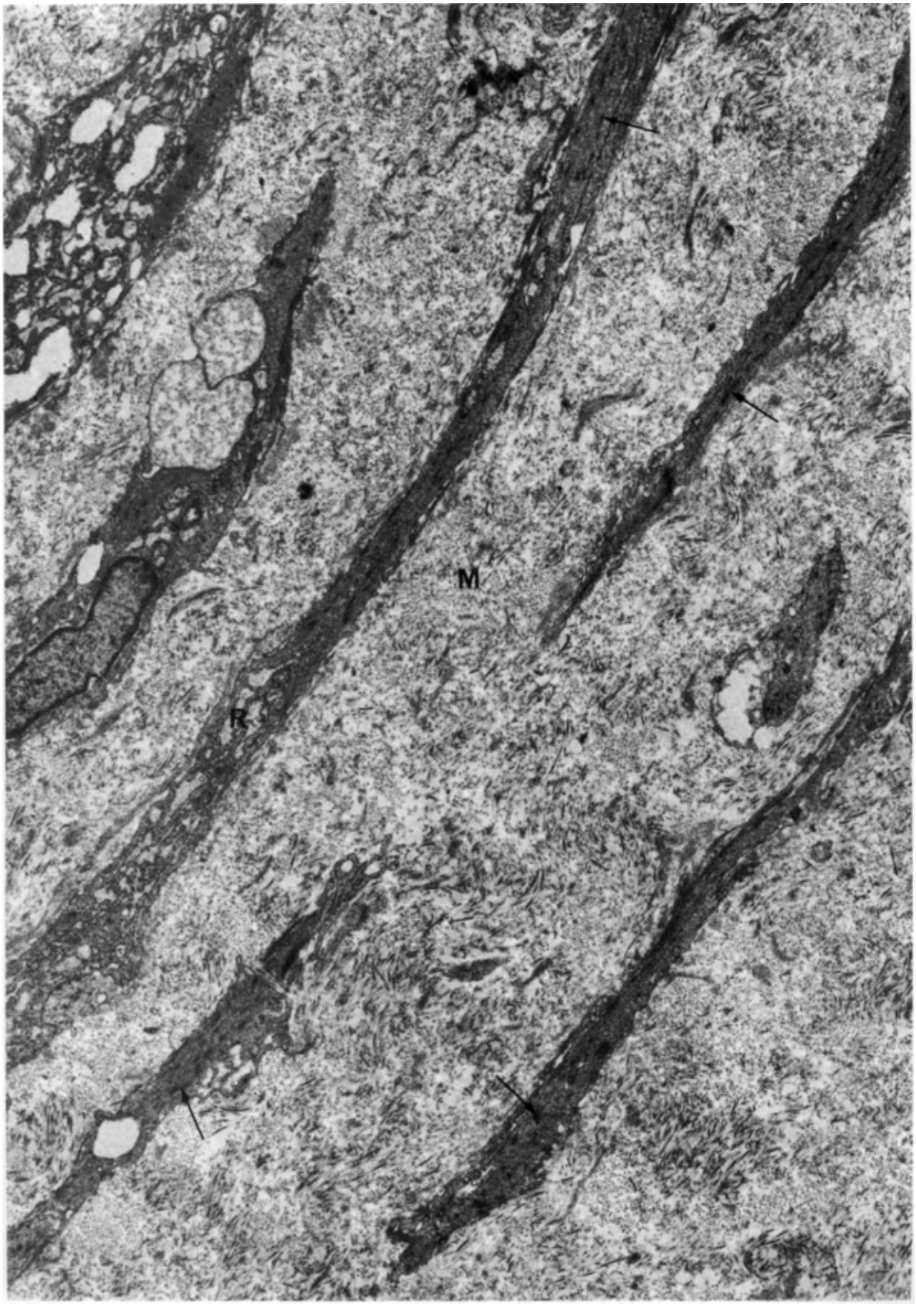
*These structures which are commonly referred to as junctions or junction-like structures or desmosomes or desmosome-like structures are in fact subplasmalemmal densities. When a solitary subplasmalemmal density is present it looks like a hemidesmosome, when a pair of such densities is present in apposing cells, the structure looks like a desmosome. No sharply defined plaques containing desmoplakins are present as in true desmosomes. A corollary to this is that one should not now (i.e. after the advent of immunohistochemistry) call these structures 'dense plaques'. One may call them 'subplasmalemmal densities' or by the popular terms 'desmosome-like structures' and 'hemidesmosome-like structures'.

†Focal densities do not appear to be present along the filaments in these cells but prominent densities are evident along the cell membrane.

‡Focal densities are not apparent along the filaments in these cells but densities are seen along the cell membrane. The filaments have been shown to bind antiactin antibodies.

Plate 371

Portion of ganglion wall. The surface facing the ganglion cavity is not included, but it lay just beyond the top left corner of the picture. Numerous strap-like cells are seen lying in the collagen-rich intercellular matrix (M). The cytoplasmic matrix appears dense because it is packed with fine myofilaments which are difficult to resolve. However, focal densities can be detected quite frequently (arrows). Dilated cisternae of the rough endoplasmic reticulum (R) are seen in one cell, and another placed closer to the surface than the rest shows numerous vacuoles and a disorganized internal structure. Note the absence of cells acceptable as fibroblasts. × 8700 (*From Ghadially and Mehta, 1971*)


M
R

(Gabbiani and Majno, 1972; Hueston *et al.*, 1976); (16) intrinsic fibrosis of muscle and in stenosing tenosynovitis (Madden, 1973; Madden *et al.*, 1975); (17) pseudomalignant myositis ossificans (Povýšil and Matějovský, 1979); (18) ganglia of wrist* (Ghadially and Mehta, 1971) (*Plates 371* and *372*); (19) hypertrophic scars (Kischer,1974; Baur *et al.*, 1975); (20) plaque of anterior capsular cataract (Novotny and Pau, 1984); (21) fibrous capsule around silicone implants (Ryan *et al.*, 1974; Rudolph *et al.*, 1978); (22) atherosclerotic lesions in man and experimental animals (Thomas *et al.*, 1963; Flora *et al.*, 1967; Knieriem,1967; Wissler, 1967; Gabbiani *et al.*, 1975); (23) cirrhotic human liver (Bhathal, 1972; Peacock and Van Winkle, 1976; Grimaud and Borojevic, 1977); (24) nodular hyperplasia of liver associated with oral contraceptives (Callea *et al.*, 1982); (25) liver of baboons where fatty change and perivenular fibrosis had occurred after prolonged alcohol consumption (Mak and Lieber, 1986); (26) subcutaneous tissue in scleroderma (Nakanishi *et al.*, 1981); (27) fibrous heart plaque in carcinoid syndrome (Lagacé *et al.*, 1975); (28) fibrous capsule around a cardiac pacemaker (Rudolph *et al.*, 1981); (29) torn human menisci (Ghadially *et al.*, 1980) (*Plate 373, Fig. 1*); (30) haemophilic synovial membrane (Ghadially, unpublished observations); (31) repair tissue of unknown aetiology which was clinically thought to be a sarcoma (*Plate 373, Fig.2*) (Ghadially, 1985); (32) pseudointima developing in the lumen of Dacron grafts inserted in canine aorta (Sottiurai and Batson, 1983); (33) epiretinal membranes and vitreous membranes from eyes with various disorders (Kampick *et al.*, 1981); (34) experimentally produced vitral fibrous membranes in rabbit (Grierson and Rahi, 1981); (35) peridontal ligament of rat monoinfected with *Actinomyces naeslundii* (Garant, 1976); (36) renal interstitial tissue after experimentally produced obstructive nephropathy in the rabbit (Nagle *et al.*, 1973); (37) desmoid fibromatosis (Stiller and Katenkamp, 1975); (38) plexiform tumour of uterus (Fisher *et al.*, 1978); (39) circumscribed fibromatosis (Feiner and Kaye, 1976); (40) nodular fasciitis (Wirman, 1976); (41) juvenile nasopharyngeal angiofibroma (Taxy, 1977); (42) fibroxanthoma (Weedon and Kerr, 1975); (43) dermatofibroma (Katenkamp and Stiller, 1975); (44) giant cell fibromas of the oral mucosa (Weathers and Campbell, 1974);(45) pseudosarcoma of skin (Woyke *et al.*, 1974); (46) infantile digital fibroma (Bhawan *et al.*, 1979; Iwaski *et al.*, 1980); (47) collagen naevus (Shelley *et al.*, 1985); (48) fibroma of tendon sheath (Smith *et al.*, 1982; Lundgren and Kindblom, 1984; Hashimoto *et al.*, 1985); (49) proliferative fasciitis (Craver and McDivitt, 1981); (50) congenital generalized fibromatosis (Kobayashi *et al.*, 1981; Liew and Haynes, 1981; Wiswell *et al.*, 1985); (51) non-ossifying fibroma of bone (Llombart Bosch *et al.*, 1974; Steiner,1974); (52) giant cell granuloma of the jaw (El-Labban and Lee, 1983); (53) desmoplastic fibroma of bone (Lagacé *et al.*, 1979); (54) sclerosing liposarcoma (Lagacé *et al.*, 1980); (55) disseminated fibrosing deciduosis (also called 'leiomyomatosis peritonealis disseminata') (Pieslor *et al.*, 1979); (56) malignant fibrous histiocytoma (Churg and Kahn, 1977; Harris, 1980; Lagacé *et al.*, 1980; Nakanishi *et al.*, 1981); and (57) epithelioid sarcoma (Blewitt *et al.*, 1983).

Myofibroblasts have also been found in the stroma of various carcinomas (primary and secondary) and other tumours particularly when there is a desmoplastic reaction. For example: (58) myofibroblasts have been seen in carcinoma of the breast, follicular thyroid carcinoma, carcinoma of the colon and basal cell carcinoma (Ghosh *et al.*, 1980; Nakanishi *et al.*, 1981;

*The old erroneous idea that ganglia are lined by synovial cells or cells showing fibroblastic differentiation has been supported by some ultrastructural studies. Fortunately the illustrations in these studies effectively belie such contentions. For a critique *see* Ghadially, 1983a.

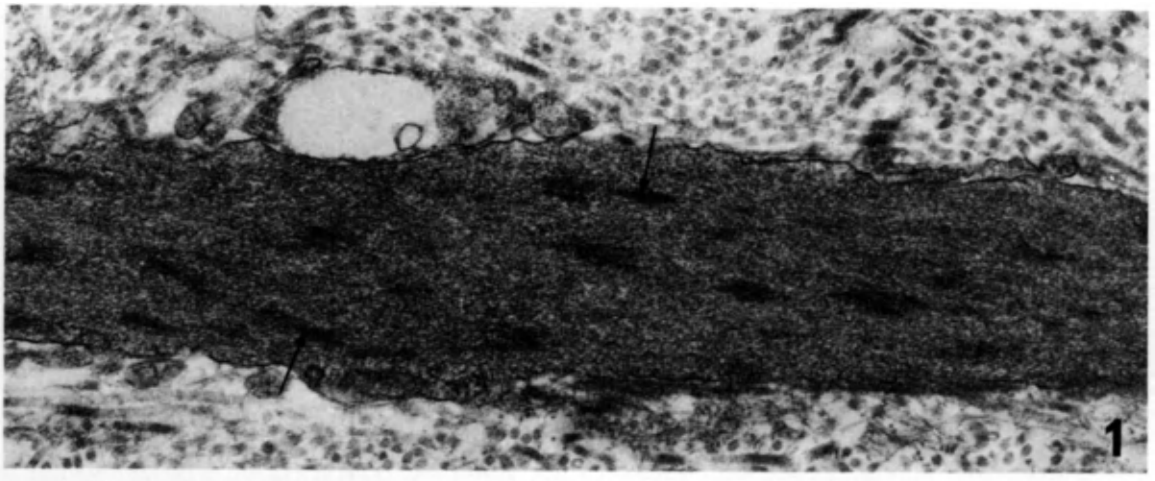
Plate 372

Myofibroblasts from ganglion wall. (*From Ghadially and Mehta, 1971*)

Fig. 1. High-power view, showing cytoplasm packed with filaments that are difficult to resolve and numerous focal densities (arrows). × 36 000

Fig. 2. Dilated cisternae (C) of the rough endoplasmic reticulum and a dense myofilament-rich (M) cytoplasm. × 16 000

Fig. 3. The cytoplasmic matrix is packed with myofilaments (M). Golgi complex (G) and numerous smooth-walled vacuoles (V), some apparently about to open or opening into the matrix (arrows). × 20 000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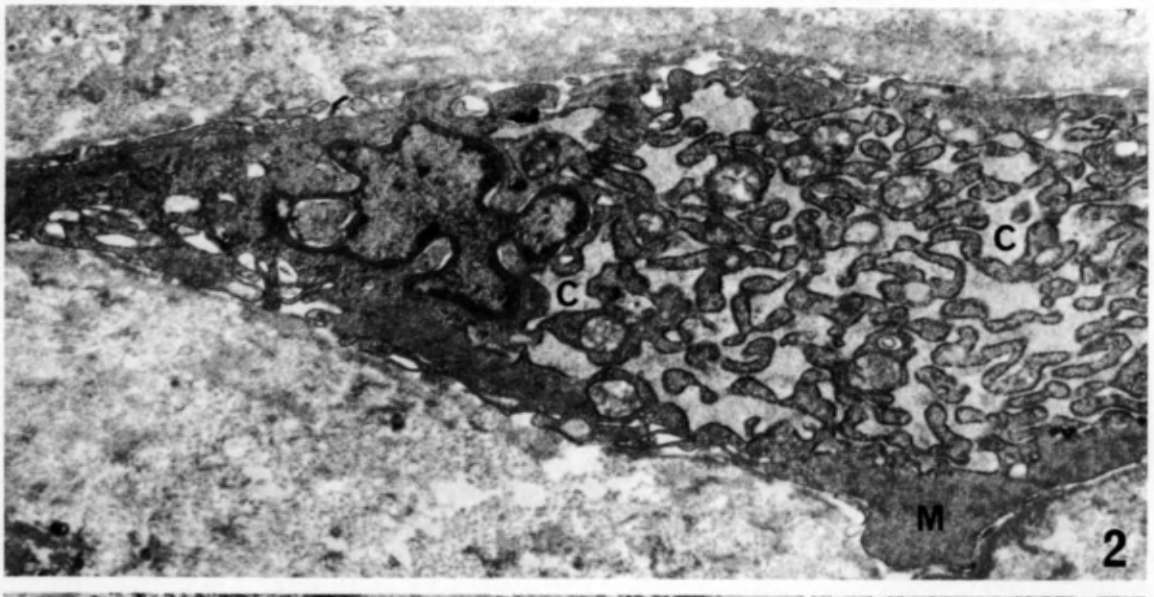
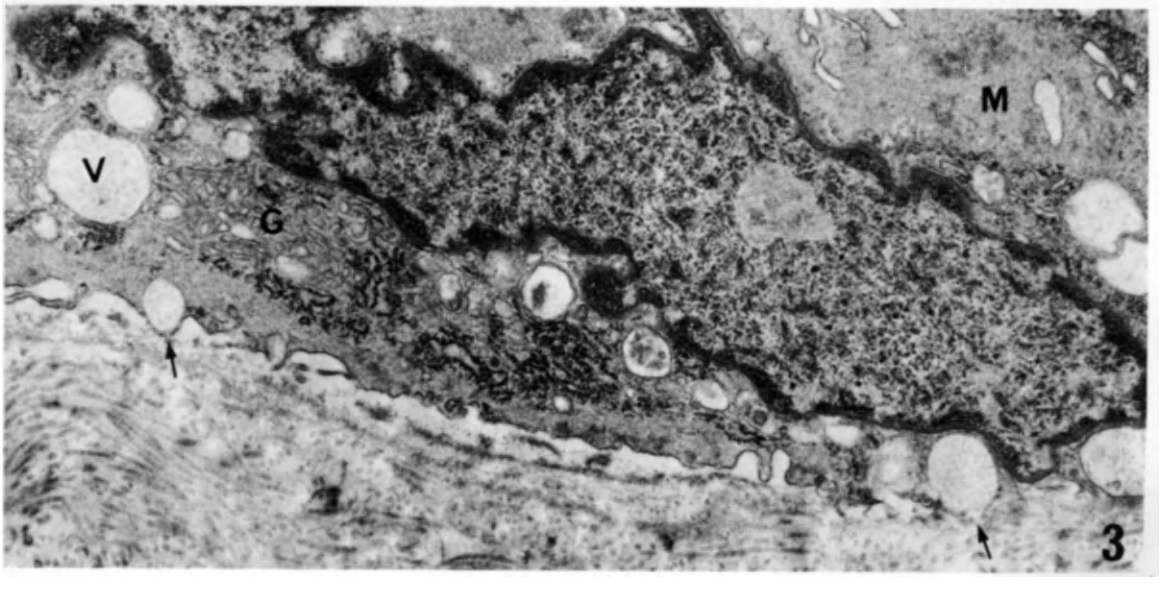

C
C
M
2


M
V
G
3

Balazs and Kovacs, 1982; Katenkamp *et al.*, 1984; Lagacé *et al.*, 1985; Sharaf, 1985); and (59) sclerosing Hodgkin's lymph nodes (Seemayer *et al.*, 1980).

Besides the above: (60) several cases histologically considered to be fibrosarcomas of a low grade malignancy have been found to be composed entirely or almost entirely of myofibroblasts (Crocker and Murad, 1969; Stiller and Katenkamp, 1975; Vasudev and Harris, 1978; Hashimoto *et al.*, 1982; Ghadially *et al.*, 1983), and hence have been referred to as 'tumours of myofibroblasts' or 'myofibroblastoma'.

Let us look at the function of myofibroblasts. In normal tissues (items 1–12) myofibroblasts subserve a contractile function and in some instances a secretory function also. Thus, in the gut they are thought to regulate the vascular and stromal spaces in the villus (Güldner *et al.*, 1972); and in the lung (Kapanci *et al.*, 1974) they have been implicated in regulation of ventilation. A contractile function is also suggested by their occurrence in the theca externa of the ovarian follicles, adrenal capsule and the testicular capsule of the rat, which is known to be contractile. One could, in fact, look upon these myofibroblasts as modified smooth muscle cells. Particularly interesting are the ultrastructural studies of Parry (1970) who showed that Wharton's jelly contains myofibroblasts and that typical fibroblasts are not present here. The classic 'stellate fibroblast' appearance presented by the cells of Wharton's jelly at light microscopy is produced when the cord collapses after cutting and release of intravascular pressure. Since myofibroblasts are virtually the only cells present here and since these cells are well endowed with rough endoplasmic reticulum and Golgi complex, it is evident that Wharton's jelly is produced by these cells, i.e. they have a secretory and a contractile role. The role of myofibroblasts in granulation tissue has been extensively studied, and it is now evident that wound contraction is engendered by myofibroblasts. Pharmacological agents which cause smooth muscle to contract or relax affect granulation tissue in the same way. That this is not due to the smooth muscle in the vessels in this tissue is attested by the fact that avascular repair tissue which forms around intraperitoneal haematomas in the rat also behaves in a similar fashion. The occurrence of myofibroblasts in pathological contractile states such as Dupuytren's contracture and hepatic fibrosis is of dual interest because on the one hand they explain the underlying pathogenic mechanism and on the other hand if a pharmacological way of inhibiting the contraction of these cells could be found this would be of therapeutic value (Madden, 1973).

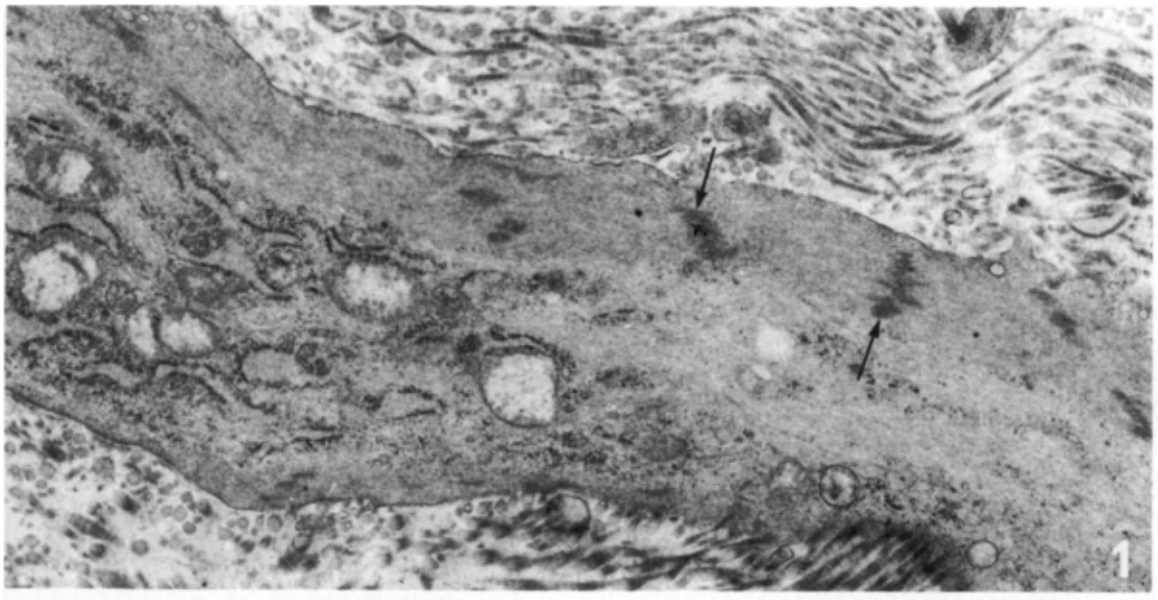
Various theories regarding the genesis and status of myofibroblasts have been considered in the literature. They have been looked upon as: (1) a distinct and different race of cells; (2) modified fibroblasts; (3) modified smooth muscle cells; or; (4) a modification of some other cell type such as a macrophage.

There is ample evidence to support the idea that fibroblasts can transform into myofibroblasts. For example, fibroblasts in culture develop bundles of intracytoplasmic filaments (shown to be actin) and intercellular connections (Goldberg and Green, 1964; Goldman and Follett, 1969; Rash *et al.*, 1972; Ryan *et al.*, 1973). Myofibroblasts are not seen in normal menisci of experimental animals (Silva, 1969; Ghadially *et al.*, 1978a); nor are they seen in uninjured portions of human torn semilunar cartilages but they are seen in tissue adjacent to the site of injury (Ghadially *et al.*, 1980) (*Plate 373, Fig. 1*). It is therefore likely that they arise from pre-existing fibroblasts for the only other cells present in this region are fibroblasts and chondrocytes.

Plate 373

Fig. 1. A part of a myofibroblast from a human torn semilunar cartilage. Note the rough endoplasmic reticulum and filaments with focal densities (arrows). × 17 500 (*From Ghadially, Lalonde and Yong, 1980*)

Fig. 2. A myofibroblast from repair tissue in the thigh of a 14-year-old boy. Note the abundant rough endoplasmic reticulum. Golgi complex and filaments with focal densities (arrows). × 24 000 (*From Ghadially, 1980*)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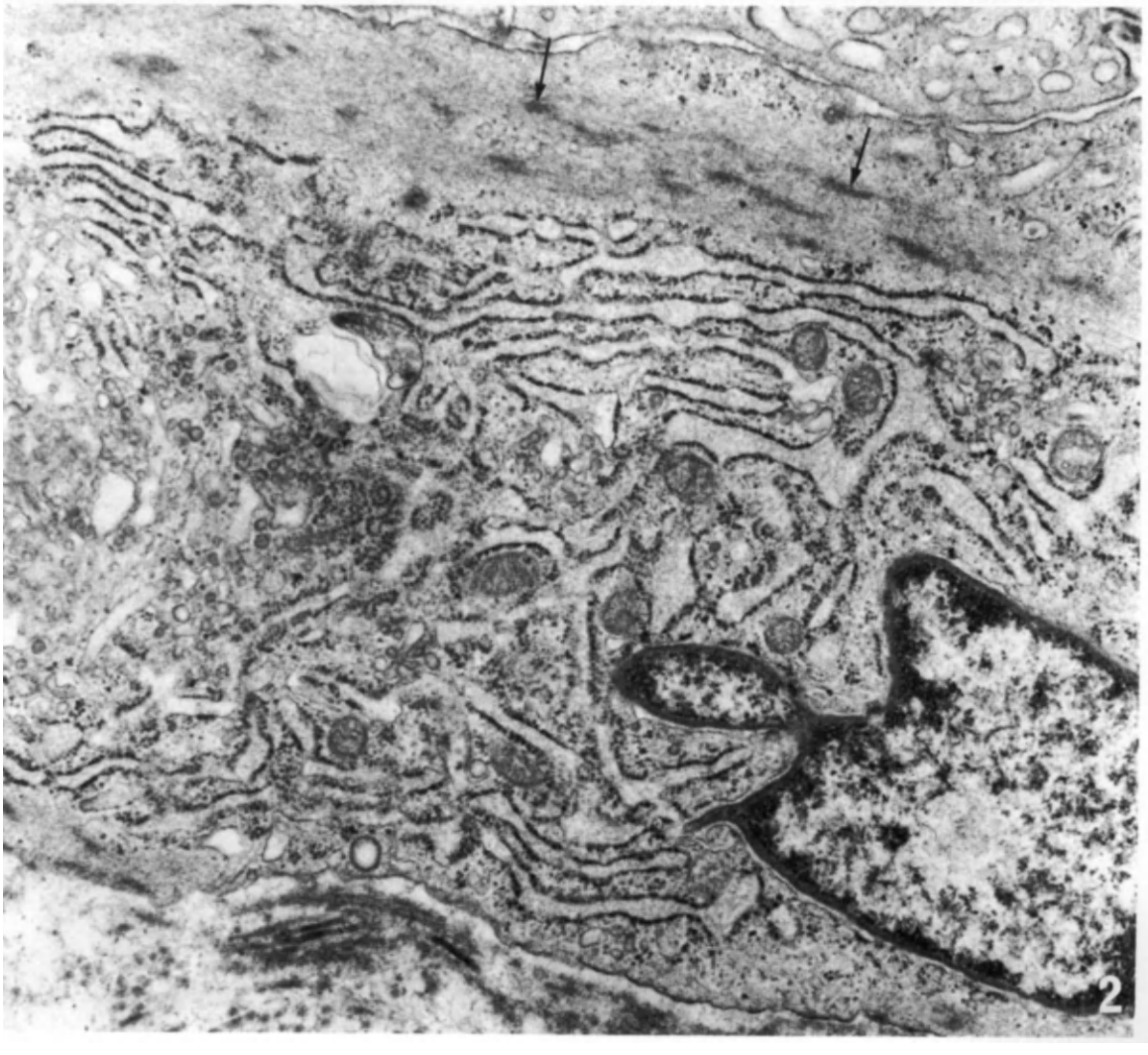

2

In the atheromatous plaque, however, it seems likely that the myofibroblast* may be a modified smooth muscle cell. It is not difficult to see that if some situation leads to a hypertrophy of the rough endoplasmic reticulum in a smooth muscle cell it will come to resemble a myofibroblast and indeed such a change can be produced in the muscle of the rat uterus by oestrogens (Ross, 1968).

On the other hand, since fibroblasts and muscle cells both derive from the mesenchyme it is possible to think of an intermediate line of development which gives rise to the myofibroblast. Myofibroblasts found in various normal sites may well fall into this category.

Finally the possibility that myofibroblasts can at times derive from cells other than smooth muscle cells and fibroblasts has been considered (Ryan *et al.*, 1973). It has been found that some of the blood clots placed in the peritoneal cavity of rats do not become adherent to adjacent peritoneal surfaces but become covered by layers of myofibroblasts and mesothelial cells. It is difficult to see how smooth muscle cells or fibroblasts can arrive at this site and the possibility that macrophages may turn into fibroblasts and then into myofibroblasts has been considered (Ryan *et al.*, 1973), but no firm conclusion has been reached on this point.

It would appear that the various hypotheses about the genesis of myofibroblasts are not mutually exclusive and that the term 'myofibroblasts' encompasses a heterogeneous collection of cells with subtle morphological differences and varying modes of genesis.

As noted earlier (item 58) myofibroblasts are found in the stroma of some carcinomas (particularly when there is a desmoplastic reaction) and in Hodgkin's disease (item 59). This stromal reaction may be looked at as a host defence mechanism to invading neoplastic cells and it has been suggested that myofibroblasts may constitute a unique expression of host response to neoplasia (Seemayer *et al.*, 1981; Batsakis *et al.*, 1982).

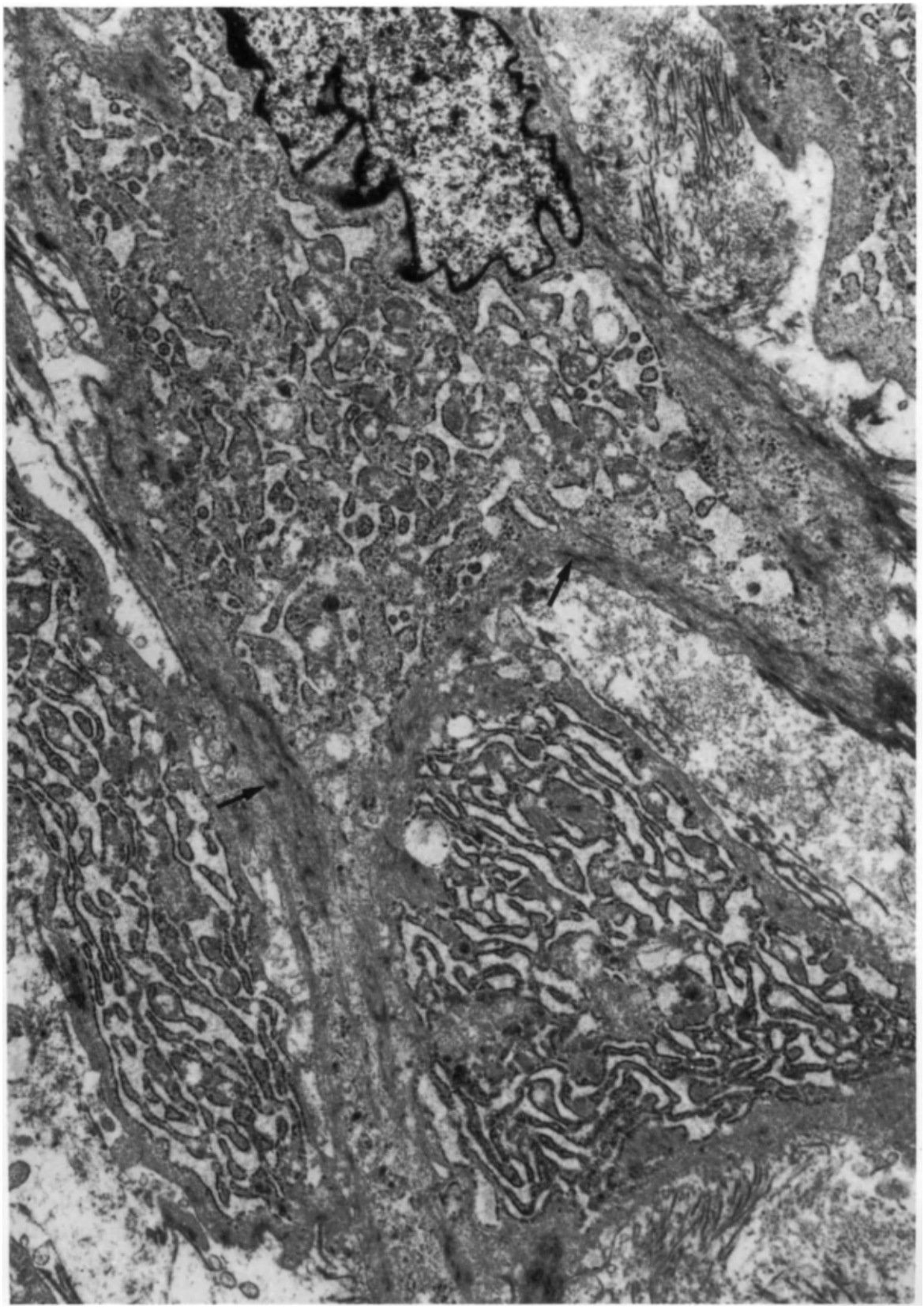
The occurrence of myofibroblasts in various fibrous tumours or tumour-like lesions (items 37–55) designated by various terms including 'fibromatosis' has led to renaming some of them as 'myofibromatosis'. For example 'congenital generalized fibromatosis' has been renamed 'infantile myofibromatosis' by Chung and Enzinger (1981). However, Fletcher *et al.* (1987) found the cells in most but not all cases of this condition to be desmin positive and hence they state that 'this entity shows true smooth muscle differentiation, rather than it being of a fibroblastic nature'. In contrast to this myofibroblasts in repair tissues and in desmoid fibromatosis are said to be desmin negative (Schurch *et al.*, 1984). I would look upon these findings as little more than evidence supporting the idea that some myofibroblasts are of smooth muscle lineage, while others are of fibroblastic lineage†.

*Terms such as 'multifunctional mesenchymal cells' and 'myofibroblasts' were once used to describe cells in atheromatous plaques, but now they are usually referred to as 'modified smooth muscle cells' or 'atheromatous smooth muscle cells', despite the fact that they morphologically and functionally more resemble myofibroblasts than smooth muscle cells. That these cells do derive from smooth muscle cells is not doubted, because: (1) they contain the α-isoform of actin which occurs in smooth muscle cells but not in non-muscle cells and wound myofibroblasts; and (2) they contain desmin which also does not occur in wound fibroblasts which presumably derive from fibroblasts (Gabbiani *et al.*, 1984; Kocher and Gabbiani, 1986).

†The credibility of identifying cell type by immunohistochemical filament typing is at the moment rather low (*see* page 900). Desmin positivity has been found even in a case of malignant fibrous histiocytoma (Miettinen *et al.*, 1982). Surely this does not make it a myogenic tumour.

Plate 374

Myofibroblastoma. Myofibroblasts containing abundant rough endoplasmic reticulum and tracts of filaments bearing focal densities (arrows). × 10000



Some but not all fibrosarcomas (item 60) contain a few or many myofibroblasts even when this feature is specifically looked for (Jakobiec and Tannenbaum, 1974; and Ghadially unpublished observations). It has been suggested (Stiller and Katenkamp, 1975) that the presence of numerous myofibroblasts* may be an indicator of a low level of malignancy and better prognosis. To date, tumours diagnosed as low grade fibrosarcoma by light microscopy but found to be myofibroblastomas† by electron microscopy have not metastasized except for one case reported by d'Andiran and Gabbiani, (1980) which the authors call 'A metastasizing sarcoma of the pleura composed of myofibroblasts'. Barring this rare exception one can say that in sites and situations where total excision of a myofibroblastoma is possible an uneventful recovery ensues, but where this is not possible the outcome can be fatal (as in the case of an intra-abdominal myofibroblastoma described by us‡), even in the absence of metastasis (Ghadially *et al.*, 1983). Thus, the myofibroblastoma appears to be an essentially benign tumour which may be locally aggressive but one which is incapable of metastasizing except in very rare instances.

The histogenesis of myofibroblastomas is not clearly established, but on the basis of what we know about the occurrence and genesis of myofibroblasts in normal and pathological tissues we may speculate that myofibroblastomas may derive from: (1) pre-existing myofibroblasts which occur normally in several tissues; (2) modified smooth muscle cells; (3) modified fibroblasts; or (4) primitive mesenchymal cells.

We have seen that myofibroblasts are of common occurrence in granulation tissue and repair tissue, and it is thought that in such instances they derive from fibroblasts; a contention supported also by tissue culture studies. Because of this (and the fact that tumours composed of a mixture of fibroblasts and myofibroblasts are quite common) the speculation that myofibroblastomas are composed of fibroblasts which have modulated or differentiated into myofibroblasts seems most attractive. Further, since one associates differentiation with less aggressive behaviour and anaplasia with malignancy, one would expect that a fibrosarcoma composed of anaplastic fibroblasts would be more likely to metastasize than a tumour composed of fibroblasts that have differentiated into myofibroblasts.

Finally, it is perhaps worth pointing out that the mere presence of some myofibroblasts in a tumour does not merit the diagnosis of myofibroblastoma. In fact, as we have seen, myofibroblasts are found in a variety of mesenchymal tumours and they have been seen in the stroma of some carcinomas also. Because of this widespread occurrence of myofibroblasts some doubt has been expressed in the literature as to whether a true tumour of myofibroblasts in fact exists. The logic behind this is incomprehensible; after all, fibroblasts occur in several mesenchymal tumours and in the stroma of carcinomas but nobody doubts the neoplastic potential of the fibroblasts or denies the existence of fibrosarcomas.

*According to Lipper *et al.* (1980), 90 per cent of the cells in the rare parosteal osteosarcoma are myofibroblasts. The parosteal osteosarcoma has a markedly indolent biological behaviour as compared to the highly virulent behaviour of the more common general group of osteosarcomas.

†Ultrastructural studies have delineated a class of neoplasms composed principally or almost entirely of myofibroblasts. Such tumours have been described under titles such as 'sarcoma of myofibroblasts', 'tumour of myofibroblasts' and 'myofibroblastic tumour', but I prefer to call them 'myofibroblastomas', because it is brief and in keeping with classic principles of tumour nomenclature. Since at least one malignant metastasizing version of this tumour has been described, one can speak about benign and malignant myofibroblastoma or in analogy with fibroma and fibrosarcoma, call these tumours myofibroma and myofibrosarcoma.

‡This tumour which occurred in a 15-year-old boy was diagnosed as low grade fibrosarcoma by light microscopy. Ultrastructurally it was composed almost entirely of myofibroblasts and a few macrophages. The patient died of cachexia and bleeding in the gastro-intestinal tract, but there was no distant metastasis nor was there a noticeable infiltration of abdominal organs.

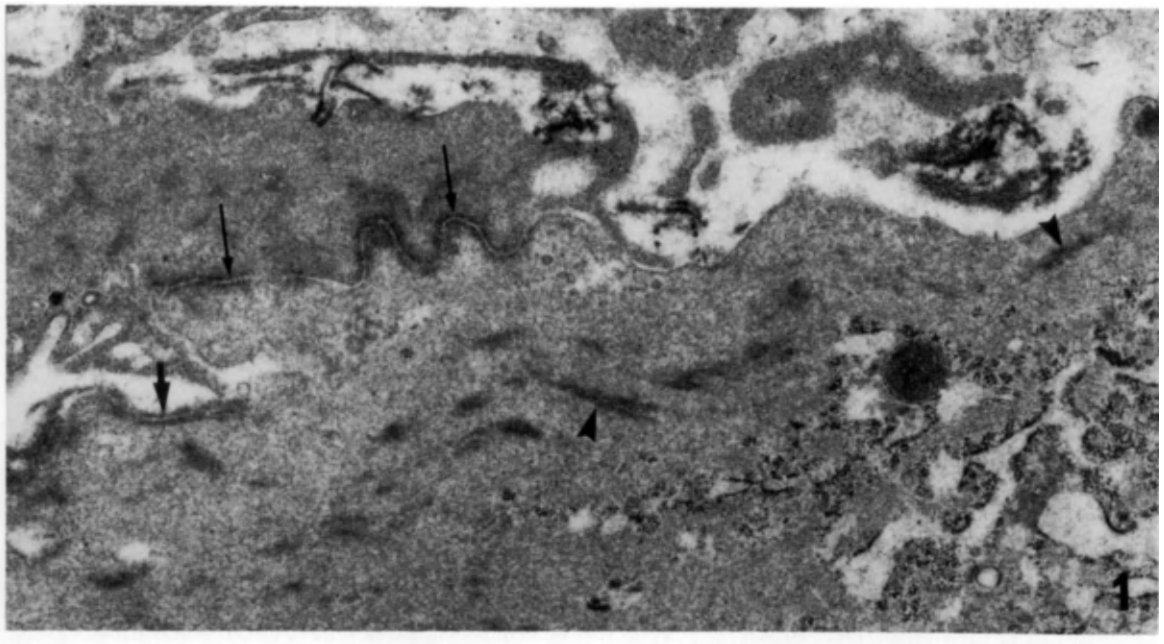
Plate 375

Myofibroblastoma. Same case as *Plate 374*

Fig. 1. Myofibroblasts showing a hemidesmosome-like structure (thick arrow), desmosome-like structures (thin arrows) and filaments with focal densities (arrowheads). × 20 000 (*From Ghadially, McNaughton and Lalonde, 1983*)

Fig. 2. Higher-power view of a hemidesmosome-like structure found in a myofibroblast. × 40 000

Fig. 3. Higher-power view of desmosome-like structures between two myofibroblasts. × 49 000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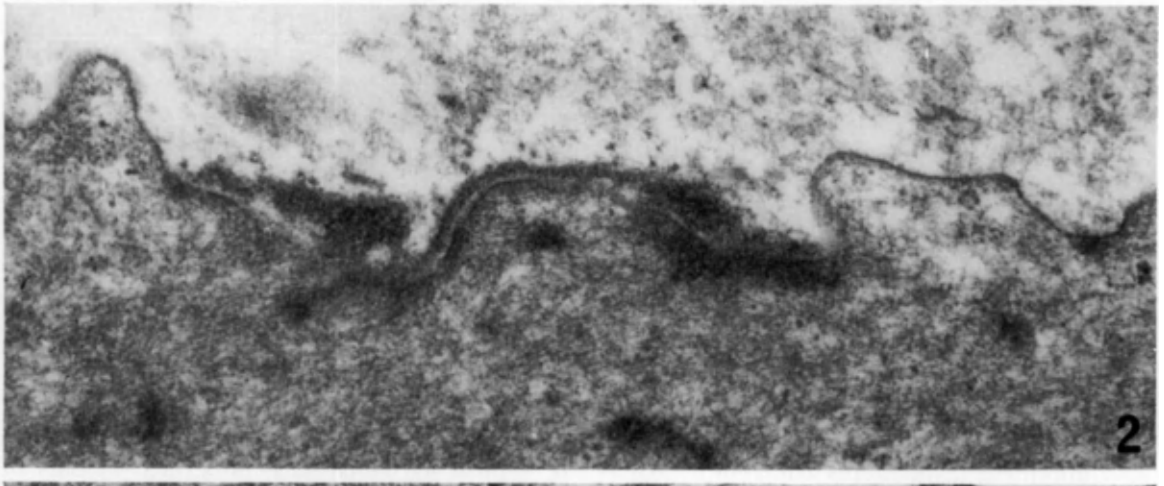
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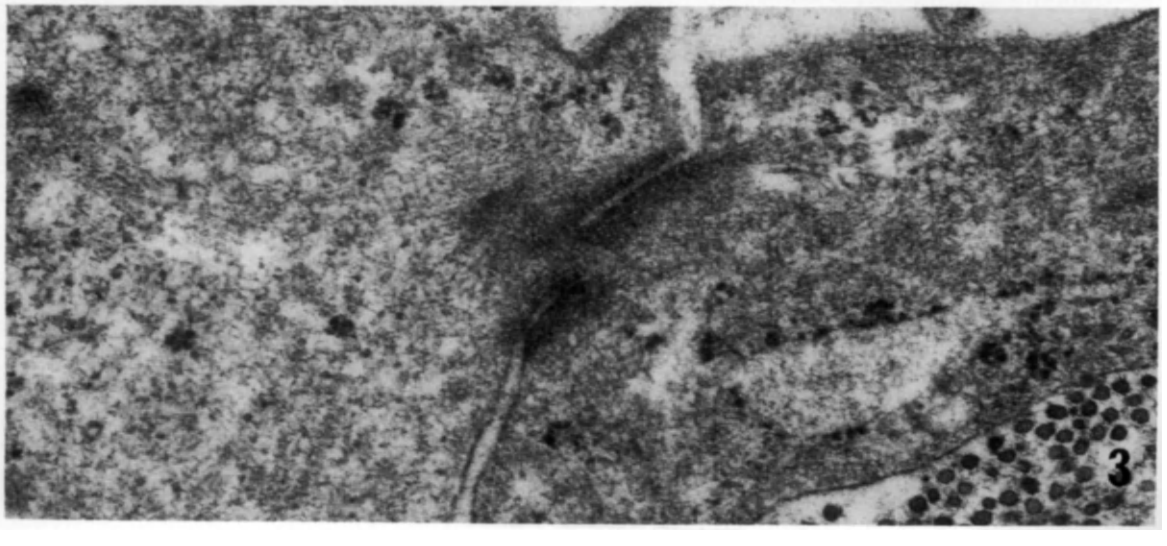

3

Intermediate filaments in normal and pathological states (including neoplastic)

Terms such as '100 Å filaments', '10 nm filaments', 'intermediate-sized filaments ' or 'intermediate filaments' are used to describe intracytoplasmic filaments comprising a major filamentous system in the cytoplasm of cells. As their name (intermediate filaments) implies the approximately 10 nm diameter (6–12 nm) of these filaments is between that of the thin (4–7 nm) filaments containing actin and the thick (11–16 nm) filaments containing myosin.

In the past these intermediate filaments were referred to as 'other intracytoplasmic filaments' that is to say filaments different from those containing actin or myosin, though the feeling persisted that some of them might be actin or myosin filaments, after all. Myofilaments subserve a contractile function, but the general consensus of opinion now is that intermediate filaments have a cytoskeletal function. Lazarides (1980) looks upon intermediate filaments as 'integrators of cellular space'. This view is in keeping with the morphology and distribution of these filaments, particularly in muscle cells (*see below*). One may look upon intermediate filaments as forming a framework or skeleton within the cell which helps to maintain the position and distribution of various organelles and inclusions within the cytoplasm.

Biochemical and immunological studies have cleared up many past doubts about the nature of these intermediate filaments. On the basis of the principal proteins (or polypeptides) present in these filaments five major types of intermediate filaments have been identified. These include: (1) intermediate filaments containing prekeratin or prekeratin-like proteins called cytokeratins (Franke *et al.*, 1978a, b). These filaments have long been known as tonofilaments or prekeratin filaments; (2) intermediate filaments containing the protein vimentin (Franke *et al.*, 1978a, b) which has a molecular weight of about 57 000 (Gordon *et al.*, 1978; Tuszynski *et al.*, 1979; Osborn *et al.*, 1980) These filaments are the predominant but not the only class of filaments found in many mesenchymal cells, such as chondrocytes* and vascular endothelial cells. However, these filaments have also been found in cultured cells of epithelial, myogenic and neurogenic origin. Furthur, it is thought that in some cases at least vimentin behaves as a maturation marker. For example, immature muscle cells and glial cells express vimentin, which is later replaced by the mature cell-specific intermediate filaments, namely the filaments containing desmin and glial fibrillary acid protein respectively; (3) intermediate filaments containing the protein desmin (also called 'skeletin') which has a molecular weight of 50 000–55 000. These are characteristically found in the muscle cells (skeletal, cardiac and smooth), but they are absent in some vascular smooth muscle cells (for references *see* page 900); (4) intermediate filaments containing neurofilament proteins which have molecular weights of 200 000, 150 000 and 68 000 (for references *see* page 888). Neurofilaments occur in most but not all neurons; and (5) intermediate filaments containing glial fibrillary acid protein which has a molecular weight of 51 000. They are found in astrocytes (for references *see* page 888).

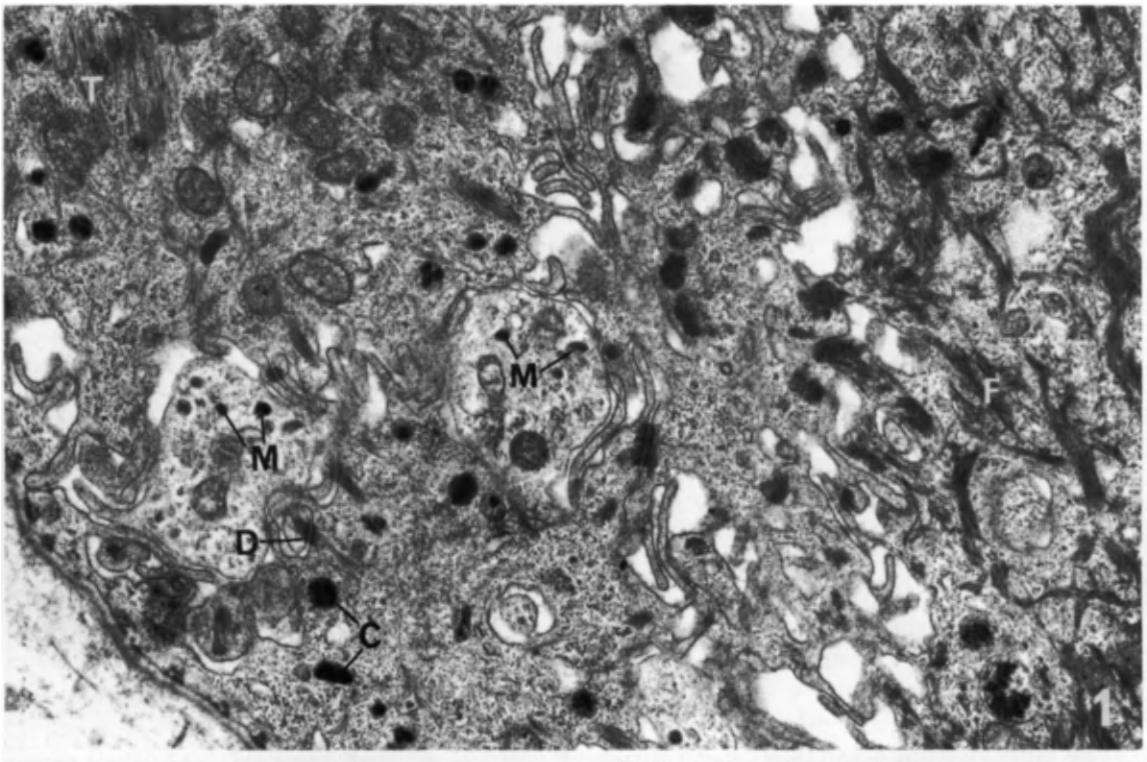
*In much of the literature, identity with one or the other of the five basic types of filaments is assumed simply on the basis of where such filaments are found. This is what I have often done in this chapter for there is no other sensible workable alternative. For example, in the legend to *Plate 381* the filaments in chondrocytes are described as vimentin filaments, not on the basis of immunocytochemical studies on that particular specimen but on the basis of current knowledge about the subject. The situation here is analogous to that which prevails with lysosomes. At first, it was mandatory to demonstrate at least one hydrolytic enzyme (i.e. acid phosphatase) before a structure could be labelled a lysosome. Today, however, we identify them on the basis of morphology, site of occurrence and knowledge acquired from past studies.

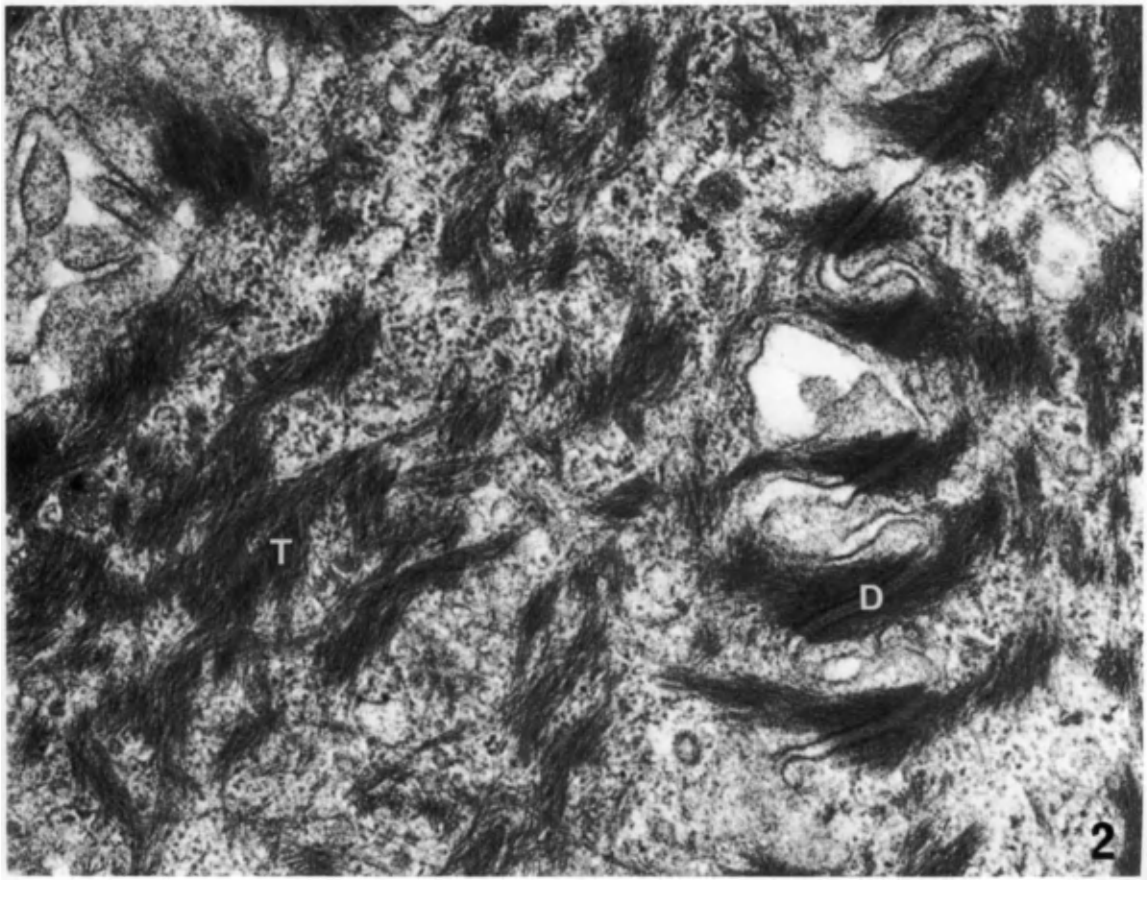
Plate 376

From normal human skin.

Fig. 1. This electron micrograph shows a basal cell containing loosely arranged tonofilaments (T) and a prickle cell containing tonofibrils (F) where the individual filaments are difficult to discern. Desmosomes (D) are also evident. Note the transversely cut melanocyte dendrites containing melanosomes (M) and the much larger, compound melanosomes (C) in the keratinocytes. (*See also Plate 334*) × 18 500

Fig. 2. High-power view of tonofilaments (T) and desmosomes (D). × 47 000


T
M
M
F
D
C
1


T
D
2

Although some small morphological differences do exist (*see below*) between different types of intermediate filaments, they are not constant or characteristic enough to permit an electron microscopist to unequivocally identify the different types of intermediate filaments in every instance. Indeed at times it is difficult even to be certain as to whether one is looking at intermediate filaments or not. For example, if faced with filaments of about 7-8 nm diameter one would wonder whether they were rather thick actin filaments or rather slender intermediate filaments. In this connection it is worth noting that preparative procedures probably affect the thickness of the filament★. The problem is also compounded by the fact that at high magnifications sections through filaments do not have a sharp margin (a point often aggravated by imperfect focusing) so that accurate objective measurements are difficult to obtain. (Thus observer differences also affect the reported values of filament thickness.) An important prerequisite is that the electron optical magnification be monitored and the optical enlargement checked by suitable methods (for details about this *see* Ghadially *et al.*, 1972). Often this is not done (or not mentioned), so that the literature is plagued with doubt and dubious data about the diameter of various filaments.

However, various other factors besides the diameter of the filament give a clue about the nature of intracytoplasmic filaments, and it is matters such as these, and the changes engendered by pathological processes which form the topic of this section.

The myofilaments† of striated muscle are easily recognized by their highly ordered arrangement (this is what produces the striated appearance). The myofilaments of smooth muscle are characterized by their orderly arrangement and the occurrence of focal densities along their course.

Tonofilaments particularly tonofilaments of squamous epithelia (*Plates 376* and *377*) tend to be more electron dense than other varieties of intermediate filaments. They also have a strong tendency to form bundles or sheaves (i.e. tonofibrils). Tonofilaments are often seen converging upon and looping through the plaques of desmosomes‡ (Kelly, 1966).

Tonofilaments are called 'keratin filaments' or 'keratin-like filaments' by some authors because of the mistaken notion that they are composed of keratin or resemble keratin (*see* for example *Fig. 1.8* and accompanying legend in Carr *et al.*, (1977) where obvious tonofibrils converging on desmosomes in thymic epithelial cells are described as 'aggregates of microfibrils resembling keratin'). This is not correct, for tonofibrils are not rich in sulphur as is keratin, and such filaments occur even in non-keratinizing epithelia, and indeed in virtually every variety of epithelial cell. The formation of true keratin, say for example in the skin, involves the deposition of an electron-dense substance called 'keratohyaline' (contains proline and amino

★*See* footnote on page 840.

†Myofilaments occur in cells other than muscle cells. This has been dealt with on pages 866–881.

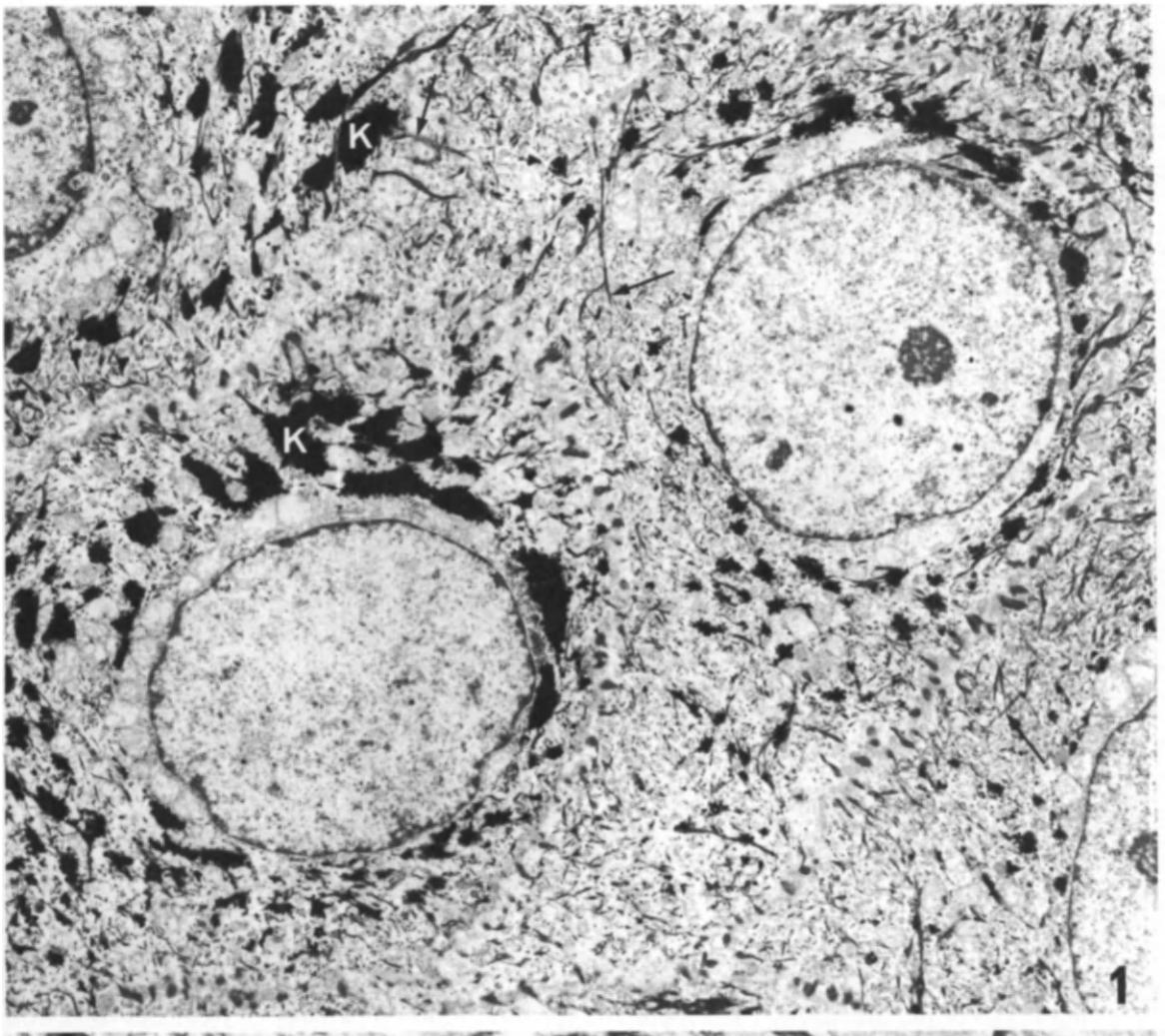
‡Some people speak about 'tonofilaments attached to desmosomes' but this is not strictly correct, for most of the filaments seem to loop through the plaques of desmosomes. Hence it is better to speak about 'tonofilaments converging upon desmosomes' as does Fawcett (1966).

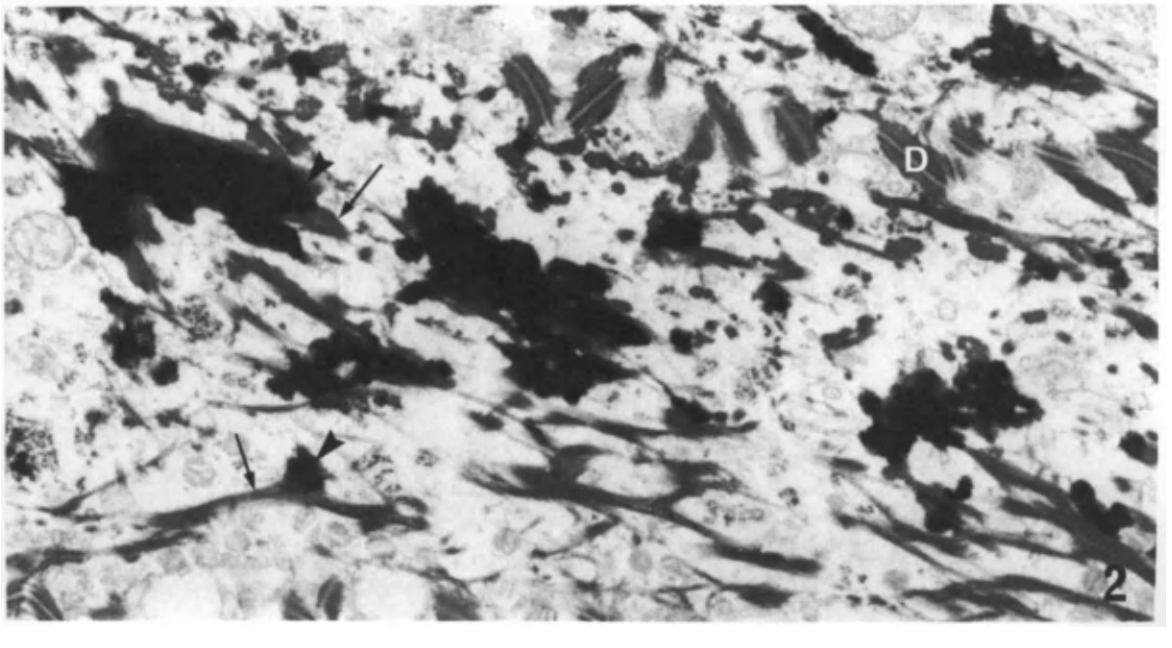
Plate 377

From a facial wart.

Fig. 1. Keratinocytes containing numerous tonofibrils (arrows) and keratohyaline granules (K). × 5500

Fig. 2. High-power view demonstrating that keratohyaline granules are formed by the deposition of electron-dense keratohyaline (arrowheads) in and around tonofibrils (arrows). Some altered desmosomes (D) are also present. × 20000


K
K
1


D
2

acids rich in sulphydryl groups) between and around tonofilaments (this forms large, irregular electron-dense masses called keratohyaline granules) which serve as a scaffolding for its deposition (for more details *see* Breathnach, 1971; Rhodin, 1974; Weiss and Greep, 1977). To circumvent such problems, the term 'prekeratin filaments' is used by some authors, while Franke *et al.* (1978a) have proposed the term 'cytokeratins' for prekeratin-like polypeptides in these filaments. They state that they 'have chosen this term in order to emphasize their nature as components of living cells that are not keratinizing'. They also point out that 'the cytokeratins are synthesized in several epithelial cell types capable of division and growth, and therefore cannot be regarded as exclusive to, and indicative of, the irreversible differentation of keratinizing cells'.

I agree with these sentiments entirely and heartily deplore those who call these filaments 'keratin filaments' or 'keratin-like filaments' (*see below*). Nevertheless, I can see no overwhelming reason for totally casting aside so time-hallowed a term as 'tonofilaments', which seems adequate as long as one remembers that keratin is not synonymous with tonofilaments.

Tonofibrils reach their maximum prominence in cells of squamous epithelia, and this characteristic usually persists (albeit in an attenuated form) in tumours derived from such epithelia. Tonofibrils are more frequently encountered and better developed in well differentiated squamous cell carcinoma (Ghadially, 1985). However, even in poorly differentiated versions of this tumour, such as those found in the nasopharynx, it is possible to find a few characteristic tonofibrils and desmosomes (Michaels and Hyams, 1977). Hence this point is of diagnostic value. In the basal cell naevus syndrome the epithelial cells come to contain quite large 'lumpy tonofilament bundles' and keratohyaline granules (Ullman *et al.*, 1972) while quite large 'rounded tonofilament aggregates' and a 'profound disturbance of the tonofilament system' have been described in the oral lesions of the white sponge naevus (leukoedema exfoliativum mucosa oris) a hereditary dyskeratotic hyperplasia affecting the mucous membranes (Frithiof and Bánóczy, 1976). Similar aggregates of tonofilaments have also been demonstrated in three cases of erythrodermia ichthyosiformis congenita bullosa Brocq (Ishibashi and Klingmüller, 1968a, b, c).

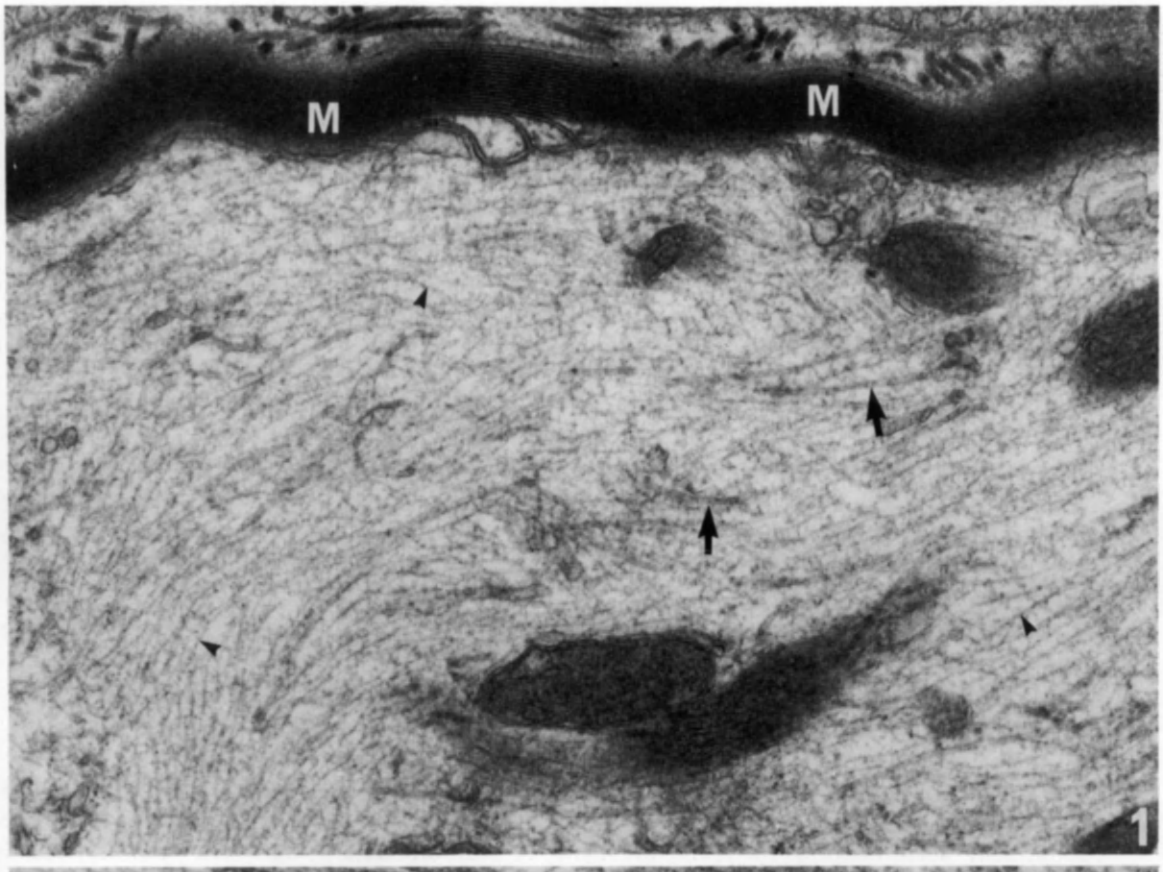
The sparse cytokeratin filaments in adenomatous epithelia as a rule do not impinge on our consciousness but in some pathological states quite large accumulations of such filaments are found. Examples of this are the irregular-shaped and rounded masses of cytokeratin filaments seen in Mallory's bodies (*see Plates 386* and *387*) and the rounded masses of filaments seen in GH-cell pituitary adenomas (*see Plate 388*).

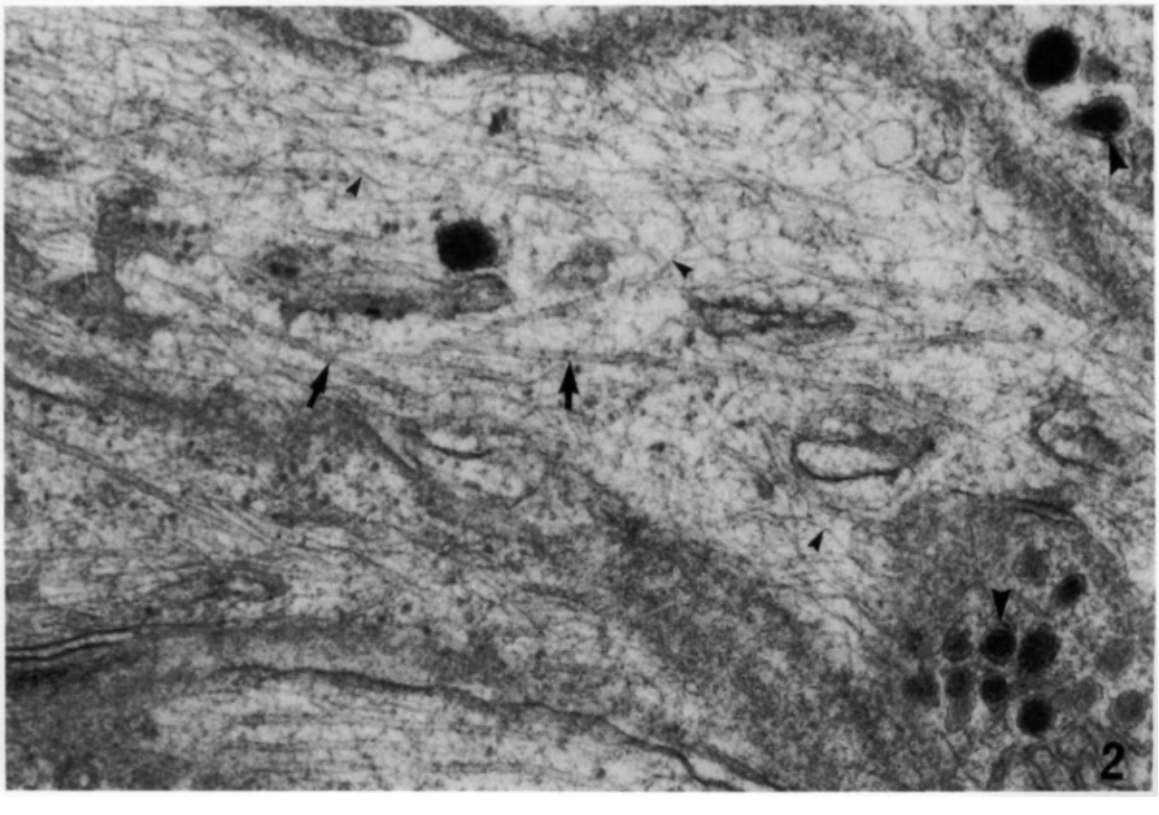
Neurofilaments (*Plate 378*), containing neurofilament protein where the major polypeptides have molecular weights 200 000, 150 000 and 68 000, occur in neurons, tumours of neurons (e.g. neuroblastoma and ganglioneuroma) and some neuroendocrinomas (e.g. Merkel cell carcinoma of skin). Some neurofilaments (about 10 nm in diameter) are seen in the body of neurons, but they occur mostly in myelinated axons and also larger dendrites. They almost invariably occur in company with microtubules (called 'neurotubules' by some). At times neurofilaments have a knobbly appearance or appear to have small side arms arising from them. However, negatively

Plate 378

Fig. 1. From oculomotor nerve of rat. Part of an obliquely or almost longitudinally-cut single myelinated nerve fibre showing myelin sheath (M), axon containing neurofilaments (arrowheads) and microtubules (arrows). × 38 000

Fig. 2. Ganglioneuroma of mediastinum. Seen here are axons containing neurofilaments (small arrowheads), microtubules (arrows) and neurosecretory granules (large arrowheads). × 52 000


M
M
1


2

stained preparations of isolated neurofilaments show them to be straight smooth filaments like intermediate filaments in other sites. In passing it is worth noting that this combination of neurofilaments, neurotubules and neurosecretory granules is helpful in distinguishing neuroblastoma from other neoplasms such as Ewing's tumour and lymphoma (for details *see* Ghadially, 1985).

An increase in the number of filaments in human neurons has been reported to occur as a result of ageing, Alzheimer's disease*, sporadic motor neuron disease, infantile neuroaxonal dystrophy, vincristine neuropathy, Pick's disease and in neurons of experimental animals in aluminium encephalopathy, spindle inhibitor encephalopathy, lathyrogenic encephalopathy, vitamin E deficiency, copper deficiency and retrograde and Wallerian degeneration (Terry and Pena, 1965; Wisniewski *et al.*, 1970; Terry, 1971; Selkoe *et al.*, 1979). The general consensus of opinion is that such an increase in filaments is a degenerative or regressive change and that endogenous (genetic) and exogenous (various noxious agents, e.g. aluminium toxicity might be responsible in some instances) factors may be responsible.

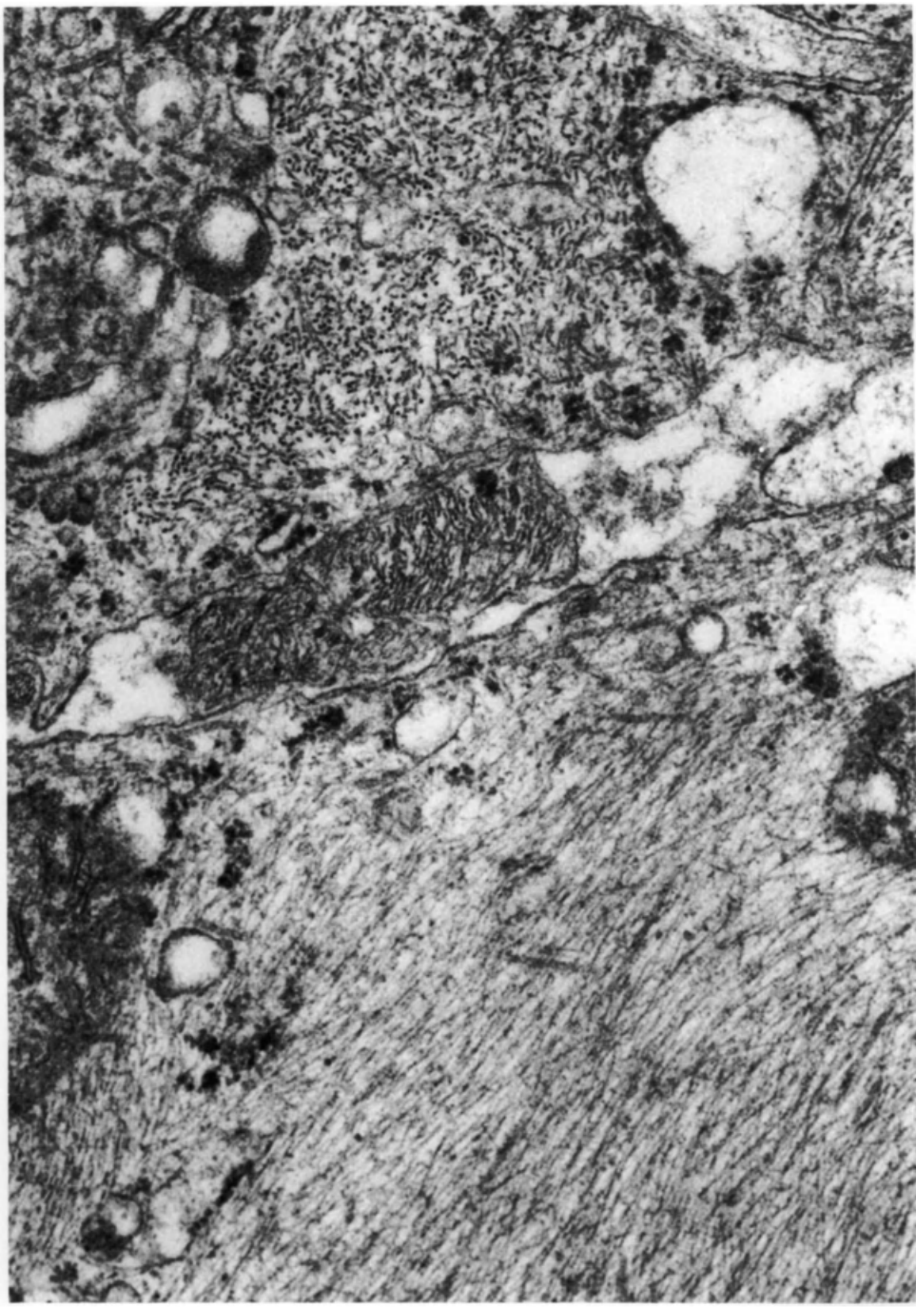
The problem of separating glial filaments from neurofilaments and raising specific antibodies against each of them produced at first a controversial literature. Earlier studies suggested that the major proteins of glial filaments were similar or identical to those in neurofilaments, but later studies with improved separation techniques indicate that such a view is incorrect and that glial filaments contain glial fibrillary acid protein (molecular weight 49 000–51 000) which is quite different from the proteins (polypeptides) found in neurofilaments (Goldman *et al.*, 1978; Selkoe *et al.*, 1979; Borenfreund *et al.*, 1980; Lazarides, 1980).

Glial filaments (*Plate 379*) occur chiefly as bundles of filaments (8–9 nm) in cell bodies and processes of astrocytes. They are a prominent component of gliosed scar tissue resulting from various pathological processes. For example, an increase in glial filaments has been seen in: (1) adrenoleucodystrophy (Schaumburg *et al.*, 1975; Goldman *et al.*, 1978); and (2) in the Jimpy and Staggerer mouse (a demyelinating disease) (Skoff, 1976, Lee *et al.*, 1977). Perhaps the most prominent accumulations of such filaments is seen in certain astrocytic gliomas called 'spongioblastomas'. They are characterized by a peculiar type of cell degeneration which leads to the accumulation of material known as 'Rosenthal fibres'. Ultrastructural studies show that these 'fibres' comprise granular and filamentous masses (intermediate filaments) (Gullotta and Fliedner, 1972) which one might presume are glial filaments. Thus as in the case of filaments in neurons an accumulation of glial filaments also indicates a degenerate cell. Tissue culture studies on spongioblastomas show that there is a stage of increased enzyme activity and protein synthesis in the stages which precede cell degeneration but once the Rosenthal fibres are formed these degenerate cells are devoid of enzyme activity (Gullotta and Fliedner, 1972).

*It is generally believed that the filaments in the neurofibrillary tangles of Alzheimer's disease are different from normal neurofilaments found in humans and other animals and also the filaments in the filamentous accumulations produced by experimental manoeuvres in animals. The basic structure seen in the Alzheimer-type of neurofibrillary tangle is referred to as 'paired helical filaments'. In ultrathin sections the pair of helical filaments present a profile 20 nm wide with constrictions about 10 nm wide at 80 nm intervals. (The 20 nm width represents places where the two spiralling 10 nm filaments are lying side by side and the 10 nm constrictions are places where the filaments are lying on top of one another in the section thickness.) Monoclonal antibodies show that neurofibrillary tangles and neurofilaments share some but not all antigenic determinants (Anderton *et al.*, 1982; Dickson *et al.*, 1985; Yen *et al.*, 1985). The species specificity (i.e. seen only in brains of humans with Alzheimer's disease) of paired helical filaments is probably not quite as absolute as some believe because paired helical filaments have been seen in: (1) very old rats (Knox *et al.*, 1980, van den Bosch de Aguilar and Goemaere-Vanneste, 1984); and (2) giant neurons of the whip spider (Foelix and Hauser, 1979).

Plate 379

Astrocytoma. The cytoplasm of these tumour cells is packed with innumerable filaments. Most of the filaments in the cell at the top are cut transversely, while longitudinally sectioned filaments are prominent in the cell occupying the bottom part of the electron micrograph. × 59 000



As mentioned earlier intermediate filaments containing desmin occur in muscle. It has long been suspected that a cytoskeletal filamentous framework which holds the myofilaments in place exists in muscle cells (skeletal, cardiac and smooth). We now know that intermediate filaments containing desmin subserve this function (Allen and Pepe, 1965; Ishikawa *et al.*, 1968, 1969; Rash *et al.*, 1970; Cooke and Chase, 1971; Uehara *et al.*, 1971; Somlyo *et al.*, 1973; Cooke, 1976). However, these filaments are quite difficult to find in routine preparations of adult skeletal muscle (Kelly, 1969), but they are more easily seen in embryonic striated muscle and in smooth muscle (adult or embryonic).

In smooth muscle these filaments form a network which links the focal densities on the actin filaments with the subplasmalemmal densities. In skeletal muscle these filaments have a mainly transverse orientation (i.e. at right angles to the myofilaments) linking the myofibrils to the cell membrane at the Z, N and M lines.

The predominant protein in this cytoskeleton of intermediate filaments in muscles is desmin (Greek noun meaning link or bond) (Lazarides and Hubbard, 1976; Hubbard and Lazarides, 1979; Lazarides, 1980). An identical protein isolated from smooth muscle was called 'skeletin' by Small and Sobieszek (1977). Intermediate filaments presumably of the desmin variety are said to be more prominent in hypertrophied human myocardial cells (Ferrans and Roberts, 1973) and in myocardial cells of animals treated with anabolic steroids (Behrendt, 1977). However, perhaps the most striking accumulation of desmin filaments (mainly in myocardium and to a much lesser extent in skeletal muscle) is that reported by Porte *et al.* (1980) in three cases of a familial cardiomyopathy. At light microscopy oval or rounded PAS positive bodies (several μm in diameter) were seen in the muscle fibres. Electron microscopy showed that the hyaline bodies were composed of fascicles or whorls of intermediate filaments intermingled with glycogen particles (*Plate 380*). There was marked destruction of neighbouring myofibrils. Immunofluorescence studies showed that the filamentous masses were positive for desmin and negative for vimentin and cytokeratin (Stoeckel *et al.*, 1981).

As noted earlier intermediate filaments composed of vimentin (L, *vimentus* meaning wavy) occur principally in cells of mesenchymal origin, particularly in chondrocytes and endothelial cells (*Plates 381* and *382*). When few, they may be diffusely scattered in the cell, but when many they may present as a wavy fascicle of filaments, as a perinuclear collar of filaments or as a juxtanuclear crescentic, triangular, or cap-shaped aggregate of filaments (called 'nuclear cap') or as a spherical mass of whorled or randomly scattered filaments (called 'globular filamentous body'*). At times vimentin filaments may appear to arise as bushy outgrowths from the nuclear envelope, a mitochondrion or a lipid droplet. Such images are explicable on the basis of sectioning geometry and superimposition but the possibility that true continuity between the filaments and such structures exists cannot be ruled out. There is no real proof that such filaments branch although appearances mimicking branching are at times seen (*see* comments on page 840).

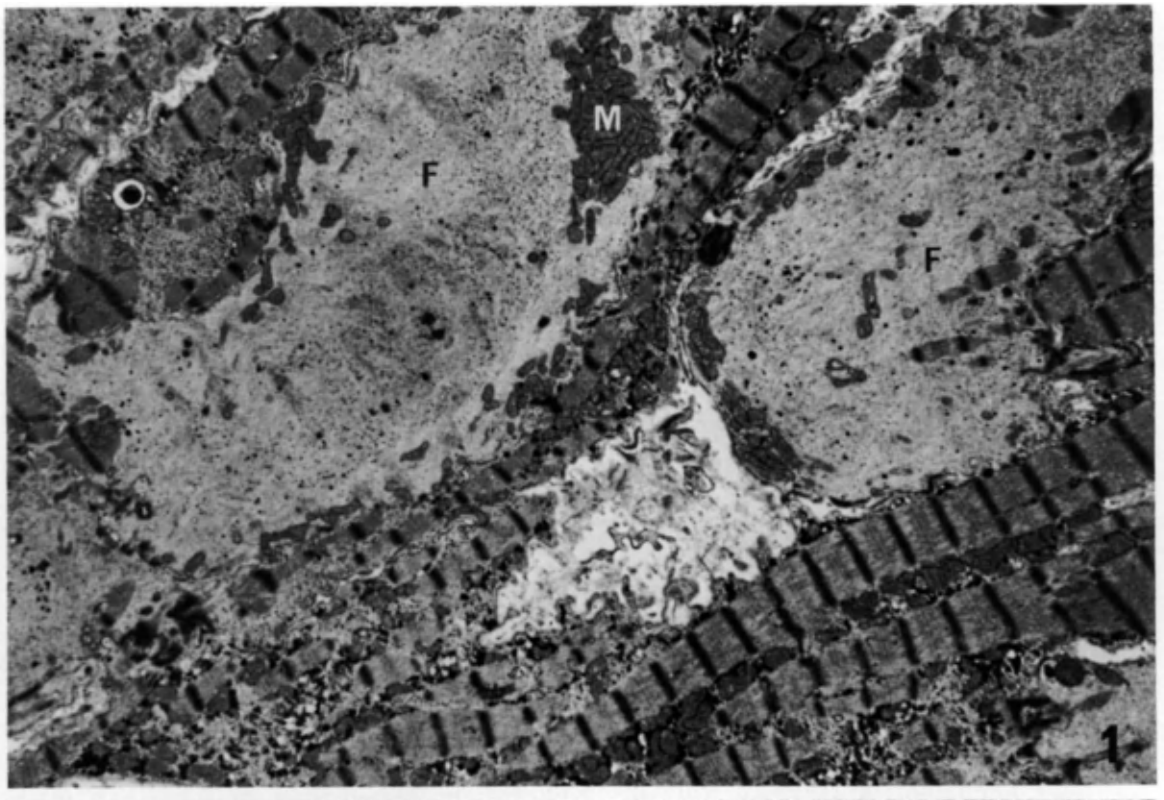
*Globular filamentous bodies are usually, but not invariably, found in the juxtanuclear position. Although these bodies are usually composed of vimentin filaments, such bodies composed of other types of filaments also occur. There has been much speculation in the literature (particularly that dealing with tumours) about the nature and diagnostic significance of these bodies. Therefore, a separate section in this chapter has been devoted to this topic (*see* pages 906–911).

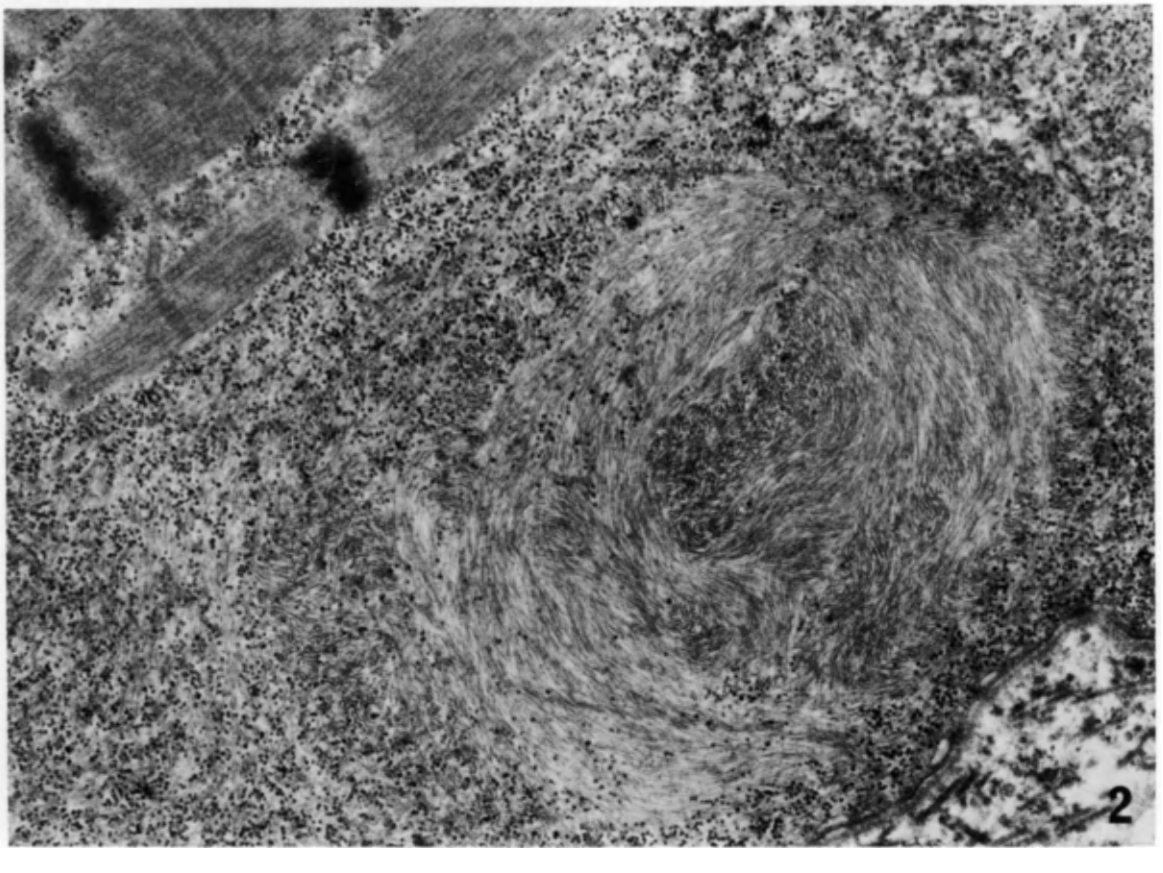
Plate 380

A case of unusual familial cardiomyopathy. (*From Porte, Stoeckel, Sacrez and Batzenschlager, 1980*)

Fig. 1. Biopsy of cardiac muscle showing large filamentous masses (F) replacing myofibrils. The mitochondria (M) are aggregated and displaced to the periphery of the filamentous aggregates. The filaments within the filamentous areas are not resolved at this low magnification. ×2500

Fig. 2. Biopsy of skeletal muscle (intercostal) showing a muscle fibre containing a subplasmalemmal globular filamentous body. × 25 000


M
F
F
1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Filaments of the above-mentioned morphology when seen in cells of mesenchymal origin (except muscle cells) may now be reasonably safely regarded as vimentin-containing intermediate filaments*. Lazarides (1980) points out 'one of the most interesting and characteristic properties of vimentin-containing intermediate filaments is their aggregation into filamentous bundles in the form of a cap or a ring in cells exposed to colcemid'. This finding originally prompted the belief that these intermediate filaments were formed from the depolymerized products of microtubules. Such an explanation is no longer tenable because we now know that the subunits of microtubules and filaments are quite different (*see below* for similar situation with regard to neurofilaments and microtubules).

We noted earlier that tonofilaments converging upon the plaques of desmosomes are composed of cytokeratins. However, an exception to this are the desmosomes found in meningiothelial meningioma where the filaments are composed of vimentin. Further, in this tumour some of the tumour cells are at times packed with vimentin filaments which decussate to form an irregular meshwork (*Plate 383*) rather than the wavy fascicles or the juxtanuclear or perinuclear aggregates or the whorls formed by vimentin filaments in other sites (*Plates 381* and *382*).

As noted earlier, vimentin-containing filaments occur not only in a wide variety of mesenchymal cells but also in cultured cells of epithelial, myogenic and neurogenic origin. Therefore it is conceivable that in certain pathological states vimentin filaments may develop in virtually any cell type. We have also seen that actin filaments occur in many cell types besides muscle cells and that they could be confused with intermediate filaments. Such considerations combined with the fact that in many pathological states the accumulations of intracytoplasmic filaments have not been characterized makes it difficult to list exactly the situations in which an increase in a particular type of filament occurs. Nevertheless we will now look at a variety of quite interesting observations from the pathological point of view in which one might reasonably suppose that an increase in intermediate filaments (usually but not invariably vimentin) had occurred.

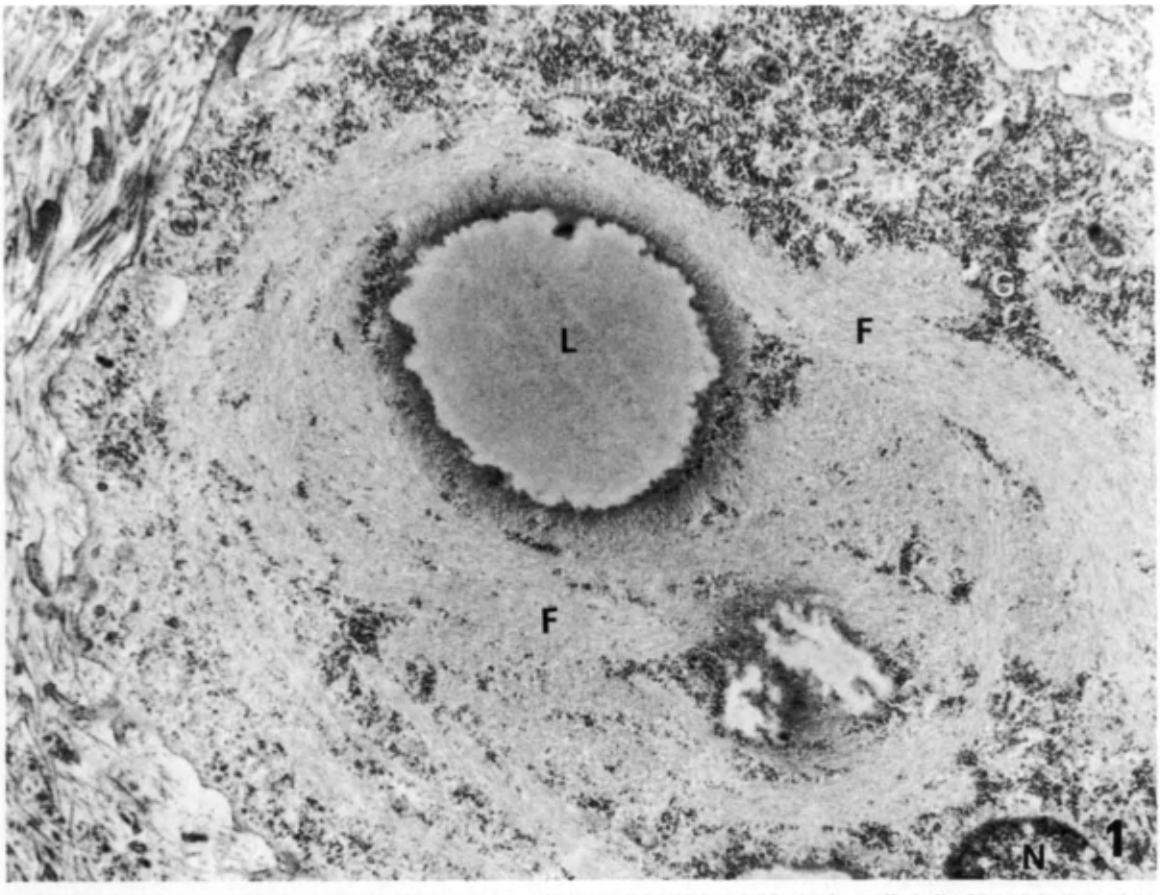
An increase in intermediate filaments (presumably vimentin) has been noted in: (1) chondrocytes of rabbit articular cartilage associated with ageing (Barnett *et al.*, 1963); (2) chondrocytes of rabbit articular cartilage after experimentally produced chronic haemarthrosis (Roy, 1968); (3) chondrocytes of rabbit articular cartilage after experimentally produced lipoarthrosis (Ghadially *et al.*, 1970); (4) chondrocytes of rabbit articular cartilage after repeated injections of blood and autologous fat (from the mesentery) into the joint (Ghadially and Mehta, unpublished observation) (*Plate 381*); (5) chondrocytes of rabbit articular cartilage after injection of sodium aurothiomalate into the joint (Ghadially and Lalonde, 1978); (6) chondrocytes of rabbit articular cartilage after production of superficial defects (Fuller and Ghadially, 1972); (7) chondrocytes of human articular cartilage associated with age, osteoarthritis and chondromalacia (Meachim and Roy, 1967; Zimny and Redler, 1969; Ghadially, 1983); (8) synovial intimal cells in rheumatoid arthritis (for references *see* Ghadially and Roy, 1967, 1969); (9) vascular endothelial cells of synovial vessels and cutaneous vessels in

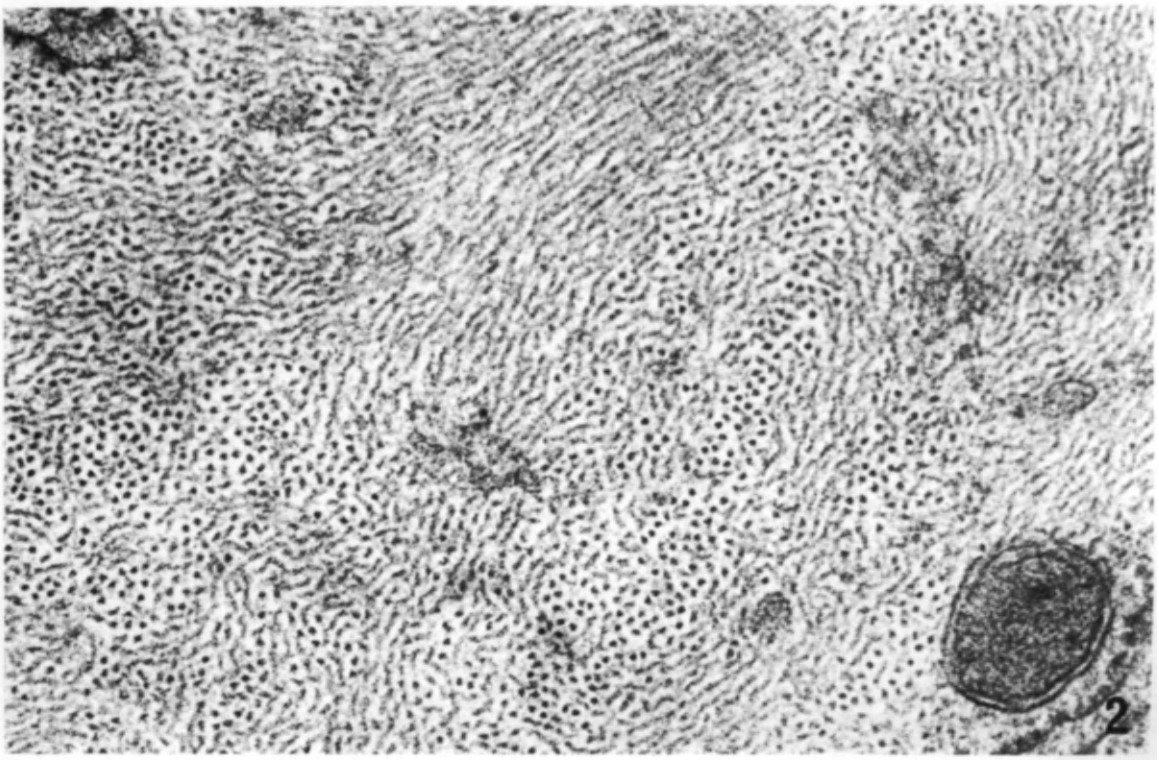
*Some studies (for references *see* Lazarides, 1980) show that the caps (i.e. juxtanuclear aggregates of filaments) contain both vimentin and desmin. It is not known whether vimentin and desmin copolymerize into a single filament or whether two distinct types (vimentin and desmin) of filaments are present.

Plate 381

Fig. 1. Chondrocyte from articular cartilage of a rabbit that had received repeated injections of blood and autologous fat into the knee joint. The cytoplasm is packed with vimentin filaments (F), some of which appear to radiate from a lipid droplet (L). Also seen are many glycogen particles (G) and part of the nucleus (N). The usual cell organelles are not evident in this plane of sectioning. × 15 000

Fig. 2. High-power view of vimentin filaments in a chondrocyte. From rabbit articular cartilage where a partial thickness defect had been made three months prior to collection of the specimen. × 85 000


L
F
F
N
1


2

rheumatoid arthritis (Ghadially and Roy, 1969; Ghadially *et al.*, 1978b) (*Plate 382*). Here again the consensus of opinion is that an increase in intermediate filaments in chondrocytes and synovial cells is a sign of regressive or degenerative changes engendered by age or noxious influences.

The idea that an increase in filaments is a degenerative change is also supported by their reported increase in: (10) human lens epithelial cells related to ageing and pathological conditions (cataract) (Perry *et al.*, 1979); (11) ageing human cutaneous fibroblasts (Pieraggi *et al.*, 1984).

An increase in intermediate filaments (presumably vimentin) has been seen in: (12) human lymphocytes from thoracic duct as compared to those in blood (Zucker-Franklin, 1963); (13) activated lymphocytes from mixed lymphocyte cultures but not those activated by phytohaemagglutinin (Parker *et al.*, 1967); (14) lymphomas and leukaemias (including plasma cell and myeloid leukaemia) (for references, *see* Rangan *et al.*, 1971; Beltran and Stuckey, 1972). Indeed, filaments have been noted in a wide variety of experimentally produced and human tumours besides leukaemias (Lehto and Virtanen, 1978). Prominent juxtanuclear filamentous aggregates (cytokeratin) and psammoma bodies were found in a papillary adenocarcinoma of the endometrium by Hameed and Morgan (1972). These authors suggest that such aggregates of filaments provide the scaffolding for calcification and psammoma body formation, not only in the tumour studied by them but also in other tumours such as meningiomas. Furthermore, they and others who have studied filaments (vimentin) in lymphomas and leukaemias have proposed that such an increase indicates a state of heightened metabolic activity associated with an active or neoplastic state.

However, many workers have taken the contrary view and suggested that the accumulation of intermediate filaments even in tumours is a sign of degeneration and impending necrosis. Such a view has been expressed about: (15) pituitary adenomas where focal whorls of filaments (cytokeratin) occur (Cardell and Knighton, 1966; Peillon *et al.*, 1970; Schochet *et al.*, 1972; Roy, 1978); (16) Chang cells cultured with activated lymphocytes (Biberfeld, 1971); and (17) human breast carcinoma cells in culture where massive accumulation of 11 nm thick filaments occurs in declining cells in no longer dividing cultures (Tumilowicz and Sarkar, 1972).

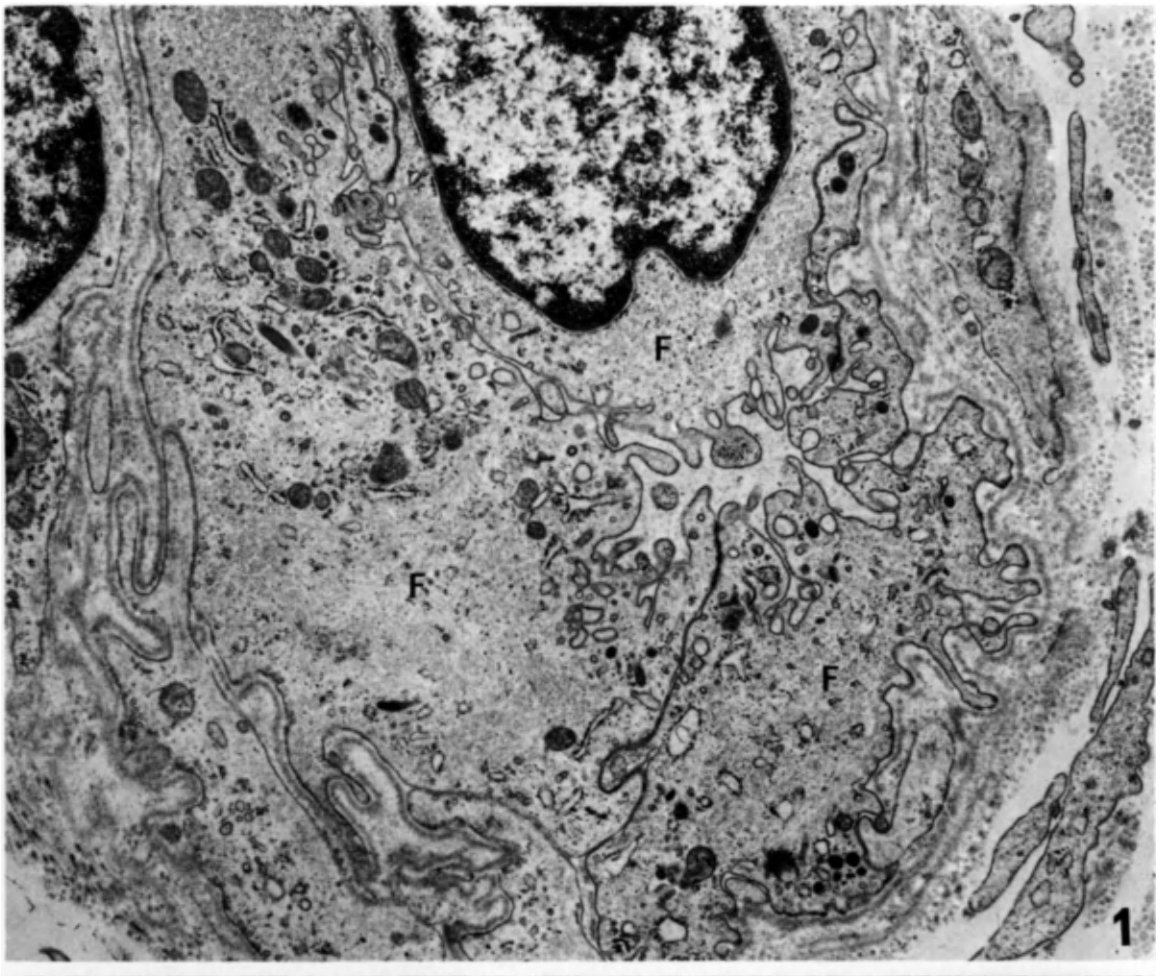
Perhaps the most compelling evidence that accumulation of filaments represents a regressive or degenerative change comes from the study of such filaments in neurons (*see below* and page 888) and here quite aptly the term 'neurofibrillary degeneration' has been coined to describe this phenomenon. My studies on articular tissues also support this idea, for the change is seen where degenerative changes may be expected or are known to occur. This is also in keeping with the observation (both in articular tissues and in neurons) that, as filaments accumulate, cell organelles are first displaced and then destroyed, so that ultimately the cytoplasm contains little besides filaments.

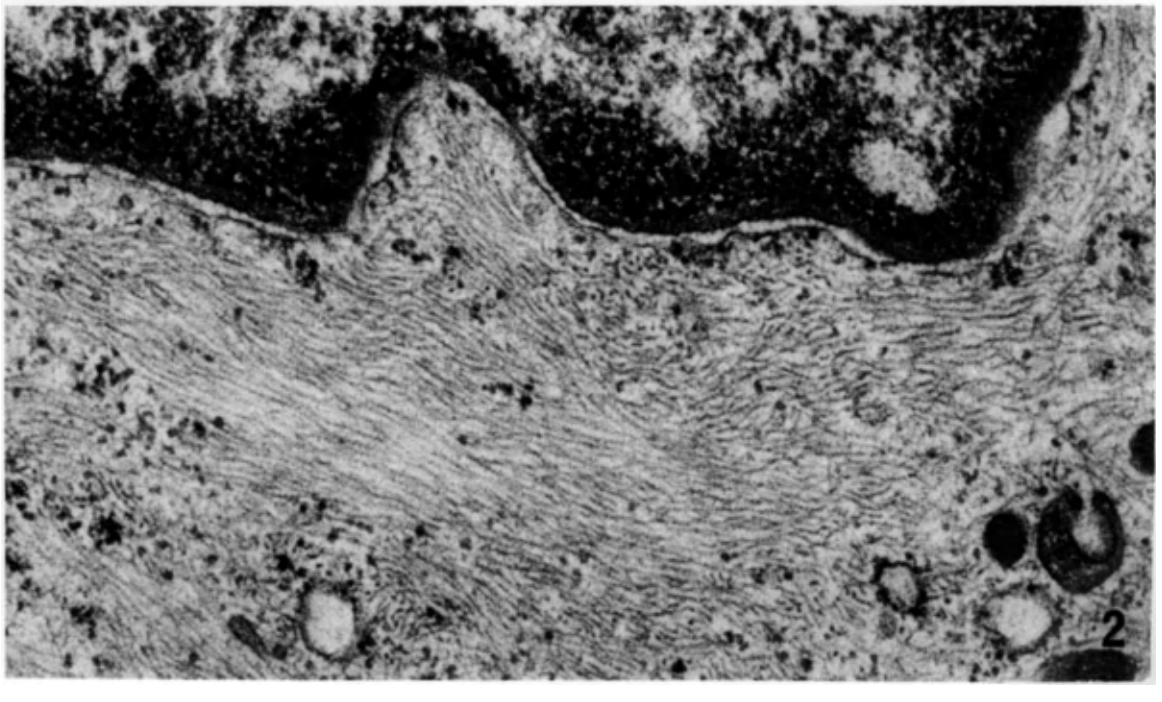
Plate 382

From the skin of a case of rheumatoid arthritis after chrysotherapy.

Fig. 1. The cytoplasmic matrix of the endothelial cells of this blood vessel contain numerous vimentin filaments (F) which are not resolved at this low magnification. Note the paucity of organelles such as mitochondria, rod-shaped microtubulated bodies and endoplasmic reticulum in these cells. × 16 500

Fig. 2. High-power view of an endothelial cell showing vimentin filaments. × 55 000


F
F
F
1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As noted earlier, the only serious dissenting vote comes from students of haemopoietic cells and their neoplasms, who consider the accumulation of filaments as a sign of heightened activity rather than degeneration. Therefore, it behoves us to re-examine the situation and see if alternative explanations can be proposed. It seems to me that in the case of lymphomas and leukaemias one can argue that, in this, as in other neoplasms, there are both populations of actively growing cells and senescent and dying cells, and it is possible that it is the latter group which accumulate intracytoplasmic filaments. The observation that 'stimulated' lymphocytes in mixed lymphocyte cultures contain increased amounts of filaments but those stimulated by phytohaemagglutinin do not, is particularly intriguing, for one can contend that this shows that stimulation *per se* is not the common operative factor which leads to filament accumulation in lymphocytes. One can also point out that the mixed lymphocyte culture is not a market garden where lymphocytes thrive and multiply but a battle ground where strains of lymphocytes are injured and killed and that it is such cells that probably accumulate filaments in their cytoplasm. Such contentions are also supported by the observation that in cultures of Chang cells and lymphocytes stimulated with phytohaemagglutinin, it is the injured and dying tumour cells which develop masses of filaments and not the attacking lymphocytes. Thus, there is now a massive body of evidence which indicates that a gross increase in intermediate filaments is a sign of regressive and degenerative changes in a variety of cell types.

An interesting observation, whose significance has long been debated, is that in encephalopathy induced by treatment with spindle inhibitors (e.g. colchicine) or in cultures of neurons treated *in vitro* with such drugs there is a prompt disappearance of microtubules and a massive replacement by neurofilaments. This change, however, is reversible, for subsequently the neurofilaments disappear and an increased population of microtubules (called 'neurotubules' by some') is seen (Wisniewski *et al.*, 1968; Terry, 1971).

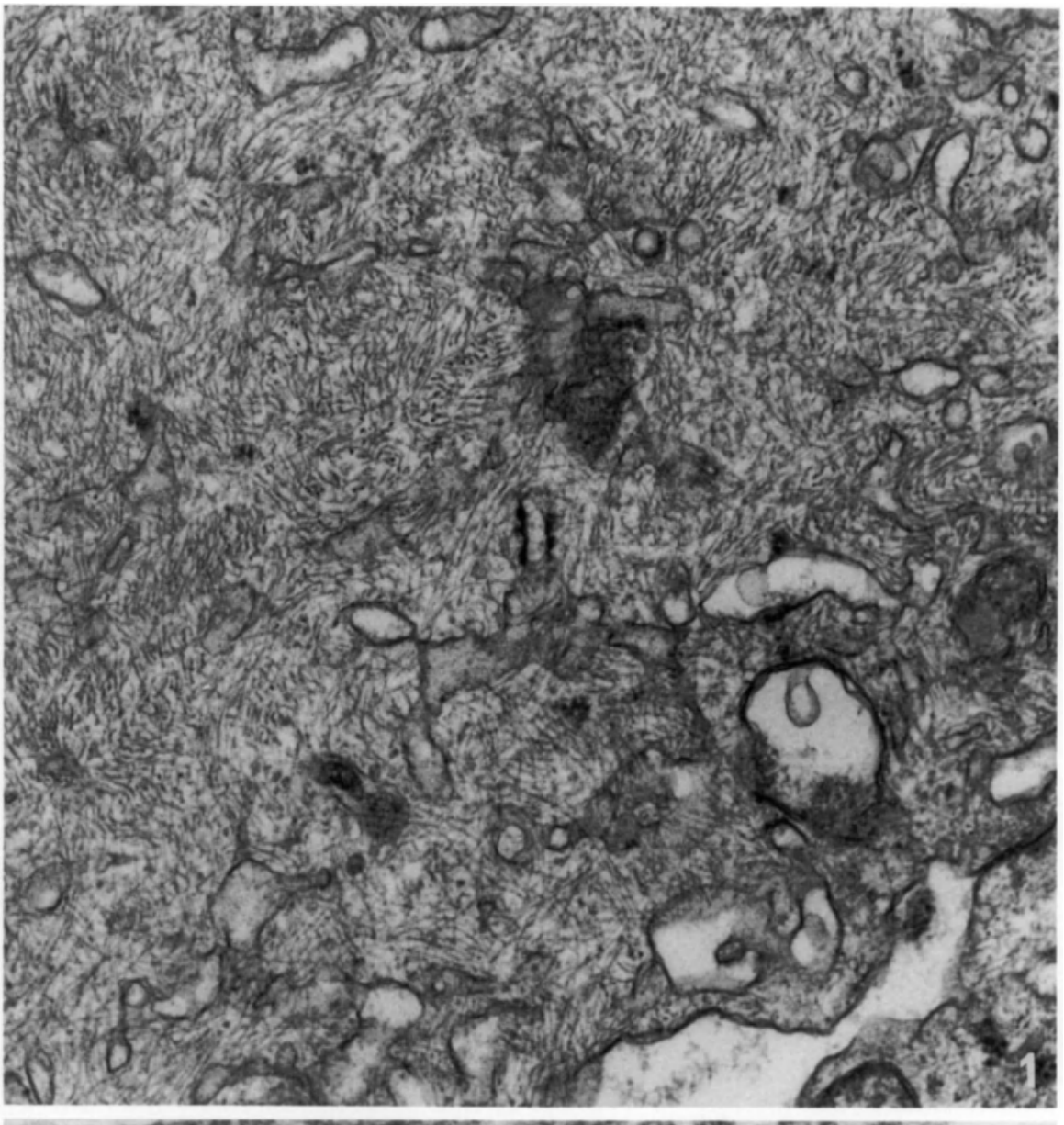
It would appear that HeLa cells in culture respond similarly to neurons when treated with colchicine (Robbins and Gonatas, 1964; Bensch and Malawista, 1969). Here too, filamentous accumulations are seen and it has been shown that the antimitotic effect of this drug is due to binding with tubulin dimers, which prevents their polymerization to form spindle microtubules. The capacity of this drug to prevent the formation of microtubules or lead to their disappearance in other cell types has also been amply demonstrated (Borisy and Taylor, 1967). The conclusion from such studies in the past was that the subunits of intermediate filaments and microtubules are probably identical and interchangeable. We now know that this is not true, and that the subunits of microtubules and intermediate filaments are quite different. More attractive is the idea that depolymerization of microtubules leads to a collapse of the cytoskeleton* and that this in turn leads to an aggregation of intermediate filaments, creating the impression that an increase in filaments has occurred.

*The cytoskeleton of most, perhaps all, cells is now envisaged as a meshwork composed of microtubules radiating from the cell centre (i.e. the juxtanuclear zone housing the centrioles and Golgi complex) intermingled with ramifying bundles of intermediate filaments and some actin filaments. The latter (actin filaments) are located principally under the cell membrane.

Plate 383

Fig. 1. Meningioma. A tumour cell containing vimentin filaments. × 45 000

Fig. 2. Meningioma. Same specimen as *Fig. 1.* A higher-power view showing hemidesmosomes (arrows) and associated vimentin filaments. × 110 000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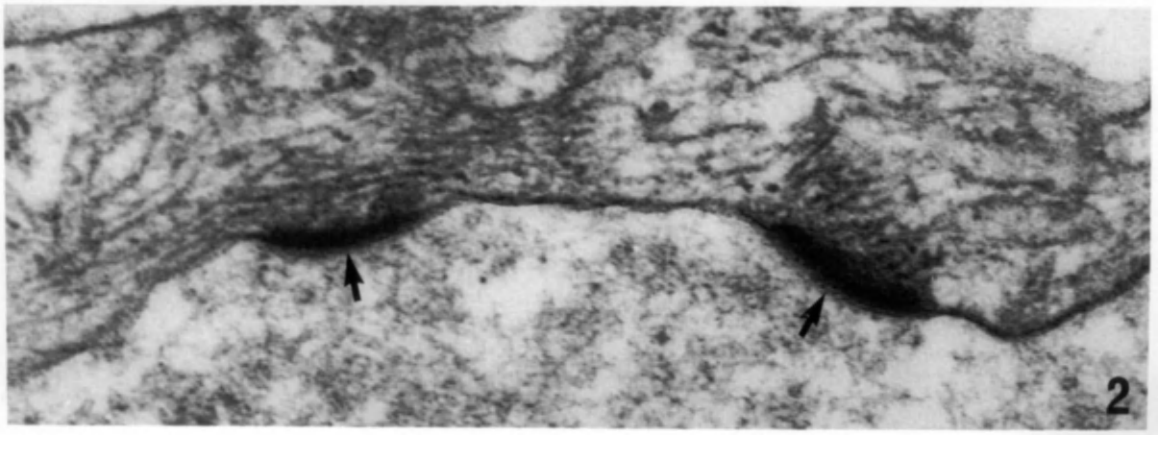
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A few intermediate filaments are at times seen in monocytes and macrophages. These filaments have a wavy disposition and generally lie in the juxtanuclear position. This morphology is in keeping with the fact that they are vimentin filaments. However, at times these cells contain fibrils composed of quite straight rigid-looking filaments (of unknown nature) in their cytoplasm (*Plate 384*). I have seen such fibrils in macrophages from malignant ascites produced by a variety of tumours and also in the circulating monocytes of an apparently normal individual (intracytoplasmic and intranuclear, *see Plate 58*). More recently, I have seen somewhat similar (i.e. rigid-looking and electron-dense) fibrils (*Plate 385*) in neutrophil leucocytes in the peripheral blood of an apparently normal individual and in the semen of a person with immotile sperms. The electron density of some of these fibrils suggests that they may be composed of fibrin. However, despite much searching I have not seen any banding pattern, let alone one that would identify this material as fibrin. In a personal communication, Zucker-Franklin reports that she has also seen these fibrils in neutrophils in occasional specimens of peripheral blood. The nature of these peculiar fibrils seen sporadically in monocytes and neutrophils is obscure.

With the advent of immunohistochemistry intermediate filament typing has become a useful adjunct in research and diagnostic oncology. We will deal briefly with this topic now. In the previous section of the text we noted that five major types of intermediate filaments occur, and that they are markers for certain specific cell types. We also noted that since all these filaments measure about 8–11 nm in diameter and since it is very difficult to accurately measure the thickness of filaments, there are many occasions when on morphological grounds alone we may not be able to distinguish between them.

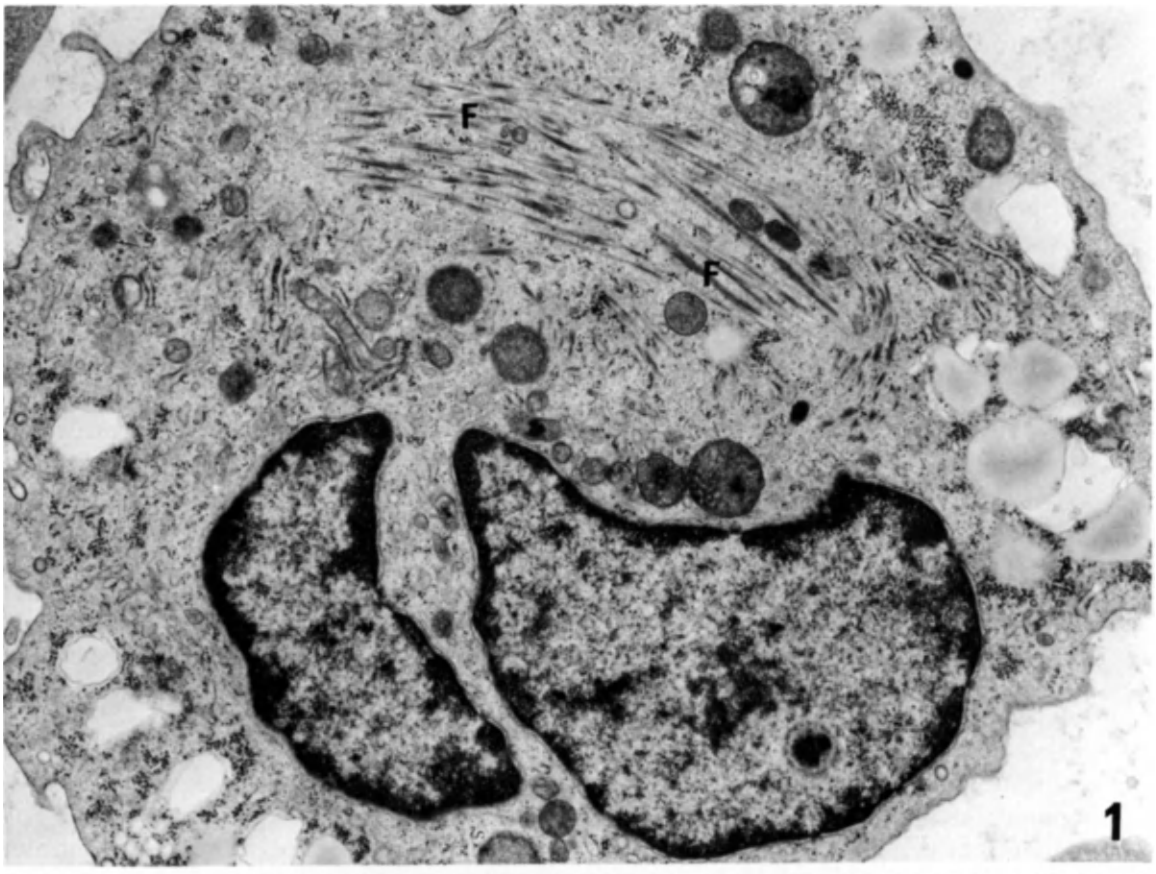
However, immunohistochemical techniques using antibodies against these filament proteins can help to identify the type of filament present and hence the cell type (normal or neoplastic) and this in turn can be of value in distinguishing major tumour groups. As one might expect, the intermediate filament type characteristic of the normal cell of origin is 'inherited' not only by the primary tumour but usually also by its metastasis. As a rule, tumours do not acquire 'additional' or 'new' types of intermediate filaments (*see below* for exceptions).

This is the theoretical basis on which tumour diagnosis by filament-typing rests. It is beyond the scope of this book to analyse the massive literature on this subject. Fortunately, an excellent short review (Osborn and Weber, 1982) and a longer review with 148 references (Osborn and Weber, 1983) have been published from which one can trace the literature on this subject.

Plate 384

Fig. 1. Macrophage from a malignant ascites produced by secondary deposits of malignant melanoma showing an intracytoplasmic focal aggregate of fibrils (F). × 16 500

Fig. 2. Higher-power view of fibrils shown in *Fig. 1* demonstrates that they are sheaves of rigid-looking electron-dense filaments. × 50 000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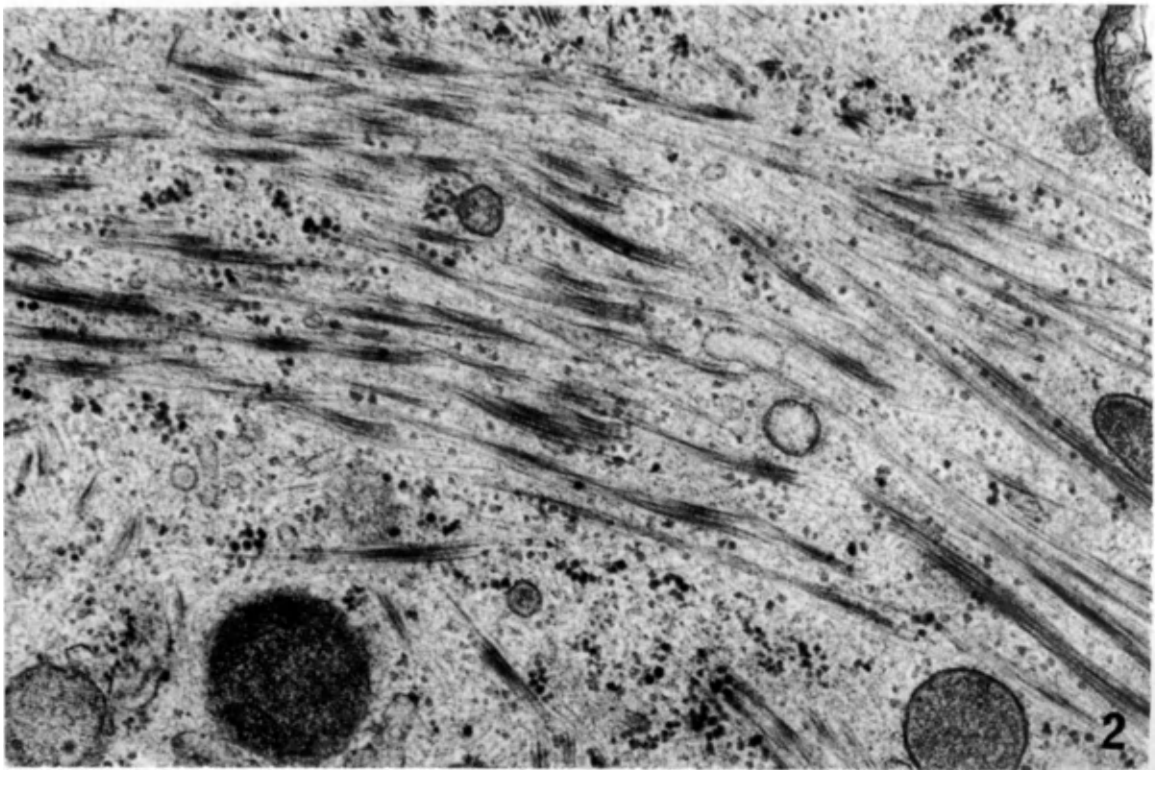
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Briefly, the situation may be summarized as follows: (1) carcinomas are characterized by the presence of cytokeratins, and can often be subtyped by studying their cytokeratin profiles; (2) a variety of sarcomas contain vimentin; (3) myosarcomas also contain some vimentin but they are characterized by the presence of desmin; (4) ganglioneuroblastomas contain neurofilament proteins. Some, but perhaps not all, neuroblastomas contain neurofilament proteins. In keeping with this is the observation that not all neurons contain neurofilaments; (5) some neuroendocrine tumours like phaeochromocytoma, oat cell carcinoma of lung (i.e. anaplastic carcinoid) and Merkel cell tumour of the skin contain neurofilaments (Lehto *et al.*, 1983; Miettinen *et al.*, 1983b; Osborn and Weber, 1983). However, pigmented naevi and melanomas contain only vimentin filaments (Miettinen *et al.*, 1983c); and (6) gliomas (astrocytomas) are characterized by glial fibrillary acidic protein.

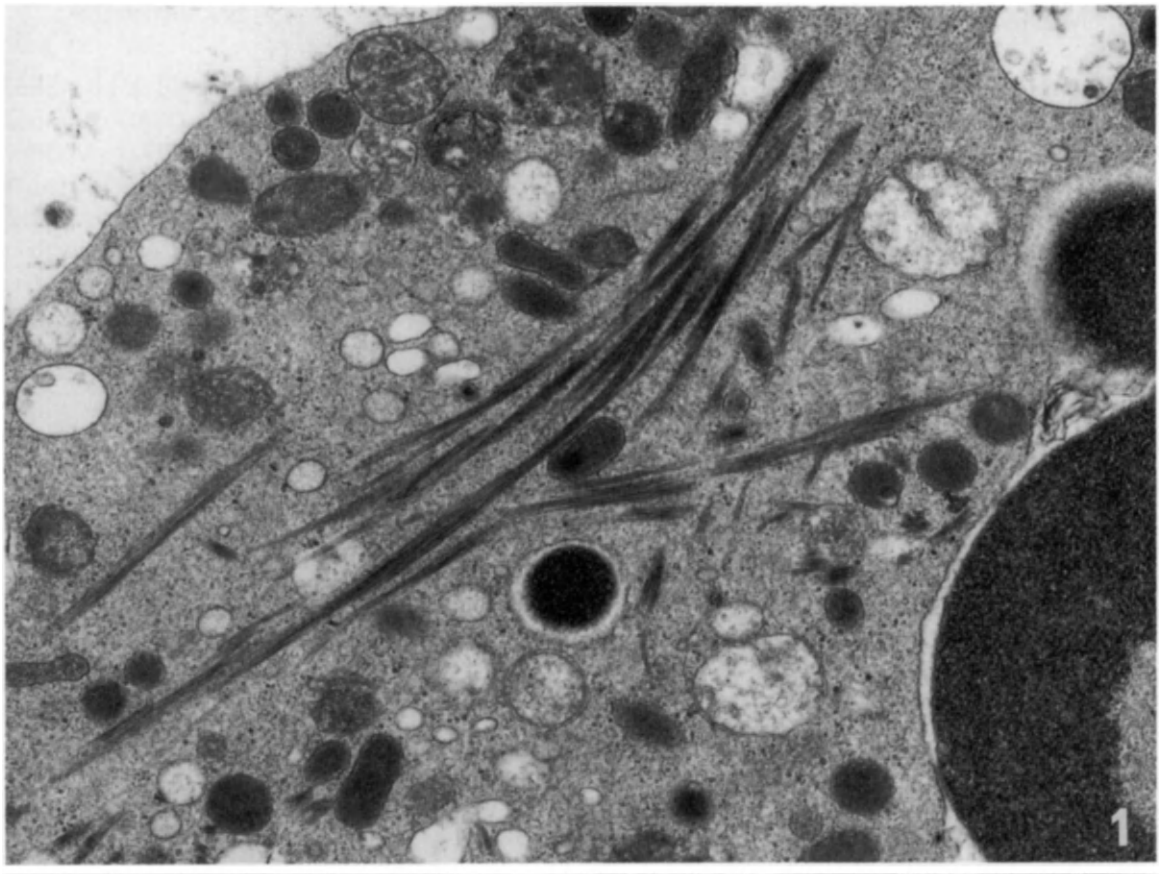
However, one has to admit that tumour diagnosis by filament-typing is not as simple as portrayed in the previous paragraph because coexpression of two or even three different classes of filament-proteins may be found in a tumour. For example: (1) a solid metastasis from a carcinoma as a rule expresses only cytokeratins but in the ascities form it coexpresses cytokeratin and vimentin. In keeping with this is the fact that epithelial cells in culture coexpress cytokeratin and vimentin (Franke *et al.*, 1982); (2) certain thyroid and renal cell carcinomas coexpress cytokeratin and vimentin (Holthöfer *et al.*, 1983; Miettinen *et al.*, 1984); (3) coexpression of cytokeratin and neurofilament proteins has been found in several neuroendocrine neoplasms (e.g. islet cell tumours, bronchial carcinoids, Merkel cell tumours and parathyroid adenomas) (Broers *et al.*, 1985; Gould *et al.*, 1985; Lehto *et al.*, 1985; Miettinen *et al.*, 1985a, b); and (4) coexpression of vimentin, cytokeratin and glial filament proteins has been seen in pleomorphic adenomas of parotid gland (Gould, 1985).

Some interesting findings regarding the histogenesis of some controversial neoplasms are also emerging as a result of filament-typing. For example: (1) alveolar soft part sarcoma, a tumour of unknown histogenesis contains vimentin and desmin. This supports the idea that this tumour is of myogenic origin (Denk *et al.*, 1983); (2) some leiomyosarcomas contain vimentin only (no desmin). This reflects their possible derivation from a certain type of vascular smooth muscle cell which contains vimentin only (Denk *et al.*, 1983); and (3) the enigmatical synovial sarcoma whose nature and histogenesis has been the subject of endless debate has been found to contain cytokeratin in the epithelioid component and vimentin in the spindle cell component (Miettinen *et al.*, 1982) unlike normal or inflamed synovial membranes which do not contain cytokeratin (Miettinen *et al.*, 1983a). This among much other evidence makes it unlikely that the tumour has anything to do with the synovial membrane or even synovial-type of differentiation in a mesenchymal tumour. The idea that this is a carcino-sarcoma gains support from these observations, but the histogenesis of this tumour still remains unknown (for more details *see* Ghadially, 1985, 1987).

Plate 385

Fig. 1. A neutrophil leucocyte containing electron-dense fibrils. From the blood of an apparently normal individual. × 33 000

Fig. 2. Massive deposits of electron-dense fibrils are seen in this neutrophil leucocyte. From a specimen of semen of an infertile man with immotile sperms due to an absence of dynein arms. × 31 000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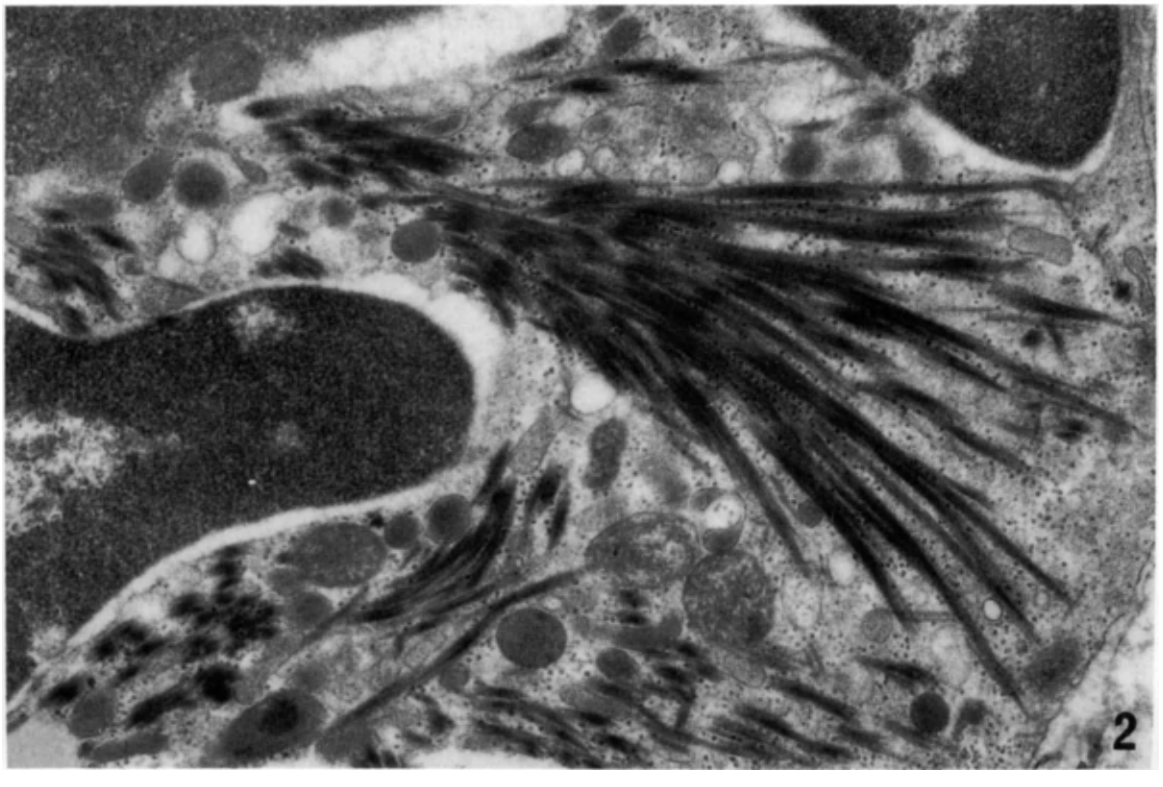
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Mallory's bodies

Because of perennial debates as to which structure or structures should or should not be called 'Mallory's bodies', I will begin by quoting the relevant passage where Mallory (1911) described what he called 'alcoholic hyalin' and what we now call 'Mallory's bodies'. In 1911, Mallory wrote: 'In the chronic, progressive, so-called alcoholic, type of cirrhosis there occurs a peculiar form of necrosis of the liver cells, which seem to be characteristic of it. The cytoplasm of the cells first undergoes a degenerative change in consequence of which an irregular, coarse, hyaline meshwork appears in it. This meshwork stains deeply with eosin and with phosphotungstic acid hematoxylin after fixation in Zenker's fluid'.

Thus the classic Mallory's body is an irregular-shaped hyalin structure found in the hepatocytes of alcoholics, and purists argue that this and only this can be called a 'Mallory's body'. This stance is neither sensible nor practical because: (1) rounded hyalin bodies which differ from the classic Mallory's bodies only in shape (but which otherwise have the same light and electron microscopic appearance and histochemical and immunohistochemical properties) are also found in the hepatocytes of alcoholics; and (2) irregular and rounded bodies of the type seen in alcoholic cirrhosis are also seen in hepatocytes altered by other diseases and experimental manoeuvres. Therefore, all these are now accepted as 'Mallory's bodies' by most people.

Mallory's bodies have been found not only in alcoholic hepatitis of man (*Plate 386*) and baboon but also in diverse other pathological states (*Plate 387*) and experimental situations such as: (1) infantile cirrhosis (Smetana *et al.*, 1961); (2) Indian childhood cirrhosis (Nayak *et al.*, 1969); (3) primary biliary cirrhosis (MacSween, 1973); (4) Wilson's disease (Schaffner *et al.*, 1962; Scheuer, 1970); (5) (a) after intestinal resection, (b) repeated fasting in obesity, (c) obesity with and without diabetes, (d) Christian–Weber disease, (e) abetalipoproteinaemia, and (f) chronic active hepatitis (French, 1983); (6) human hepatomas and hepatocellular carcinomas (Norkin and Campagna-Pinto, 1968; Keeley *et al.*, 1972); and (7) hepatocytes or hepatoma cells of rats or mice exposed to hepatocarcinogens such as 3-methyl-diaminoazobenzene, diethylnitrosamine, griseofulvin and dieldrine (for references *see* Borenfreund and Bendich, 1978; Franke *et al.*, 1979; Meierhenry *et al.*, 1981).

Early ultrastructural studies aimed at establishing the ultrastructural equivalent of Mallory's bodies were confusing and erroneous. Thus, it was claimed that Mallory's bodies are: (1) swollen mitochondria (Schaffner *et al.*, 1963); (2) altered endoplasmic reticulum (Biava, 1964); (3) autolysosomes containing mitochondria and other organelles (Flax and Tisdale, 1964); and (4) a central lipid droplet surrounded by endoplasmic reticulum (Reppart *et al.*, 1963).

Even when it was realized that Mallory's bodies comprise an accumulation of filaments, the filaments were wrongly characterized by immunofluorescence as actin filaments (Nenci, 1975). It was the studies of French and his co-workers (for references *see* French, 1983) which first showed that the filaments were intermediate filaments and the immunological studies of Franke

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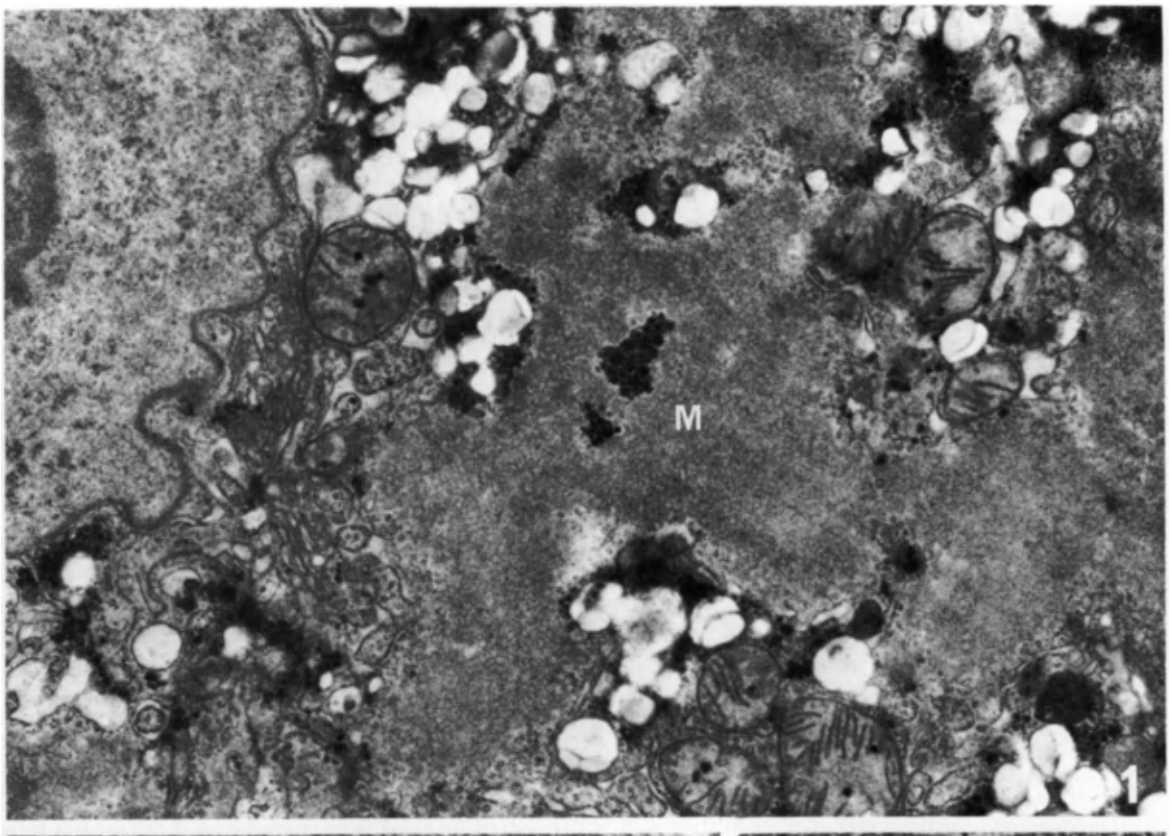
Plate 386

From a case of alcoholic hepatitis. (*Pfeifer and Altmann, unpublished electron micrographs*)

Fig. 1. A large irregular shaped Mallory's body (M) is seen in the cytoplasm of a hepatocyte. × 20 000

Fig. 2. High-power view from a Mallory's body showing short randomly orientated filaments forming a meshwork (type 2 filaments). × 48 000

Fig. 3. High-power view from a Mallory's body showing parallel filaments (type 1 filaments). × 45 000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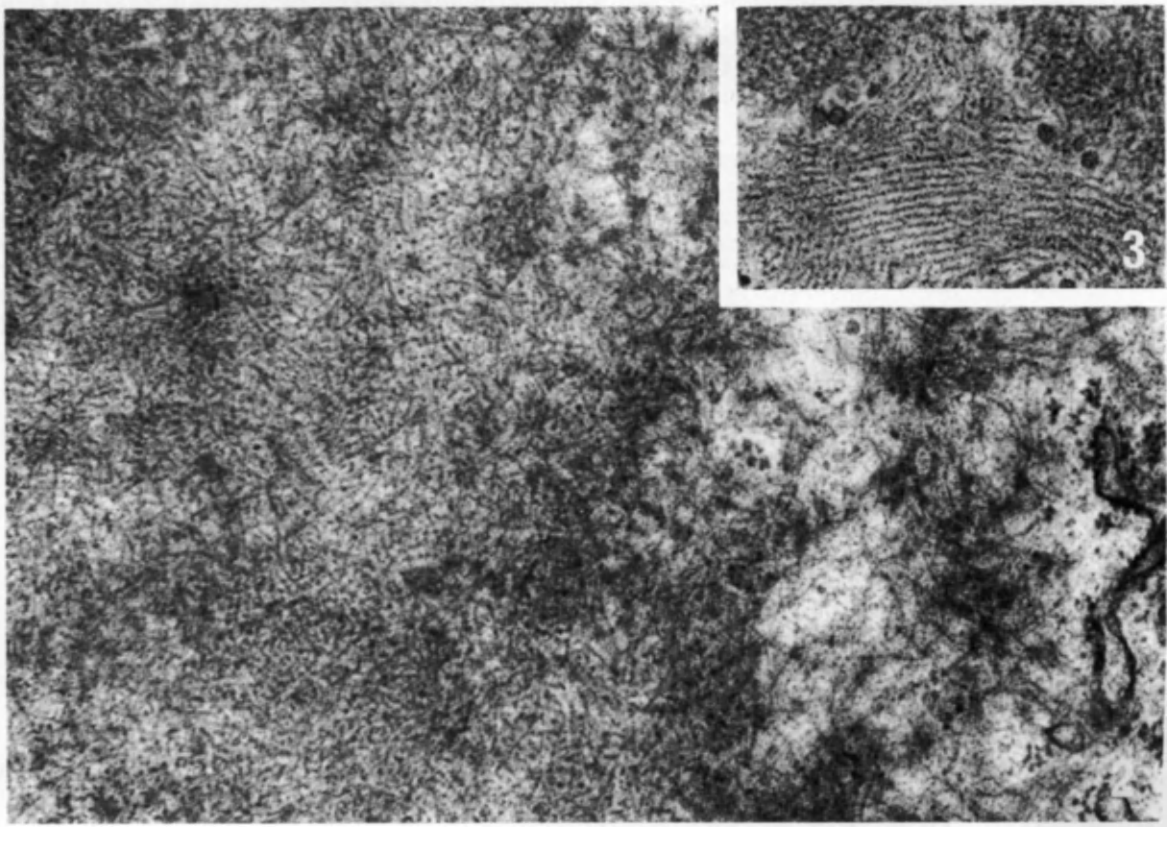

3

et al. (1979) which established unequivocally that these filaments contain prekeratin-like or cytokeratin-like polypeptides★.

In ultrathin sections, filaments of human Mallory's bodies appear to be considerably thicker (up to about 21nm thick) than the usual cytokeratin filaments found in epithelial cells†. This is because other proteins are associated with them. Filaments of Mallory's bodies isolated from grisoflavine-fed mice have a fuzzy coat but this is not apparent in human material. Reconstituted human and rodent filaments of Mallory's bodies resemble normal cytokeratin filaments in thickness and solubility characteristics.

The manner in which Mallory's bodies form is not clear. Denk *et al.* (1981) found (as revealed by immunofluorescence microscopy) that cells that contained Mallory's bodies had a severely deranged or virtually absent cytokeratin meshwork compared with neighbouring normal-looking hepatocytes. They therefore suggested that Mallory's bodies grow by incorporation of filaments from the cytokeratin meshwork. Kimoff and Huang (1981) obtained similar results (immunocytochemical and immunoelectron microscopic studies) with human and mouse Mallory's bodies and hence reached the same conclusion. However, morphometric studies (Irie *et al.*, 1982) seem to suggest that the amount of cytoplasmic cytokeratin filaments is not altered in cells containing Mallory's bodies.

The dynamics of Mallory's body formation has also been studied (Borenfreund and Bendich, 1978) in cultures of hepatocytes from rats treated with the hepatocarcinogen diethylnitrosamine. After three months *in vitro* these cells come to contain Mallory's bodies which persist in carcinomas raised by inoculating the cultured cells into young rats or nude mice. Here also immunofluorescence studies show a disturbance of the cytoskeleton in cells containing Mallory's bodies.

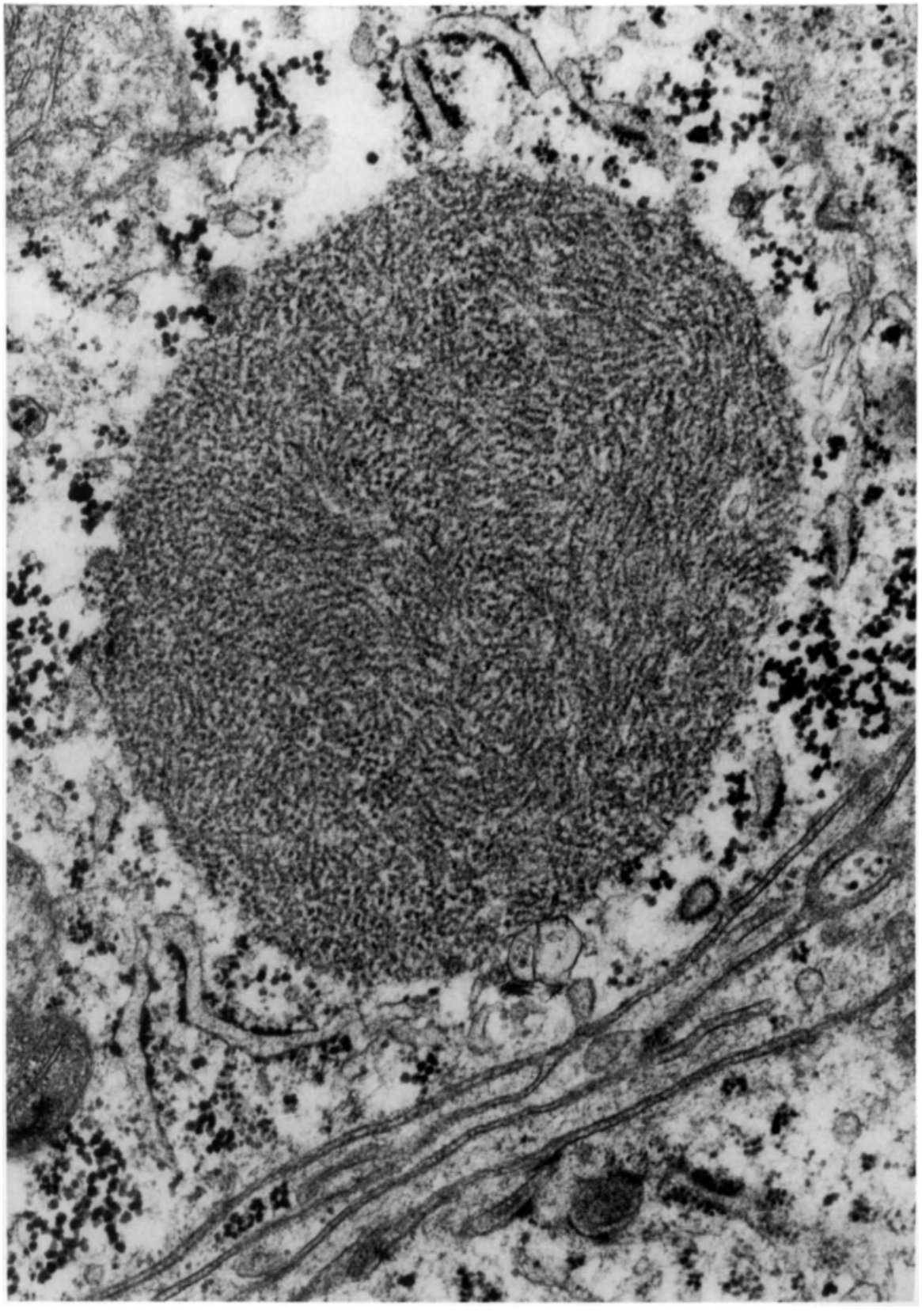
Finally, it is worth pointing out that the Mallory's body is not the only hyaline body known to contain cytokeratin filaments. For example, such bodies are seen in GH-cell adenoma of the pituitary, some neuroendocrinomas and some carcinomas of the lung (*see* page 906). There is nothing to be gained by calling all these bodies, 'Mallory's bodies', yet this is precisely what some authors have done. Such extension of nomenclature brings in its wake confusion and a temptation to list all filamentous bodies be they composed of cytokeratin or other intermediate filaments, as Mallory's bodies. The term 'Mallory's bodies' is best restricted to describe the irregular and spherical cytokeratin-containing bodies found in pathological hepatocytes.

★Yokoo *et al.* (1972) speak about 'three types of hyalin' (perhaps better described as three morphological components that may be found in Mallory's bodies): (1) Type 1 hyalin (some authors refer to this as Type 1 filaments). Filaments with a mean diameter of 14.1 nm (range 9–21 nm). They are said to have an indistinct boundary, exhibit irregular densities and occur in parallel arrays or whorls; (2) Type 2 hyalin (at times referred to as 'Type 2 filaments'). Filaments with a mean diameter of 15.2 nm (range 11.5–20 nm). This, the principal component of Mallory's bodies, presents as short, straight or slightly curved, randomly orientated filaments which form a close-meshed network; and (3) Type 3 hyalin which presents as an amorphous electron-dense material (probably derived from disintegrating filaments).

†Human and murine Mallory's body-filaments contain not only lower molecular weight cytokeratin polypeptides (molecular weight 48 000, 45 000 and 41 000), as do normal cytokeratin filaments in non-keratinizing epithelia, but also higher molecular weight cytokeratin polypeptides (molecular weights from 55 000 to 66 000) reminiscent of epidermal cytokeratins (Denk *et al.*, 1981).

Plate 387

Hepatocellular carcinoma. A rounded Mallory's body is seen in the cytoplasm of a tumour cell. × 52 000



Globular filamentous bodies

Of the various configurations of intermediate filaments seen in cells, none has attracted more attention than spherical, globular or 'ball-like' aggregates of filaments which are best referred to as 'globular filamentous bodies'*. The filaments in such bodies are either randomly scattered or deployed in an orderly fashion to form a concentric whorl or whorls. They usually, but not invariably, lie in the juxtanuclear position. When they occur in the juxtanuclear region they tend to indent the nucleus. Some of these bodies are large enough to be seen by the light microscope where they present as rounded, hyaline, weakly eosinophilic inclusions in the cell cytoplasm. When the inclusion is very large and fills the cell cytoplasm we get the appearance called 'hyaline cell' or 'ground-glass cell' by the light microscopist. However, it must be remembered that some but not all spherical hyaline inclusions are composed of filaments. For example, in altered hepatocytes spherical hyaline inclusions may be composed of: (1) cytokeratin filaments (hence acceptable as a variety of Mallory's body) (*Plate 387*); (2) dilated rough endoplasmic reticulum containing alpha-fetoprotein, alpha 1-antitrypsin or fibrinogen; (3) hypertrophied smooth endoplasmic reticulum; and (4) giant mitochondria.

Globular filamentous bodies have been seen in diverse situations, including normal and neoplastic cells. However, most reports deal with their occurrence in tumours. The type of filament involved in the formation of the globules has been immunohistologically characterized in only a few instances, as will be apparent from the list presented below.

Globular filamentous bodies have been seen in: (1) GH-cells (stands for growth hormone) and GH-cell adenomas of the pituitary (bodies contain cytokeratin filaments) (Schochet *et al.*, 1972; Kovacs *et al.*, 1984; Slowik *et al.*, 1979; Hofler *et al.*, 1984; Neumann *et al.*, 1985) (*Plate 388*); (2) articular chondrocytes in ageing (man and rabbit) and osteoarthritic joints and after experimental manoeuvres such as injection of autologus blood, fat or gold salts into the joint (for details and references *see* Ghadially, 1983); (3) enchondroma (Alonso *et al.*, 1978); (4) altered hepatocytes (including neoplastic) (*Plate 387*) (Ichida, 1983; Ordónez and Mackay, 1983; Huntrakoon and Bhatia, 1984); (5) trabecular (Merkel cell) carcinoma of skin (bodies contain cytokeratin and/or neurofilaments) (Zak *et al.*, 1982; Haneke, 1985; McMahon *et al.*, 1985); (6) duodenal carcinoid (Carstens and Broghamer, 1978); (7) mediastinal (thymic?) carcinoid (Rosai *et al.*, 1972); (8) a primary liver cell tumour with a carcinoid-islet cell morphology (Warner and Seo, 1980); (9) an insulinoma (Warner and Seo, 1980); (10) medullary carcinoma of thyroid (Johannessen *et al.*, 1978); (11) neuroblastoma and ganglioneuroma (bodies contain microtubules and neurofilaments) (Bender and Ghatak, 1978; Yunis *et al.*, 1979; Pearl *et al.*, 1981); (12) mesothelioma (Kawai *et al.*, 1981) (*Plate 389*); (13) neurons in aluminium encephalopathy (Terry and Pena, 1965; Selkoe *et al.*, 1979); (14) granular cell myoblastoma (Fisher and Wechsler, 1962); (15) basal cell carcinoma (Warner and Seo, 1980); (16) giant cell carcinoma of lung (Wang *et al.*, 1976); (17) adenocarcinoma of endometrium (Ferenczy, 1976); (18) pleomorphic adenoma of salivary gland (Chen, 1979; Buchner *et al.*, 1981); (19) lobular *in situ* carcinoma of breast (Carter *et al.*, 1969); (20) small cell (follicular) carcinomas of thyroid

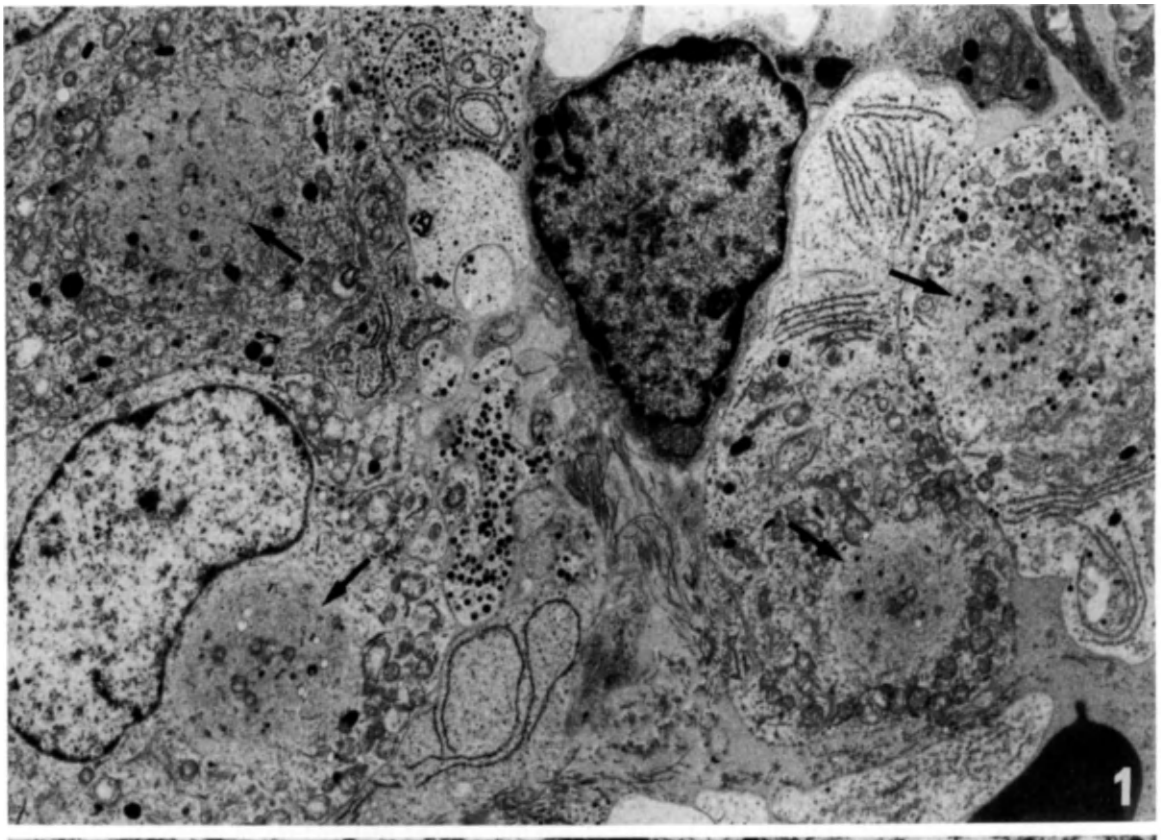
*No specific name has been given to these bodies, but among others they have been called 'globular bodies' and 'fibrous bodies'. The term 'globular bodies' is rather non-specific. It can apply to any number of structures and in fact it was at one time used by some (and is still used by some who practise scientific caution with more zeal than common sense) to describe lipid droplets in cells. 'Fibrous bodies' is inaccurate because these bodies are composed of filaments and not fibres (for differences between filaments and fibres, *see* pages 1215–1221). I have therefore coined the term 'globular filamentous bodies' which epitomizes their morphology, and makes it difficult to confuse them with any other structure.

Plate 388

Sparsely granulated GH-cell adenoma. (*From a block of tissue supplied by Dr E. Horvath and Dr K. Kovacs*)

Fig. 1. A low-power view showing a group of cells containing globular filamentous bodies (arrows). About 70 per cent of the tumour cells in this section contained these bodies. × 6000

Fig. 2. Higher-power view of a globular filamentous body. × 21 000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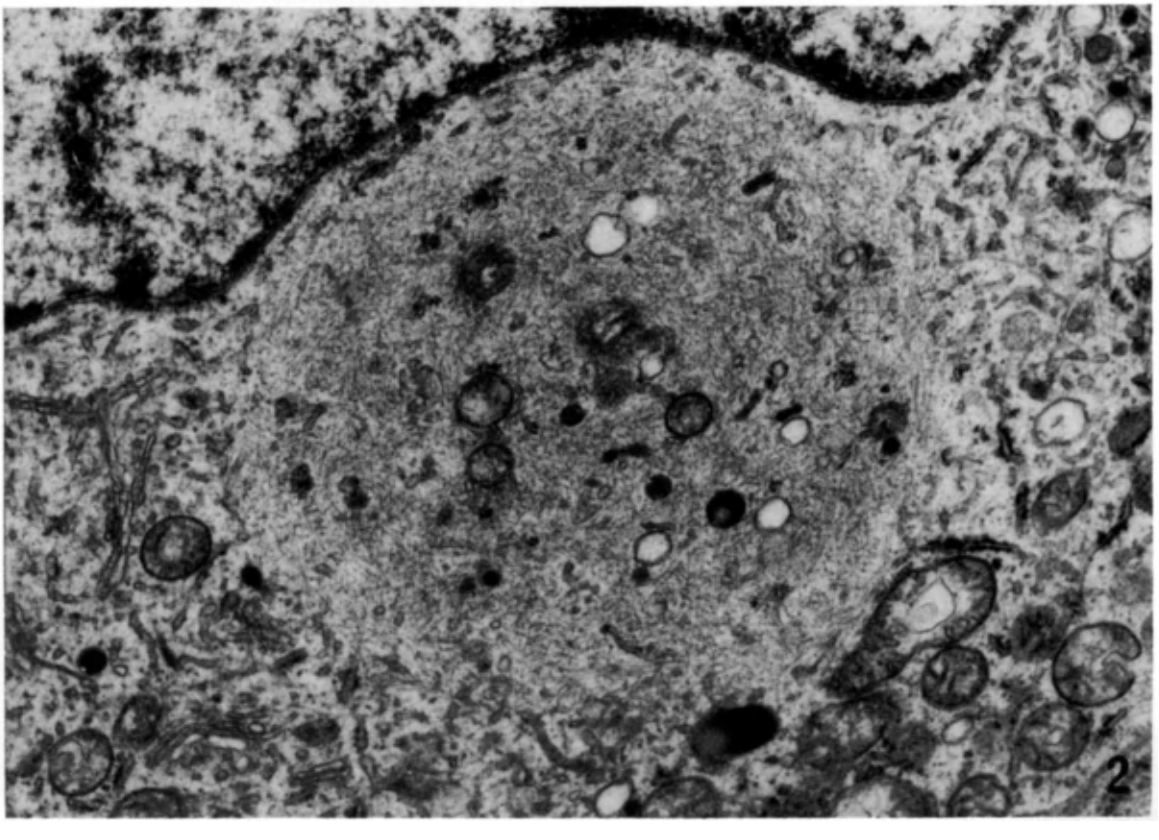
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(Gonçalves *et al.*, 1978); (20) metastatic granulosa-theca cell tumour (Gondas, 1969); (21) epithelioid sarcoma (Schmidt and Mackay, 1982; Mukai *et al.*, 1985); (22) malignant epithelioid haemangioendothelioma (Fukayama *et al.*, 1984); (23) haemangiosarcomas induced in mice by strontium-90 (Ash and Loutit, 1977); (24) endometrial sarcomas (Böcker and Stegner, 1975; Paulsen *et al.*, 1982); (25) rhabdomyoma (Konrad *et al.*, 1982; Konrad and Hübner, 1983); (26) rhabdomyosarcoma (Diaz-Flores *et al.*, 1978; Yagishita *et al.*, 1979; Hilderbrand *et al.*, 1980; Mierau and Favara, 1980); (27) malignant rhabdoid tumour of kidney (bodies composed of vimentin filaments) (Schmidt *et al.*, 1982; Rousseau-Merck *et al.*, 1983); (28) bizarre leiomyoblastoma (Min *et al.*, 1979); (29) a case of unusual familial myopathy (bodies composed of desmin filaments, *see Plate 376*) (Porte *et al.*, 1980); and (30) infantile digital fibromatosis (Battifora and Hines, 1971; Iwasaki *et al.*, 1974, 1980; Bhawan *et al.*, 1979).

The crucial feature which distinguishes the sparsely granulated GH-cell adenoma (item 1) from other pituitary adenomas is the presence of numerous globular filamentous bodies composed of intermediate filaments (cytokeratin) about 11 nm in diameter. The nuclei of the cells of this tumour are often crescent-shaped and these bodies lie adjacent to the concave face of the nucleus (i.e. in the Golgi zone). The filaments are randomly dispersed to form aggregates rather than whorls. Enmeshed in the filaments are various cytoplasmic organelles and inclusions such as mitochondria and secretory granules. Smooth endoplasmic reticulum is always associated with the filamentous bodies and is at times quite prominent.

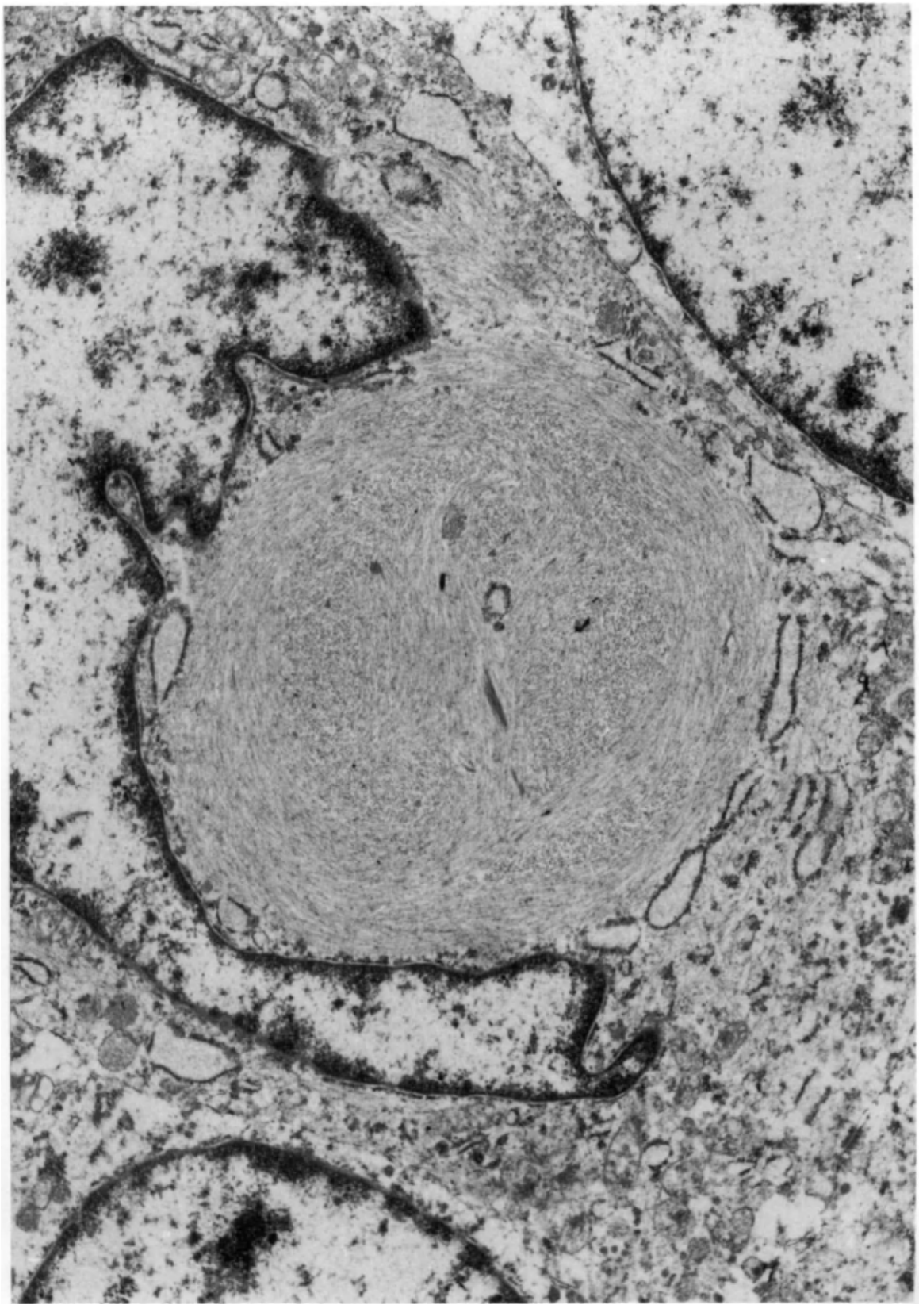
Supernumerary centrioles are commonly seen in the tumour cells, usually situated adjacent to or within the spherical filamentous bodies. The frequency of these bodies varies from tumour to tumour but they are often quite numerous and at times detectable in virtually every tumour cell. Kovacs *et al.* (1984) state: 'Fibrous bodies are diagnostic for the acidophilic cell line, indicating that the adenoma originates in growth hormone cells or their precursors'.

As will be apparent from the list presented above, globular filamentous bodies have been seen in many varieties of tumours. Thus, only when one knows that one is dealing with a pituitary tumour does the presence of numerous filamentous bodies become diagnostic of the sparsely granulated GH-cell adenoma.

Globular filamentous bodies have been seen in hepatocellular carcinomas (item 4) and there is argument in the literature as to whether they are composed of cytokeratin filaments (and hence akin to or a variety of Mallory's body) or actin filaments. I am inclined to the former view because the morphology of the filaments is in keeping with intermediate filaments and certainly not actin filaments, and because close proximity and continuity with reticular or diffuse Mallory's bodies has been noted by Keeley *et al.* (1972).

Plate 389

Pleural mesothelioma. Quite a number of tumour cells contained globular filamentous bodies; one of them is shown in this electron micrograph. Tissue preparation is less than optimum because primary fixation was in formaldehyde. × 19 000



In rhabdomyoma and rhabdomyosarcoma (item 26) at least two types of spherical globular bodies are seen (Ghadially, personal observations). One variety is composed of a felted mass of thin actin filaments, while the other is composed of a jumbled mass of sarcomeres (i.e. thick and thin filaments and Z-lines) (*Plate 390*). However, quite often it is difficult to be certain about such matters and all that one can say is that the globular body is composed of myofilaments.

It would appear that globular filamentous bodies in undifferentiated carcinomas of the endometrium (item 16) are of some diagnostic import, because it permits one to distinguish these carcinomas from other gynaecological neoplasms. About this Ferenczy (1976) states: 'Although intracytoplasmic microfilaments* vary quantitatively from one tumour to another, as well as from one neoplastic cell to another, they represent the most constant ultrastructural feature of endometrial carcinomas, and are even present in the least differentiated, anaplastic growth of endometrial origin'.

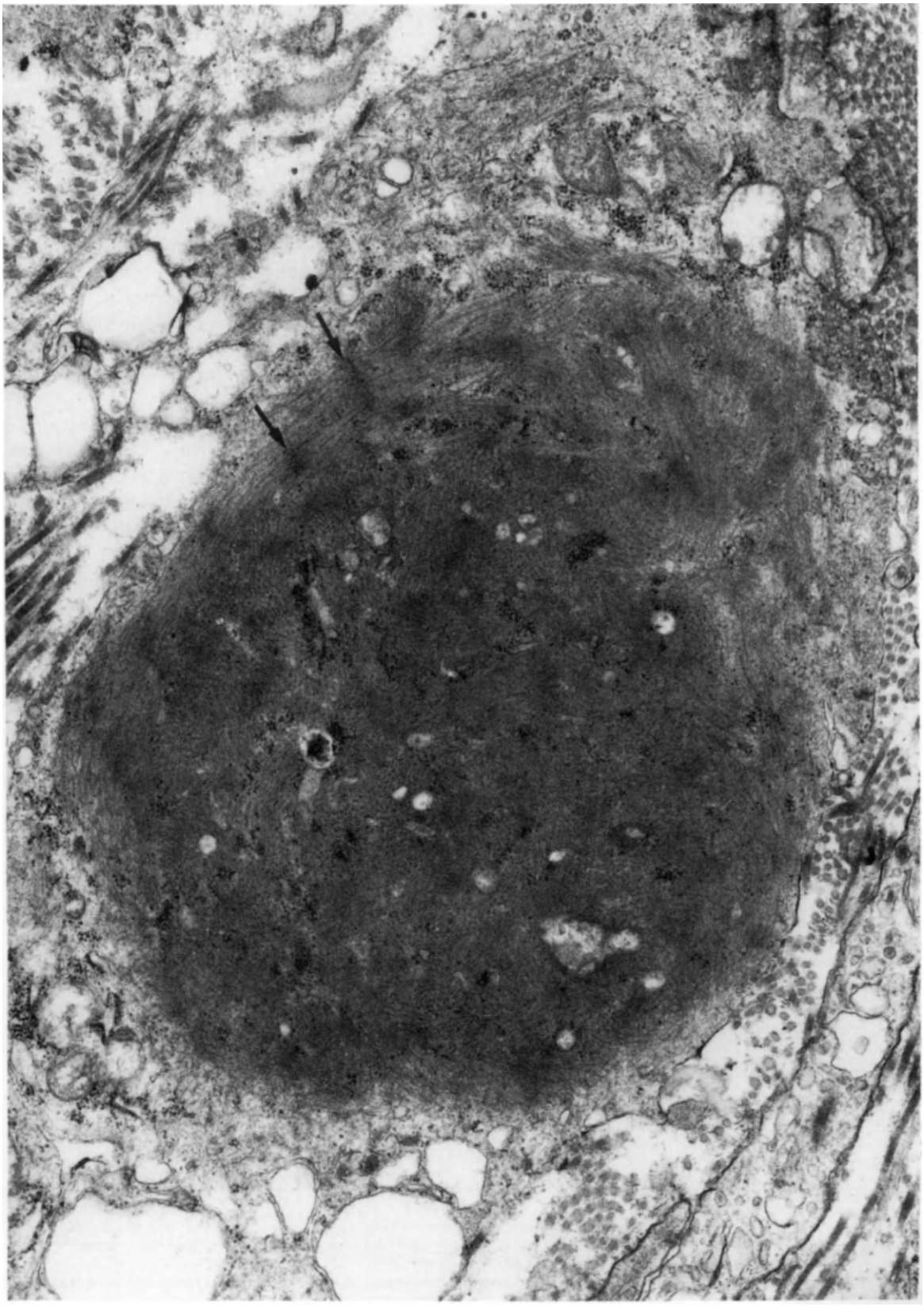
The globular filamentous bodies seen in infantile digital fibromatosis are somewhat different from those seen in other tumours in that they are markedly eosinophilic with the light microscope and much more electron-dense with the electron microscope than other filamentous bodies. These bodies are composed of a felted mass of fine filaments. The cells in these lesions are myofibroblasts and it is believed (Battifora and Hines, 1971; Bhawan *et al.*, 1979) that the globular filamentous bodies most probably derive from disorganized or broken-down myofilaments.

The significance and mechanism of globular spherical body formation is not known. However, a clue may perhaps be found in the fact that when cultured cells are treated with colchicine, perinuclear and juxtanuclear aggregates (called 'caps') of intermediate filaments develop (Goldman, 1971; Lazarides, 1980). Since microtubules as well as intermediate filaments are a part of the cytoskeleton and since microtubules are depolymerized by colchicine, one may speculate that disintegration of microtubules perhaps leads to the collapse and aggregation of intermediate filaments to form caps; and one may further speculate that such a derangement can occur in neoplastic cells, as well as non-neoplastic cells as a result of ageing or various noxious influences. In this connection it is worth recalling the case reported by Sajjad and Mackay (1982) where numerous globular filamentous bodies developed in a synovial sarcoma after intra-arterial administration of chemotherapeutic agents, where none existed before. The thesis proposed above is similar to that expounded on pages 892–896 where a substantial body of evidence is presented in support of the idea that prominent collections of intermediate filaments in cells are an indicator of cellular regression or degeneration.

*The term 'microfilaments' is used by some in a loose fashion for just about any intracytoplasmic filament, while others use it specifically to describe the thin actin filaments. The filaments in these tumours (endometrial carcinomas) are clearly not actin filaments but intermediate filaments (cytokeratin). There is no need for the prefix 'micro' when talking about filaments, intracellular or extracellular, it only leads to confusion (*see* footnote on page 870).

Plate 390

Rhabdomyosarcoma. A globular filamentous body composed of myofilaments. Myofibrils composed of thick and thin filaments are difficult to discern, but Z-lines are (arrows) easily seen. × 27 000



Crystals and crystalloids of intracytoplasmic filaments

In previous sections of this chapter we noted that intracytoplasmic filaments can assume various configurations. It remains to point out that on very rare occasions intracytoplasmic filaments can form crystalline or crystalloid structures in the cell cytoplasm. One such group of crystalline structures called 'crystalline filamentous cylinders' is sufficiently distinct and complex to merit a section on its own (*see* pages 914–919). Here we deal with the few other situations where crystals composed of intracytoplasmic filaments have been found in the cell cytoplasm (*Plate 391*).

Perhaps the best known filamentous crystal is the Charcot–Böttcher crystal of the Sertoli cell which is seen: (1) on rare occasions in apparently normal human testis and somewhat more frequently (also larger size) in pathological human testis where spermatogenesis is disturbed (Narbaitz *et al.*, 1978; Lu and Steinberger, 1978; Vydra, 1980; Aldemir *et al.*, 1980; Andres *et al.*, 1981; Terquem and Dadoune, 1981; Kaya and Turkyilmaz, 1985); (2) in azoospermic swine testis (Toyama, 1975; Toyama *et al.*, 1979); and (3) in boar testis (in segments of seminiferous tubules lacking in spermatogenesis) (Osman, 1978).

In a well illustrated study, Kaya and Turkyilmaz (1985) point out that a prominent feature in the Sertoli cell is the presence of numerous intracytoplasmic filaments. These filaments aggregate to form small clusters or bundles (1 μm long and 0.1 μm wide) which are at times called Spangaro crystals (Fawcett, 1975). It is thought that further aggregation of these bundles of filaments produces the much larger spindle-shaped Charcot–Böttcher crystals (10–25 μm long and 2–3 μm wide) composed of parallel filaments*.

It has now been shown that the Lubarsch's crystal of spermatogonia is also composed of parallel filaments (Sohval *et al.*, 1971; Nagano, 1969; Paniagua and Nistal, 1984). Hence, it is sometimes referred to as a Charcot–Böttcher-like crystal. The classic crystal of the Leydig cell is the Reinke crystal (*see Plate 415* in Chapter 13) which is composed of hexagonal microtubules. However, I have seen crystals apparently derived from intermediate filaments in a cell in a Leydig cell tumour†, but the morphology was quite different from that of Charcot–Böttcher crystals (*Plate 391, Fig. 2*).

Other instances where intracytoplasmic filaments have formed crystalline or paracrystalline structures include: (1) a sarcoma (type not mentioned) cell which contained crystals composed of vimentin filaments (*Plate 391, Fig. 1*) (Bockus *et al.*, 1985); and (2) a large cell anaplastic carcinoma of lung where crystalline and paracrystalline structures presumably composed of cytokeratin filaments were present (Dr T. Loy, personal communication).

In this section of the text, we have looked at intracytoplasmic crystals which appear to be composed of one or other of the well known intracytoplasmic filaments. Other intracytoplasmic crystals composed of unknown filamentous, microtubular, spherical or cylindrical subunits are dealt with on pages 978–985.

*The nature of the filaments is obscure but a study of the published electron micrographs suggests that they are intermediate filaments rather than the thin actin filaments.

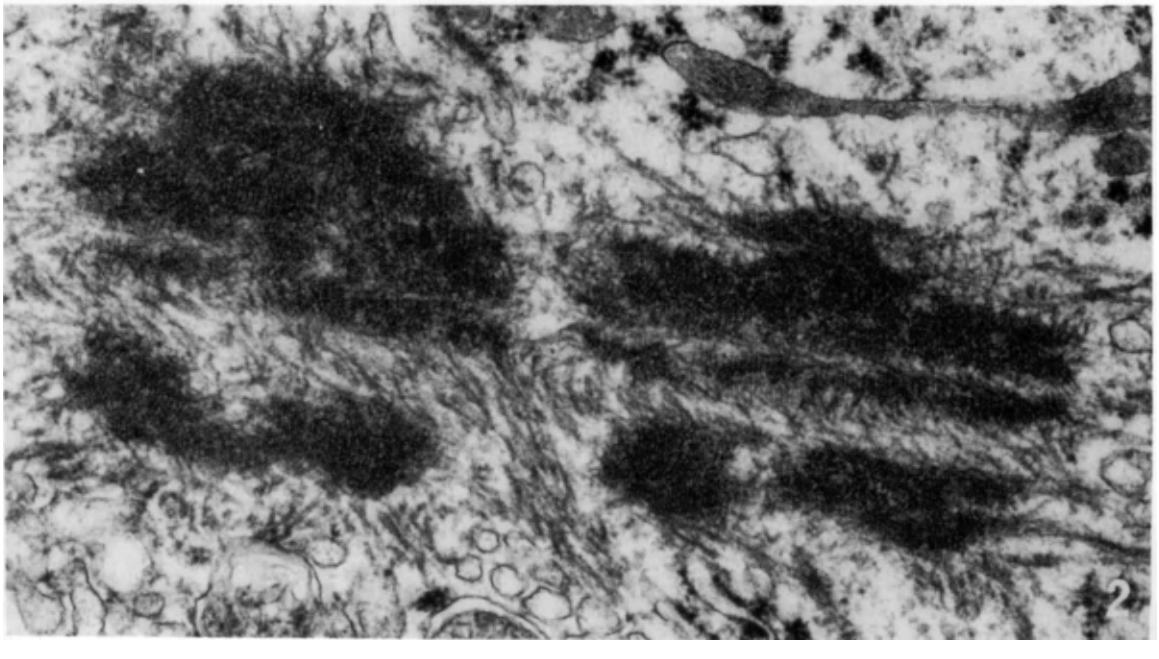
†An indubitable Leydig cell tumour (not a Sertoli cell tumour) where the cells contained much smooth endoplasmic reticulum and mitochondria with tubulovesicular cristae.

Plate 391

Fig. 1. A sarcoma cell containing crystalline structures which are clearly composed of, or derived from, intracytoplasmic filaments (most likely vimentin). Note how the filaments flow into the crystals. × 40 000 (*From Bockus, Remington, Friedman and Hammar, 1985*)

Fig. 2. Leydig cell tumour. A tumour cell containing electron-dense crystals or crystalloids and associated intracytoplasmic filaments. × 48 000


1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Crystalline filamentous cylinders

These rather rarely encountered structures have been seen mainly but not entirely in various myopathies. They have been referred to in the literature by various names such as: 'concentric laminated bodies', 'laminated convoluted bodies', 'cylindrical laminated bodies', 'concentrically laminated membranous inclusions', 'laminar cytoplasmic structures', 'concentric fibrillar lamellae', and 'subsarcolemmal filamentous cylinders'.

The term 'concentric laminated bodies' has been used to describe so many different structures* that it is best avoided. The term 'concentrically laminated membranous inclusions' is wrong because these structures are not composed of membranes (i.e. no trilaminar structure). The term 'subsarcolemmal filamentous cylinders' is best but it describes the situation in diseased muscle cells only (*Plate 392*). Since these structures have now been seen in other cells besides striated muscle, I suggest that we use the slightly modified term 'crystalline filamentous cylinders' which epitomizes their true nature and precludes confusion with any other known structure.

Like any hollow cylindrical or tubular structure (e.g. manchette of the spermatid, ribosome–lamella complex and tubular confronting cisternae) the crystalline filamentous cylinders present: (1) a circular profile when cut transversely; (2) a profile composed of parallel lines when cut longitudinally; (3) an oval profile when cut obliquely through the body of the cylinder; and (4) a test-tube-shaped or U-shaped profile when the section passes through an open end of the hollow cylinder or tube.

In transverse sections the structures seem to be composed of concentric or spiralling lamellae. The lamellae, in turn, appear to be composed of ordered arrays of filaments (hence deserving to be called 'crystalline'). At times the filaments are paired and hence they look like longitudinally sectioned microtubules, but since no circular profiles acceptable as transverse sections through microtubules are seen, one has to conclude that no microtubules are present. In some planes of sectioning linear or circularly deployed punctate or dashed profiles are seen. One may interpret these 'dots' and 'dashes' as transversely cut or slightly obliquely cut filaments. In oblique sections through the cylinder a periodically banded pattern is sometimes seen, while in other instances a crystalline lattice pattern is evident. The true three-dimensional morphology of these structures awaits clarification, but the profiles seen may perhaps be explained by assuming that these structures are composed of alternating layers of filaments running parallel to the long axis of the cylinder, and concentric or spiralling filaments running at right angles to the longitudinal filaments.

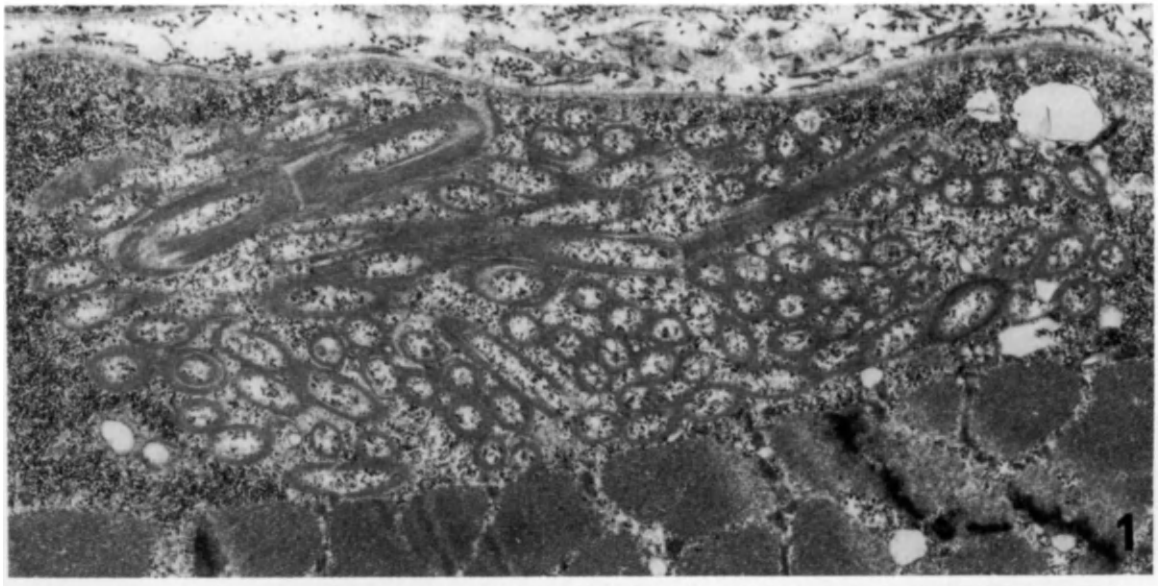
*For example, the term 'laminated bodies' or 'concentric laminated bodies' has been used to describe whorls of rough endoplasmic reticulum, myelin figures produced as an artefact of aldehyde fixation, secretory granules of type II alveolar cells, myelinosomes seen in Tay Sach's disease and drug-induced myelinosomes.

Plate 392

Muscle biopsy from a case of Duchenne's muscular dystrophy.

Fig. 1. Low-power view showing a collection of crystalline filamentous cylinders in the subsarcolemmal region of the muscle fibre. × 12 000

Fig. 2. Higher-power view showing cylinders cut in various planes. Some of the profiles mentioned in the text can be seen here. Note for example the parallel line profiles (arrow), dashed line profiles (large arrowhead) and pseudomicrotubular profiles (between small arrowheads). × 53 000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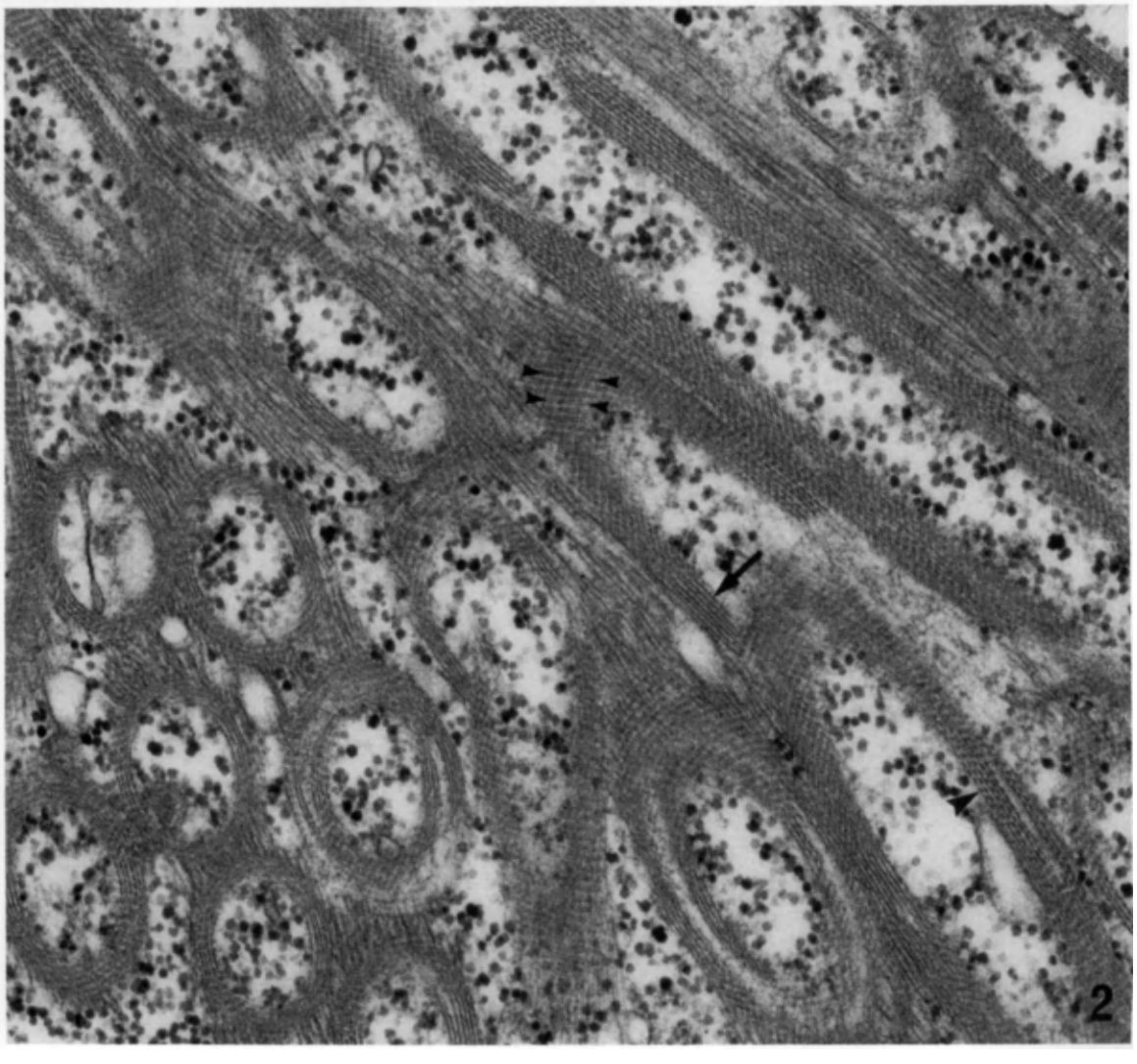

2

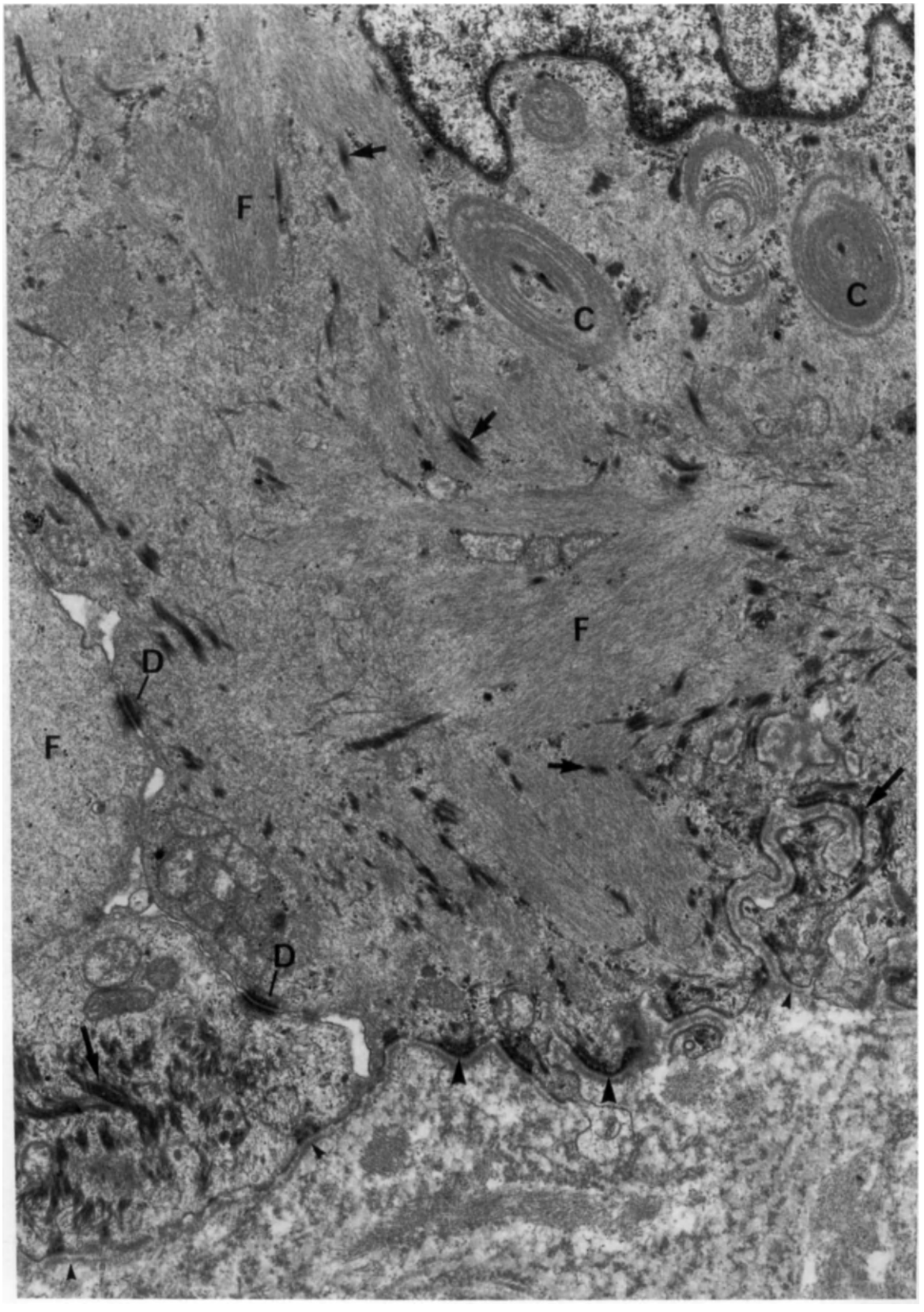
Crystalline filamentous cylinders have been seen in skeletal muscle fibres in: (1) non-thyroid hypermetabolic states (Luft *et al.*, 1962); (2) late onset glycogenosis (Engel and Dale, 1968); (3) nemaline myopathy (Shafiq *et al.*, 1967); (4) Canavan's disease (Gambetti *et al.*, 1969); (5) thyrotoxic hypokalaemic periodic paralysis (Schutta and Armitage, 1969); (6) central core disease (Dubowitz and Roy, 1970); (7) one case of van Bogaert–Bertrand disease (Gambetti *et al.*, 1969); (8) central core disease (Dubowitz and Roy, 1970); (9) alcoholic neuropathy (Tomé and Mair, 1970); (10) Duchenne's muscular dystrophy (Ketelsen *et al.*, 1970); (11) infantile neuroaxonal dystrophy (Toga *et al.*, 1971); (12) infantile spinal muscular atrophy (Roy *et al.*, 1971); (13) non-specific muscle disorders (Fisher *et al.*, 1972); (14) neurogenic atrophy (Mair and Tomé, 1972); (15) Marfan's syndrome (Goebel *et al.*, 1973); (16) acromegaly (Mastaglia, 1973); (17) congenital fibre disproportion (Neville, 1973); (18) congenital hypotonia (Hudgson and Fulthorpe, 1975); (19) children affected with muscle weakness and hypotonia with probable cerebral involvement (Payne and Curless, 1976); (20) Krabbe's disease, (neuronal ceroid lipofuscinosis) (Schochet and Lampert, 1978).

Besides the above, crystalline filamentous cylinders or structures closely resembling them have been seen in: (21) cerebellar haemangioblastoma (Cancilla and Zimmerman, 1965); (22) cortex adjacent to an expanding glial tumour (Ramsey, 1967); (23) myelinated axons from a case of progressive multifocal leucoencephalopathy (Hadfield *et al.*, 1974); (24) a pleomorphic adenoma of minor salivary gland (Fulcheri *et al.*, 1983); and (25) pleomorphic adenoma of parotid gland (Min, 1984) (*Plates 393* and *394*).

It will be noted from the list presented above that a majority of sightings of crystalline filamentous cylinders has been in diverse myopathies. Early workers in this field suggested that these structures arise from mitochondria (Luft *et al.*, 1962; Shafiq *et al.*, 1967), but there is really nothing which supports such a contention. Ketelsen *et al.* (1970) thought that the 'subplasmalemmal filamentous cylinders' represent proliferated actin, while Toga *et al.* (1971) thought that they represent accumulated actin filaments and they suggest that this might be a

Plate 393

Pleomorphic adenoma of parotid gland. Seen here are cells acceptable as neoplastic myoepithelial cells. One of them contains crystalline filamentous cylinders (C). The cells are linked by desmosomes (D). Note also the basal lamina (small arrowheads), the hemidesmosomes (large arrowheads) and tonofibrils (long arrows). The cell cytoplasm is filled with filaments (F) which are presumably actin filaments. Some of the dense bodies (short arrow) may be the focal densities of the type one sees along the course of actin filaments, others could be tonofibrils. × 17 000 (*From a block of tissue supplied by Dr K. W. Min*)


F
C
C
F
D
F
D

degenerative or regenerative change in the muscle fibre. Apparent continuity with I-band filaments has been seen by Ketelsen *et al.* (1970) and Payne and Curless (1976) and the diameter of the filaments is said to be about 6 nm (Gambarelli *et al.*, 1976; Payne and Curless, 1976). Hence it is thought that these cylindrical structures seen in skeletal muscle are composed of actin filaments.

Fulcheri *et al.* (1983) found crystalline filamentous cylinders (which they call 'laminar convoluted bodies') and large amounts of actin filaments (by immunocytochemistry) in a pleomorphic adenoma of minor salivary gland. They suggest that 'laminar convoluted bodies seen in some cells might correspond to actin'. However, measurements carried out by me (Ghadially, 1983b) on their electron micrograph (their Fig. 3) at a stated magnification of ×40 000 suggests that the filaments in the cylinders are about 10–12.5 nm in diameter. Therefore if the stated magnification is correct, they are likely to be cytokeratin filaments and not actin filaments. This is not to deny that actin filaments are present in the tumour cells, for as is well known myoepithelial cells are found in these tumours.

Min (1984) has also found crystalline filamentous cylinders in a pleomorphic adenoma of the salivary gland. With the light microscope hyaline cells (ground-glass appearance) and cells containing hyaline globules were seen. Electron microscopy showed that the tumour contained epithelial and myoepithelial cells. Only the latter contained globular filamentous bodies and more extensive deposits of filaments which produces the hyaline cells or ground-glass cells seen with the light microscope (as explained on page 906). Within this filamentous matrix lay the crystalline filamentous cylinders. The cells were united by desmosomes and at the periphery of the inclusions indubitable tonofibrils were present. Min (1984) found that the filaments in the crystalline filamentous cylinders were continuous with the filaments in the tonofibrils. This combined with the fact that varying degrees of immunoreactivity for cytokeratin was demonstrable in the hyaline inclusions leads him to suggest that the crystalline filamentous cylinders may be composed of cytokeratin filaments rather than actin filaments.

I have measured the thickness of the filaments in the cylinders and the matrix in which they lie. The filaments range in thickness from about 6–8 nm. This does not tell us much because this value is between the expected thickness of actin filaments and cytokeratin filaments.

As noted above (items 22 and 23) crystalline filamentous cylinders have also been seen in neural tissues. One may speculate that here these inclusions are probably composed of neurofilaments.

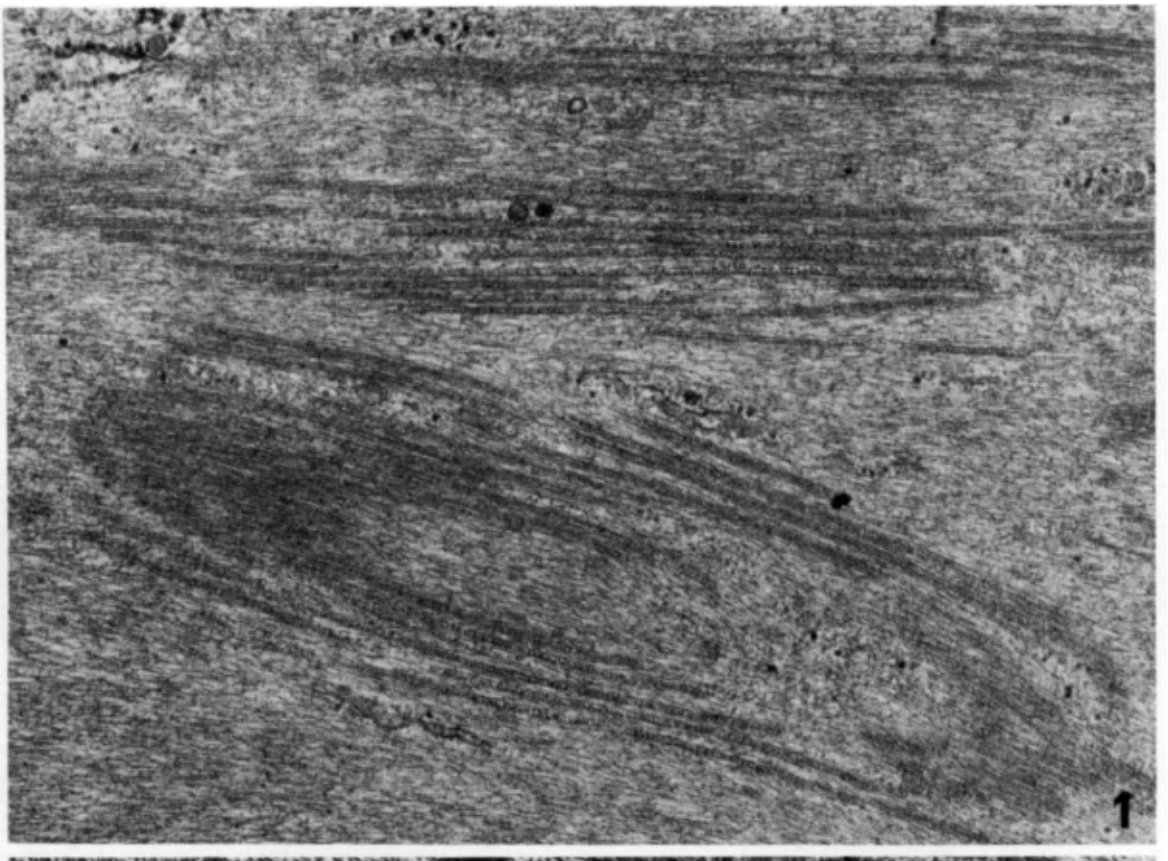
One may conclude by saying that until immuno-electron microscopy is executed on crystalline filamentous cylinders (in various sites) we cannot say which type or types of filaments are involved in their production. It may well be that a variety of filaments can form crystalline inclusions, including the ones we call 'crystalline filamentous cylinders'.

Plate 394

Pleomorphic adenoma. (*From a block of tissue supplied by Dr K. W. Min*)

Fig. 1. Longitudinally (top of picture) and obliquely (bottom of picture) cut crystalline filamentous cylinders lying in a matrix of longitudinally cut intracytoplasmic filaments. The lamellae comprising the walls of the cylinders appear to be composed of longitudinally and transversely cut filaments. This could be discerned in the original print but is unlikely to be evident in the reproduction. × 37 000

Fig. 2. Transversely cut crystalline filamentous cylinders lying in a pool of transversely cut intracytoplasmic filaments. In some places (arrowheads) the spiralling filaments in the lamellae are just discernible. × 71 000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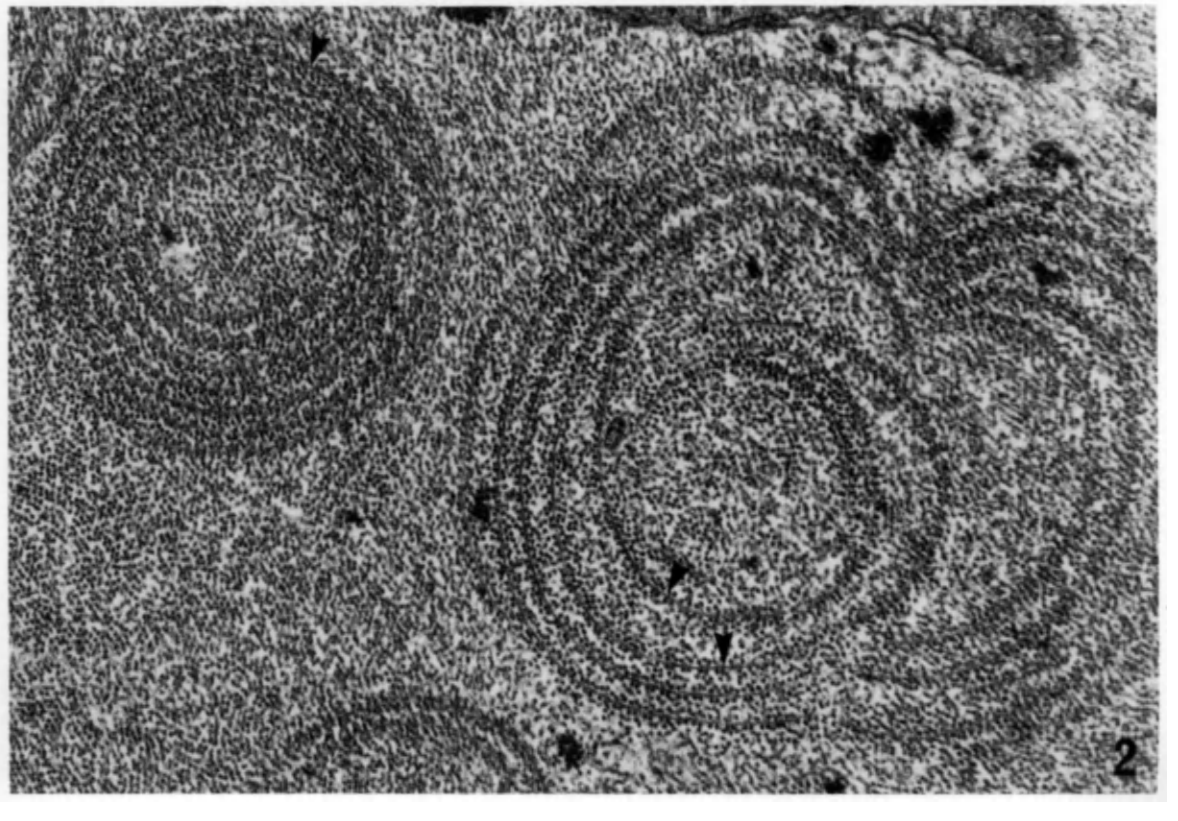
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Asteroid bodies

Asteroid bodies are irregular star-shaped inclusions found in multinucleated giant cells in sarcoidosis and other epithelioid granulomas. They were first described as 'fat needle stars' by Goldman (1890) who found them in giant cells around dermoid cysts. Light microscopically, the asteroid body is seen to lie in a clear zone or 'vacuole'.

Ultrastructurally the 'arms' or 'rays' of the asteroid body are seen to contain filaments or fibrils and some microtubules. Besides these the central part of the asteroid body also contains rounded or ring-shaped electron-dense structures believed to be procentrioles (developing centrioles) or 'postcentrioles' (degenerate or disintegrating centrioles), and at times also indubitable centrioles. The ultrastructural equivalent of the periastral clear space seen with the light microscope is an electron-lucent zone filled with whorled myelin figures, no doubt derived by hydration of lipidic material (*Plate 395*).

It has been claimed that asteroid bodies are composed of: (1) altered lipids (Goldman, 1890; Ernst, 1902); (2) proteinaceous materials (Herxheimer and Roth, 1916; Hamperl, 1940; Cunningham, 1951; Gedigk, 1954; Altmann, 1961); (3) altered fibrin (Kay, 1950); (4) trapped collagen (Azar and Lunardelli, 1969); (5) malformed cytospheres or astrospheres* due to faulty dehydration (Orsós, 1935; Altmann, 1961, 1964); (6) derivatives of the cytosphere containing filaments and microtubules (Cain and Kraus, 1977); (7) components of the cytoskeleton – predominantly vimentin filaments, and to a lesser extent microtubules (Cain and Kraus, 1983).

The most comprehensive light microscopic study of asteroid bodies is by Cunningham (1951) who states that 'in tests for collagen, Masson's trichrome stain and Mallory's connective tissue stain showed negative results. Wilder's stain for reticulum was uniformily negative'. It is therefore surprising that Azar and Lunardelli, (1969) found banded collagen fibrils (60–70 nm periodicity) in the arms of asteroid bodies. However, this was not confirmed by Cain and Kraus (1983) who did not find any collagen. It is impossible to reconcile the conflicting findings reported in these studies, but I find the studies of Cain and Kraus (1983) more convincing. Be that as it may, I will pass on the views expressed by these authors.

Azar and Lunardelli (1969) state†: 'As to the mode of formation of collagen asteroids three possible mechanisms are offered: (1) phagocytosis, (2) trapping and polymerization of collagen within performed slits of incompletely fused epithelioid cells, and (3) *in situ* formation by epithelioid cells transforming into fibroblasts during the healing of granulomas'. On the other hand, Cain and Kraus (1983), who found centrioles, vimentin filaments and microtubules in asteroid bodies, look upon them as an aggregation of elements of the cytoskeleton or cytosphere engendered by 'local fluid shifts and sol–gel transformation', and they state that: 'The stellate form of the aggregation is determined by the pre-existent radial arrangement of the elements of the cytosphere'.

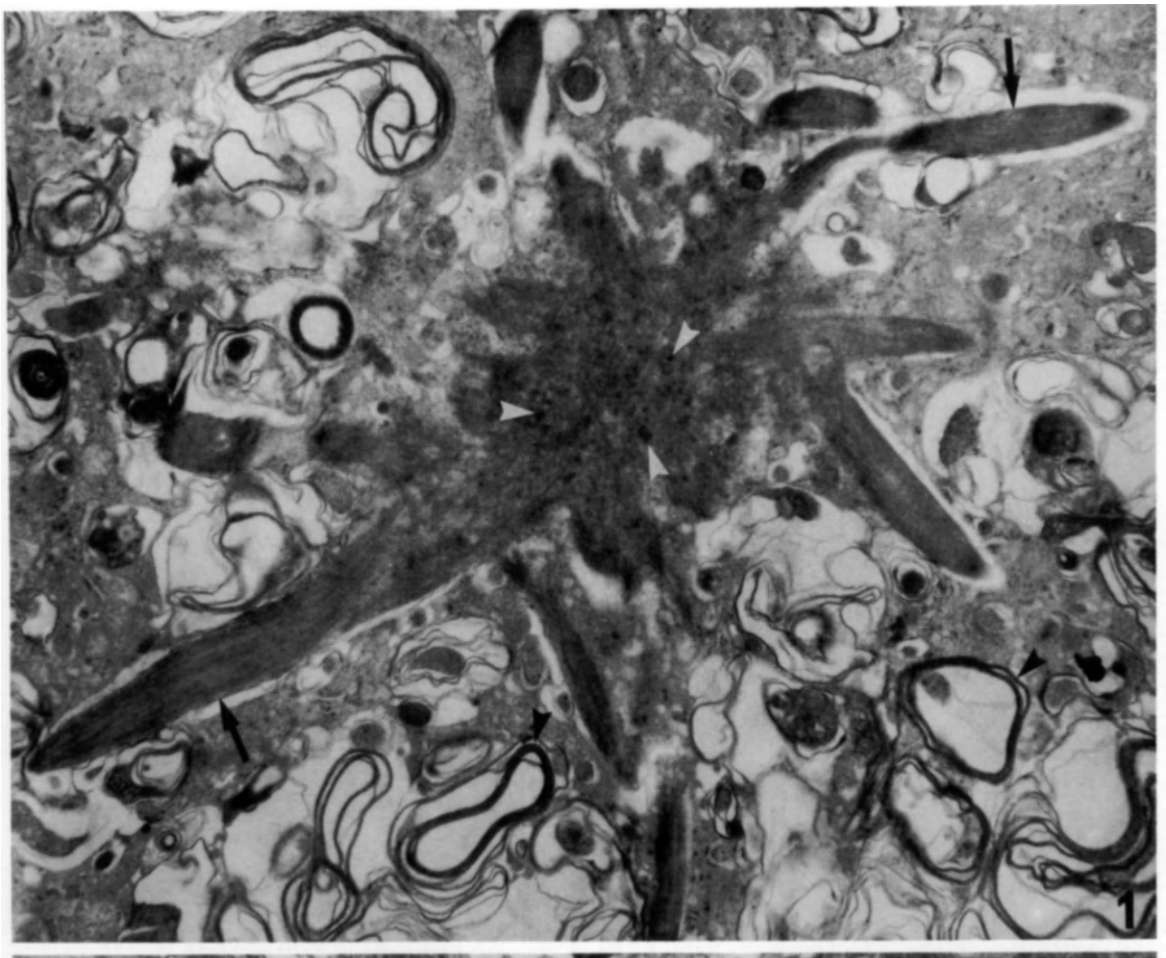
*The cytosphere or astrosphere is the star-shaped figure formed during mitosis by the astral rays (i.e. microtubules) radiating from the cytocentre (i.e. centrioles).

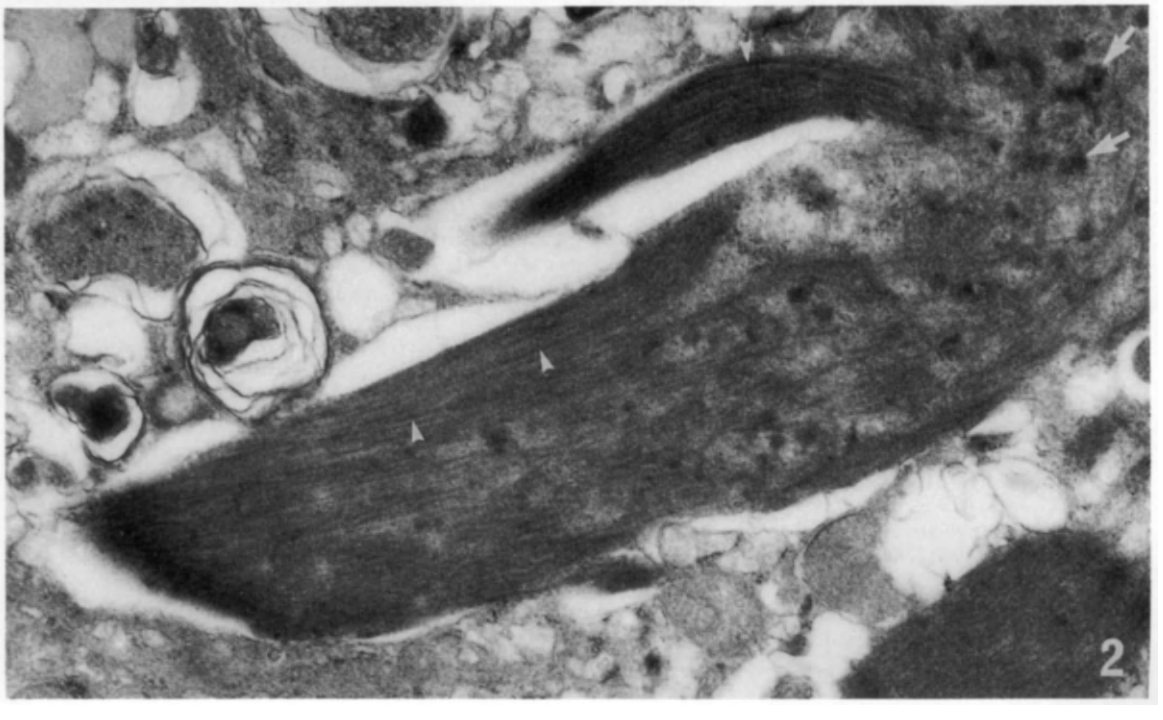
†This is roughly similar to the various theories about intracytoplasmic and intracellular collagen discussed more fully on pages 996–1001.

Plate 395

Fig. 1. Asteroid body in a giant cell from a sarcoid granuloma. Note the electron-dense structures (white arrowheads) located mainly in the body of the star, the barely discernible (because of low magnification) filaments in the arms (arrows) of the star cut in various planes and the myelin figures (black arrowheads). × 9300 (*From Cain and Kraus, 1977*)

Fig. 2. A higher-power view of a portion of an asteroid body in a sarcoidosis giant cell. Note the filaments (arrowheads) and dense structures (arrows). × 22000 (*From Cain and Kraus, 1977*)





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12

Microtubules

Introduction

Microtubules* composed of tubulin (about 24 nm in diameter, range 22–27 nm) are a virtually universal constituent of the cell cytoplasm of plants and animals. Examination of suitably prepared material has revealed that their wall contains 10–15 longitudinally orientated beaded protofilaments 3.5 nm in diameter with a repeating axial periodicity of about 8 nm (Behnke and Zelander, 1966; Behnke, 1970; Langford, 1980). A protein dimer called 'tubulin' (molecular weight 110000) composed of two monomeric subunits called 'alpha and beta tubulin' (molecular weight 55000 each), differing from each other in only a few amino acid residues, comprises the basic structural unit of microtubules. This is found to be so for microtubules from such disparate sources as human neuroblastoma cells and protozoan flagella (Olmsted *et al.*, 1961). The tubulin dimers are arranged in tandem along the length of the protofilaments and it is this which gives them a beaded appearance.

Purified preparations of tubulin obtained from brain contain small amounts of at least two other proteins (molecular weight 300000–350000) which are called 'microtubule-associated proteins'. Immunocytochemical studies show that these proteins are uniformly distributed along the surface of microtubules (Connolly *et al.*, 1978). Here they form slender lateral threads or projections exhibiting 32 nm axial periodicity (Kim *et al.*, 1979). It is thought that these projections serve as stabilizing bridges between microtubules and as sites of attachment of organelles and other particles which move along microtubules (Amos, 1979; Jensen and Smaill, 1986).

It is currently believed that cytoplasmic microtubules (polymerized state) are in a state of equilibrium with monomeric tubulin subunits (unpolymerized state) in the cytoplasm. The factors that normally regulate the equilibrium between the polymerized and unpolymerized forms are not well understood. However, low temperature, increased pressure, and various antimitotic alkaloids are known to produce a disappearance of microtubules or to prevent their formation. The antimitotic agent colchicine combines with the protein dimer of microtubules stoichiometrically (one molecule per dimer). Its antimitotic effect stems from its binding to tubulin subunits which are then unable to polymerize and form the spindle microtubules (Taylor, 1965).

Various functions have been ascribed to microtubules found in different situations and cell types. In cells such as the neurons, where they are of frequent occurrence in the dendrites and axons, they are thought to represent 'roads' (Plasmastrassen) along which intracytoplasmic substances and organelles may move. The microtubules of the mitotic spindle are responsible for the movement of chromosomes within the cell, and those in cilia and flagella for the

*This footnote is presented on the next page.

movement of these structures. In some other instances microtubules are thought to have a cytoskeletal function; that is to say, they may be involved in maintaining cell shape. An example of this is the marginal band of microtubules found in the nucleated erythrocytes of fish, amphibians, birds and human platelets. At times, microtubules appear to be involved in the development of cell shape. For example, the microtubules of the manchette of the spermatid are probably responsible for the marked elongation of the spermatid that occurs to form the neck and midpiece of the spermatozoon (*Plate 396*).

*The term 'microtubules' has come to mean microtubules composed of tubulin to many people and it is in this sense that the unqualified term 'microtubule' is used in this chapter. Such microtubules have a diameter of about 22–27 nm and a wall that is neither trilaminar nor osmiophilic like the walls of some larger (usually 30–110 nm in diameter, sometimes up to about 300 nm in diameter) tubular structures called 'tubules' derived usually but not invariably from cytomembranes.

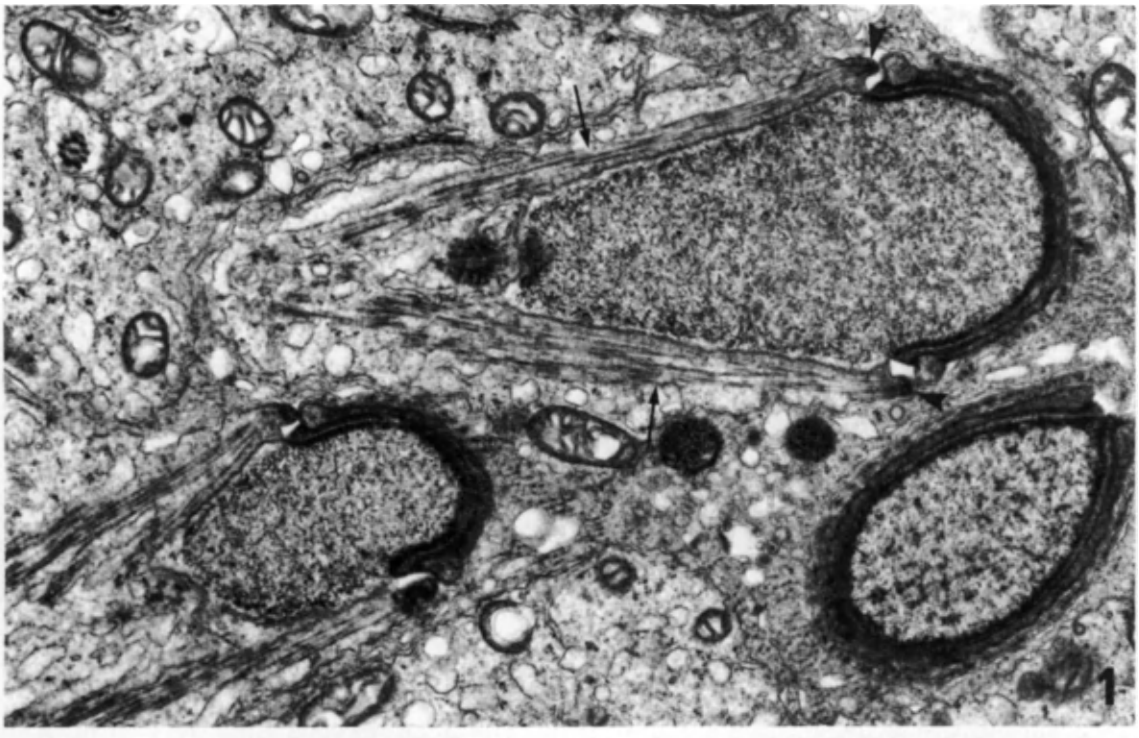
One would like to restrict the use of the term 'microtubules' for the above mentioned structures composed of tubulin but this is not a workable proposition because it deprives the morphologist of a useful term which can be applied literally to all 'small tubules' and imposes the necessity of knowing whether a given tubular structure contains tubulin or not—which is of course impossible in all but a few instances. Several structures with about the same diameter as microtubules (or smaller) found in diverse normal and pathological tissues have for long been referred to as tubules or microtubules according to the whims of various authors including the present one, as will be evident from a perusal of the first edition of this book. Some of these look like microtubules, others like tubules derived from cytomembranes and yet others bear little resemblance to either. In most cases their chemical composition is not known. To lump all these together with the large obviously membrane-bound tubules does not appear sensible. It seems far better to accept that a variety of small tubules deserving to be called 'microtubules' exist and qualify each so as to avoid ambiguity of meaning. Thus, in addition to the microtubules composed of tubulin, we may talk about: (1) viral microtubules composed of viral proteins (*Plate 68*); (2) microtubules of intranuclear rodlets and crystals which may or may not be composed of tubulin (*Plate 55*); (3) a variety of straight or slightly curved microtubules in the endoplasmic reticulum whose composition is obscure (*Plates 215–217*); (4) parallel microtubular arrays in killer lymphocytes which are thought to be composed of substances needed for the killing process (*Plates 218* and *219*); (5) microtubules in microtubuloreticular inclusions which may or may not be derived from cytomembranes (*Plates 220–226*); (6) microtubules in rod-shaped microtubulated bodies which contain Factor VIII (Chapter 10); (7) cryoglobulin microtubules in plasma cells (page 490); and (8) hexagonal microtubules in Reinke's crystals (*Plate 415*).

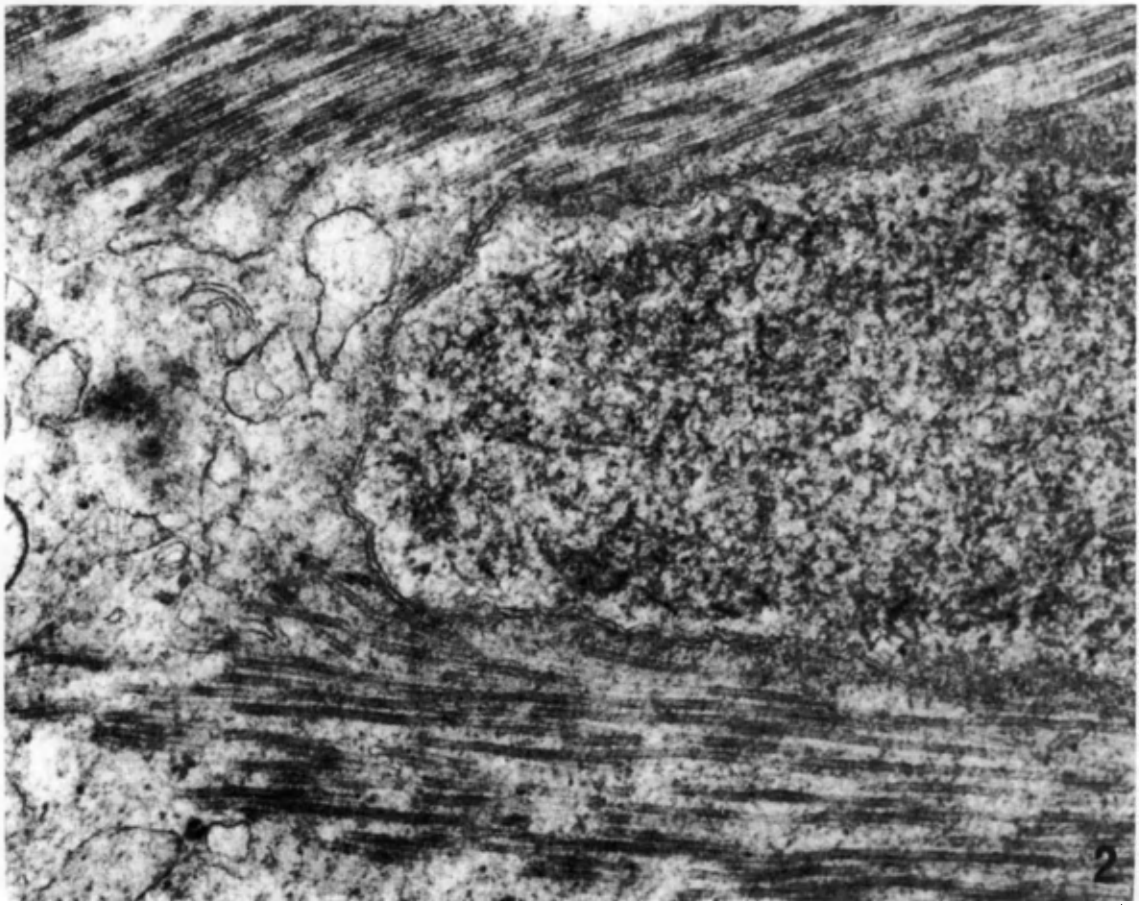
The differences in size and overall appearance of tubules and microtubules can be appreciated by comparing the microtubules mentioned above with the tubules described in this book. Such membrane-bound tubules include: (1) tubules comprising rough and smooth tubule aggregates (*Plates 211* and *212*); (2) single-walled and multiple-walled intracisternal tubules (*Plates 213* and *214*); (3) tubules in intranuclear and intranucleolar tubular inclusions (*Plates 46–49*); (4) tubular confronting cisternae (*Plates 205–209*); and (5) T-tubule networks in pathological muscle (*Plate 443*). An example of tubules which are not derived from cytomembranes is seen in Gaucher's bodies (*Plates 306–307*). Here the tubules are composed of glucocerebrosides.

In view of the above, in this book I refer to all tubular structures above 30 nm as 'tubules' and below this figure as 'microtubules' of various types. The unqualified term 'microtubules' designates what might be more fully called 'tubulin-microtubules' of the type described in this chapter. In the case of others qualifying terms are used to clarify their status.

Plate 396

Figs. 1 and 2. Spermatids from a rat testis. The manchette comprises a roughly cylindrical array (the conical appearance seen in the spermatid at the top is due to oblique sectioning) of microtubules (arrows) extending caudally from a ring-like specialization of the cell membrane (arrowheads) near the posterior margin of the acrosomal cap. Elongation of these microtubules (by addition of tubulin subunits) is probably responsible for the elongation of the spermatid, and concomitant displacement of the bulk of the cytoplasm well behind the caudal pole of the nucleus. × 15000; × 51000





Structure, function and variations

Microtubules of indefinite length but about 24 nm in diameter* have been found in a large variety of cells. They are long, smooth-walled† hollow cylindrical structures. There is no evidence that they branch or bifurcate‡. They are as a rule, not evident in osmium-fixed material: glutaraldehyde fixation is usually necessary to demonstrate them. De-The (1964) observed that 'visualization of cytoplasmic microtubules after glutaraldehyde fixation seems to be one of the main differences between this fixation and fixation with osmium tetroxide alone'. While this is true of most microtubules, certain others such as those in cilia and centrioles and, to a lesser extent, those in the mitotic spindle are seen in osmium-fixed material. It is worth noting that microtubules tend to disappear when subjected to cold. Thus, when the object is to demonstrate microtubules, it is better to fix tissues at room temperature or at 37°C rather than at the conventional 4°C. In keeping with this are immunofluorescence studies (Schliwa *et al.*, 1978) with antitubulin antibodies with which one can show an astral array of fluorescent 'fibres' (clumped microtubules) radiating from the centre to the periphery of the melanophores of the angel fish (*Pterophyllum scalare*). This system of 'fibres' is replaced by diffuse fluorescence (due to tubulin from depolymerized microtubules) of moderate intensity after cold treatment but is restored after warming the cells.

Occasional microtubules have now been demonstrated in such a large variety of cells that it would be difficult to list all the situations in which they have been seen. More fruitful would be to review those studies which indicate the role of these structures in cell economy. Perhaps the best known and most studied microtubules are those of the mitotic apparatus (*Plate 397*).

Ultrastructural studies have revealed that the spindle 'fibres' and astral 'rays' of the light microscopist are not bundles of filaments but of microtubules. However, the actual motive force which produces the movement of chromosomes remains controversial. This has already been discussed on page 186, when we dealt with centrioles and mitosis, but briefly stated: 'Some investigators favour a sliding microtubule mechanism (as seen in cilia), while others favour the idea that polarized polymerization lengthens the pole-to-pole microtubules (pushing the poles apart) and depolymerization shortens§ the pole-to-chromosome microtubules (drawing the chromosomes towards the poles)' (Mazia, 1961; Inoué and Sato, 1967; Forer, 1969; Nicklas, 1970; Brinkley and Cartwright, 1971; McIntosh *et al.*, 1979; Cande and McDonald, 1986).

*In this section of the text we deal with microtubules composed of tubulin which range in diameter from 22–27 nm. As mentioned earlier (*see* footnote on page 938) there also occur other varieties of microtubules which have a similar or smaller diameter.

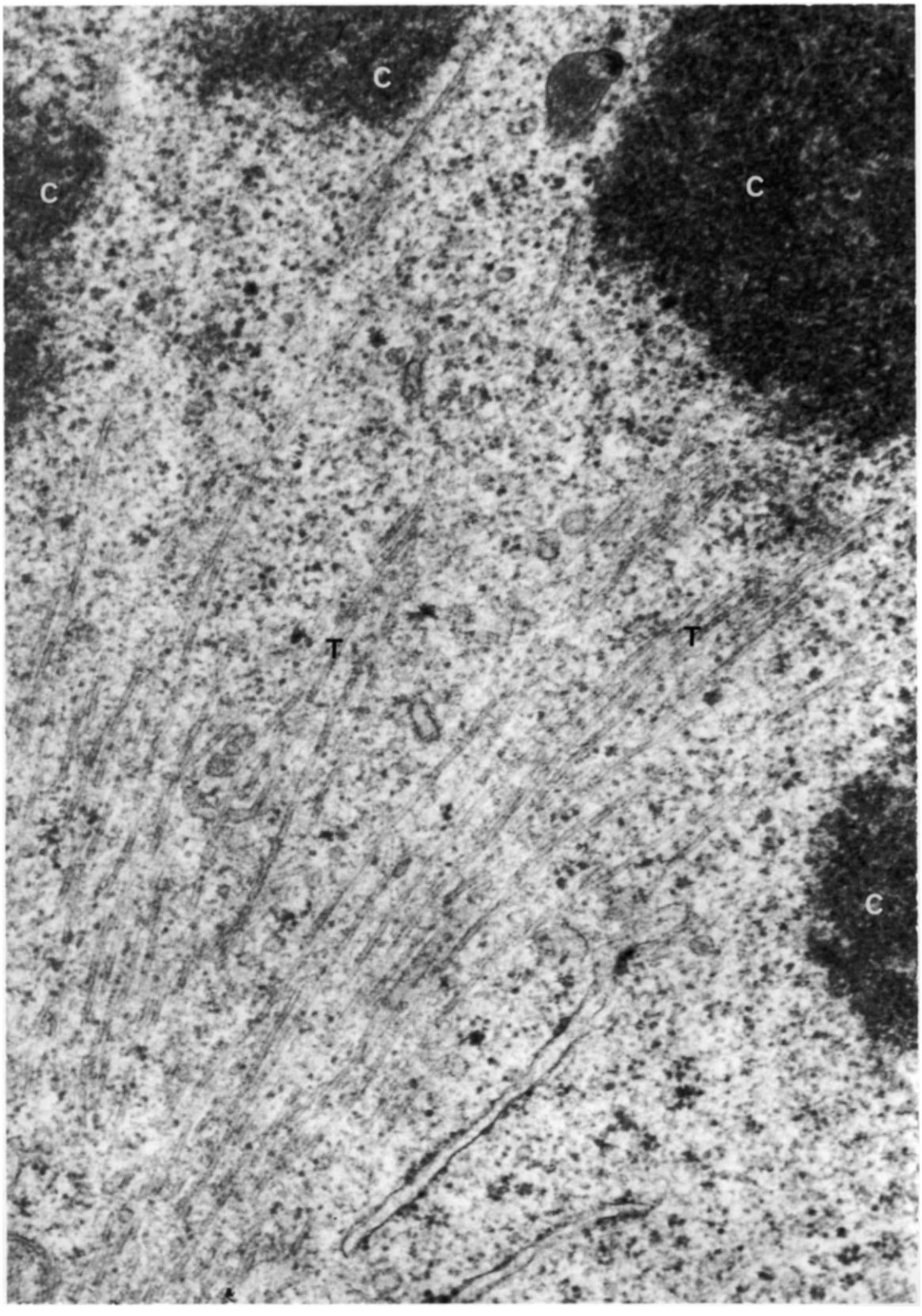
†Smooth and rough are relative terms. What appears smooth at low magnifications may not appear quite so smooth at higher magnifications. Although microtubules appear smooth in routine electron micrographs, closer examination at higher magnification often shows some filamentous or flocculent material on their surface, which presumably reflects the presence of microtubule-associated proteins (see page 937). Microtubules assembled *in vitro* in the absence of microtubule-associated proteins are indeed smooth-walled, but those assembled in the presence of microtubule-associated proteins bear slender projections (called 'bridges') on their surface.

‡Images mimicking branching can be the product of sectioning geometry and overlaying of microtubules upon or across other microtubules within the section thickness. Ignorance of such matters may lead to the erroneous conclusion that branching microtubules are present. An example of this is seen in *Fig. 2* in Carr (1972) which according to the author shows 'branching and intersecting tubules'. There is nothing in this figure to make one even remotely suspect that branching microtubules are present as the author claims, but evidence of overlaying is clear cut. Proof of branching (if it occurs at all) may be obtainable by studying the specimen with a tilting stage; or stereomicroscopy of rather thick sections with a high voltage electron microscope. *See also* comments on page 840 about this problem with filaments.

§Microtubules do not contract they shorten. When a structure contracts its mass is unchanged so that a reduction in length is accompanied by an increase in girth or thickness. Microtubules shorten by losing tubulin units (depolymerization); there is no increase in the diameter of the shortened microtubule.

Plate 397

A neoplastic cell (malignant melanoma) in mitosis showing chromosomes (C) and microtubules (T) of the mitotic spindle. × 56000


C
C
C
T
T
C

Let us now look at cilia, where there is clear evidence that sliding of microtubules and not a shortening and lengthening of microtubules produces the ciliary beat (Satir, 1968; Omoto and Kung, 1980). Ultrastructural studies on cilia have shown that the axial filament of the light microscopist is not a filament but a bundle of microtubules. This structure* now referred to as the 'axoneme' or 'axial microtubule complex' shows nine pairs of microtubules arranged in a circle around the periphery of the cilium and two microtubules in the centre. In transverse sections of cilia, the central microtubules present discrete circular profiles, but the peripheral doublets show a figure-of-eight configuration. One of these microtubules which at times appears 'solid' or 'filament-like' and bears the outer and inner dynein arms is designated 'microtubule A' or 'A-microtubule'. The other microtubule is called 'microtubule B' or 'B-microtubule'. Also seen are structures called nexin links which link adjacent microtubular doublets, and radial spokes which radiate from spoke heads in the more central parts of the cilium to each microtubular doublet.

It appears that dynein arms are instrumental in producing a sliding of the doublets relative to each other and that because of certain restraints (*see below*) the sliding is translated into a bending of the cilium to produce a ciliary beat. The spokes are thought to provide rigidity to the cilium and prevent it from buckling. The nexin links act like elastic bands holding the axoneme together, and they are thought to provide the restraint needed to translate the sliding movement into a bending movement of the cilium.

A number of observations suggest that in some instances microtubules are involved in the development and/or maintenance of cell form. The maintenance of cell form in metazoans depends upon many factors (for example, cell-to-cell attachment and mutual pressure), and microtubules play a minor, if any, role at all in most instances; but in some unicellular organisms such as the heliozoan, *Actinosphaerium nucleofilim* (Tilney, 1965), the numerous fine cell processes (axopodia) are supported by a complex array of microtubules. Such axopodia disappear and are not reformed as long as the microtubules are kept in a dissociated state with hydrostatic pressure, low temperature or colchicine; but both microtubules and axopodia promptly reappear when such influences are removed (for references, *see* Tilney *et al.*, 1966; Tilney and Porter, 1967).

A marginal band or bundle of microtubules has been observed classically† in non-mammalian vertebrate erythrocytes and in mammalian platelets (*Plate 398*) (Fawcett, 1959;

*At this point the reader should see the diagram on *Plate 507* which shows the details of the axoneme. A more detailed account of the morphology of the cilium and references are given on pages 1176–1179.

†The marginal band (Randreifen or strie bordante) was first described by Ranvier (1875) who erroneously imagined it to be a thickening of the erythrocyte membrane. Dehler (1895) interpreted it as a thickened portion of the ectoplasm. Meves (1904) showed by supravital staining that it was composed of fibrillar subunits and Weidenreich (1905) interpreted it as an artefact produced by wrinkling of the membrane surface due to shrinkage. This interpretation was widely accepted and interest in this subject declined until it was revived by the elegant studies of Fawcett (1959) and Fawcett and Witebsky (1964) which showed that the marginal band is composed of microtubules.

Plate 398

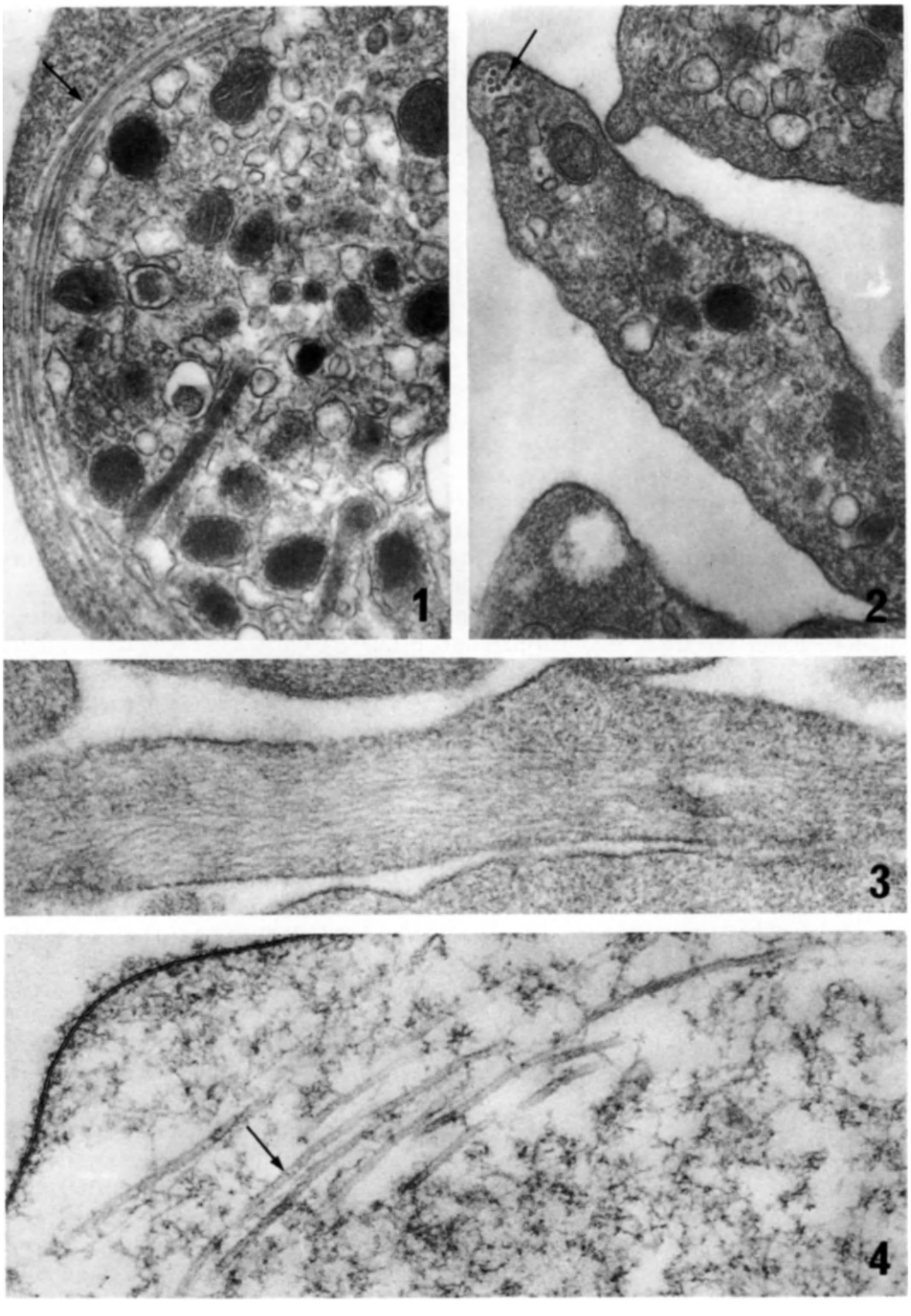
Human platelets. (*From Zucker-Franklin, 1969*)

Fig. 1. Human platelet, showing a marginal band of microtubules (arrow). × 42000

Fig. 2. A platelet showing a cross-section through the marginal band of microtubules (arrow). × 39000

Fig. 3. Fine filaments in a platelet pseudopod. × 75000

Fig. 4. Microtubules (arrow) and filamentous material which becomes apparent in the cytoplasm when platelets are incubated in distilled water at 37°C for 5 minutes. × 68000


1
2
3
4

Fawcett and Witebsky, 1964; Behnke, 1965; Haydon and Taylor, 1965; Yamamoto and Iuchi, 1975). It is thought that the flat discoid shape of the nucleated erythrocytes of fish, amphibians, reptiles and birds (i.e. non-mammalian vertebrates) is maintained by this marginal band of microtubules (for references *see* Grasso, 1966; Nemhauser *et al.*, 1980). In keeping with this is the occurrence of a marginal bundle of microtubules in the anucleate but elliptical erythrocytes (probably reticulocytes or late erythroblasts) of the camel (*Camelus dromedarius*) and llama (*Llama glama*) (Barclay, 1966; Goniakowska-Witalinska and Witalinski, 1977; Cohen and Terwilliger, 1979). The marginal band of microtubules has also been found in erythrocytes of invertebrates such as the peanut worm (*Golfingia gouldi*), the ark (*Anadara transversa*) and its close relatives ('blood clams' *Mollusca*) suggesting that the marginal bundle of microtubules has a much wider phylogenetic distribution than previously suspected (Cohen and Nemhauser, 1980; Nemhauser *et al.*, 1980). Fetal primitive erythrocytes of man and fetal erythroblasts in the liver of the rabbit are also said to show the marginal band system (Grasso, 1966; Van Deurs and Behnke, 1973) but in the adult state the erythrocytes of these species do not show the marginal band of microtubules.

It has been shown that disruption of the marginal band by colchicine in developing erythroid cells of the chicken (Barrett and Dawson, 1972) leads to spherocytosis. As mentioned before in the mature erythrocytes of man and laboratory animals such a band of microtubules has not been demonstrated. It has, however, been speculated that spherocytosis in man may be due to a defect in 'proteins of structure', because spherocytosis can be produced in normal erythrocytes by exposing them to antimitotic agents (Jacob *et al.*, 1972).

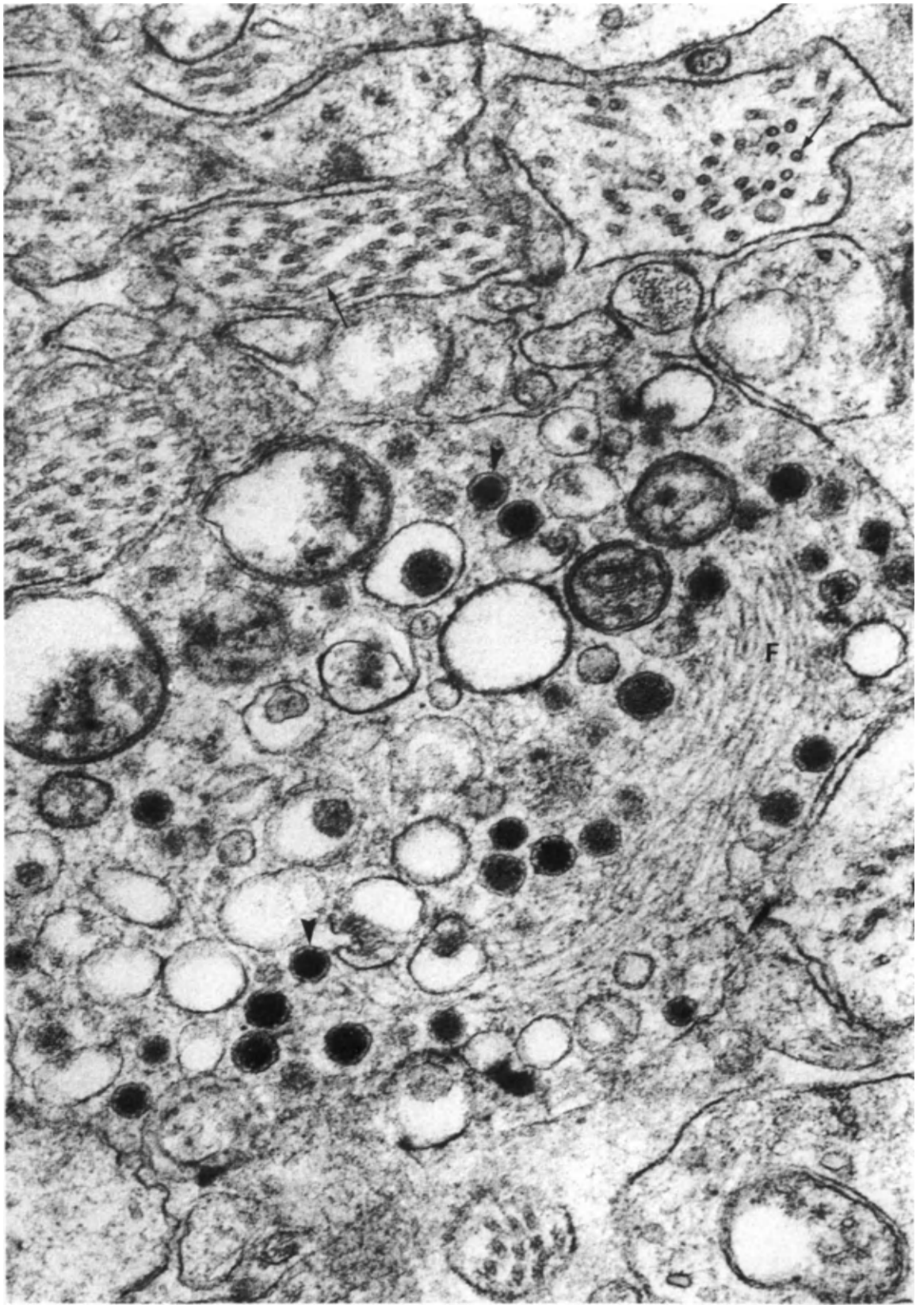
A marginal band of microtubules is found in human platelets, as well as numerous filaments in platelet pseudopodia (*Plate 398*). Furthermore, filamentous material can be demonstrated in the platelet cytoplasm when its density is reduced by incubation in distilled water (Zucker-Franklin, 1969). Brief osmotic shock at low temperature leads to a disappearance of microtubules and the appearance of filaments. Disappearance of microtubules was noted also after colchicine treatment by Zucker-Franklin (1969) but this did not interfere with clot retraction. On the basis of the above-mentioned observations Zucker-Franklin (1969) postulates that 'the contractile properties of the cells may be vested in the microfibrils, whereas the tubules may serve to maintain the highly asymmetric shape characteristic of circulating and irreversibly aggregated platelets'.

Although microtubules do not play a major role in the maintenance of cell shape in tissues of metazoans, they have been shown in some instances to be responsible for the development of cell shape. Evidence of this is found: (1) during the development of the primary mesenchyme in *Arbacia punctulata* (Tilney and Gibbins, 1969); (2) during the elongation of the primordial lens cells of the chick (Byers and Porter, 1964); and (3) in the developing spermatid (*Plate 396*) during the period of elongation to form the neck and midpiece when a cylindrical array of microtubules, called the 'caudal sheath' or 'manchette', is formed (Burgos and Fawcett, 1956; Bloom and Fawcett, 1969).

A number of observations suggest that in some instances microtubules delineate pathways along which cytoplasmic organelles and inclusions move within the cell (*see* review by Porter, 1966). For example, such a system is thought to operate in the movement of : (1) melanin granules in the melanophores of *Fundulus heteroclitus* (Bikle *et al.*, 1966) and the angel fish (*Pterophyllum scalare*) (Schliwa *et al.*, 1978); (2) Golgi vesicles at the cell plate of plant cells (Whaley and Mollenhauer, 1963; Ledbetter and Porter, 1963); (3) cytoplasmic granules in the axopods of the heliozoan, *Actinosphaerium* (Tilney *et al.*, 1966; Tilney and Porter, 1967); (4)

Plate 399

Neuroblastoma showing neurosecretory granules (arrowheads), neurofilaments (F) and microtubules (arrows). The latter are abundant in the neurites (collective term for axons and dendrites). × 63000 (*From Ghadially, 1980*)


F

endocytosed food particles along the tentacles of the suctorian, *Tokophrya infusionus* (Rudzinska, 1965); (5) lipid transport in enterocytes (absorptive cells of small intestine of rats) (Reaven and Reaven, 1977); and (6) nuclei of hamster kidney cells forming syncytia in culture after infection with para-influenza virus SV5 (Holmes and Choppin, 1968). It has also been suggested that microtubules are involved in: (1) the movement and release of secretory products—such as insulin from β-cells, histamine from mast cells and catecholamines from the adrenal medulla (Thoa *et al.*, 1972); and (2) endocytosis and exocytosis of secretory products by cultured chondrocytes (Piasek and Thyberg, 1979). This is deduced from the fact that colchicine and other antimicrotubular agents: (a) inhibit secretion of matrix components in chondrocyte cultures; (b) lead to a disorganization and dispersion of the components of the Golgi complex throughout the cytoplasm; and (c) inhibit fluid endocytosis by chondrocytes. These findings support the well-known concept that endocytosis is a process whereby the cell recovers membrane from the plasmalemma after exocytosis and it would appear that microtubules are involved in this coupling of exocytosis with endocytosis (Piasek and Thyberg, 1979).

In the above-mentioned examples there is no clear stable attachment between the moving 'particle' and microtubule akin to that seen in the case of chromosomes, although, in the case of virus-induced syncytia, rows of nuclei intimately associated with microtubules can be isolated from cell homogenates. In time-lapse movies of such cultures, Holmes and Choppin (1968) found that migrating nuclei seemed to move as if there were channels through the cytoplasm, and they state that 'the nuclei within these channels move independently of each other and may revolve end-over-end as they migrate towards the centre of the cell'. Electron microscopy showed that these channels are delineated by 25 nm diameter microtubules and 8 nm thick filaments. Upon treatment of the cells with colchicine the microtubules were destroyed and there was an increase in the number of filaments*. Such cells retain the power to fuse and form syncytia, but the nuclei do not migrate to form rows but remain randomly scattered in the syncytial cytoplasm.

The idea that microtubules in neurons also demarcate channels or 'roads' (Plasmastrassen) along which intracellular transport occurs has been frequently mooted (Andres, 1961; Bunge *et al.*, 1967; Billings, 1972). There is also evidence that, in pathological states or experimental situations (e.g. administration of colchicine, vinblastine or aluminium compounds) where microtubules are destroyed and an increase in intracytoplasmic filaments occurs, such movements are arrested.

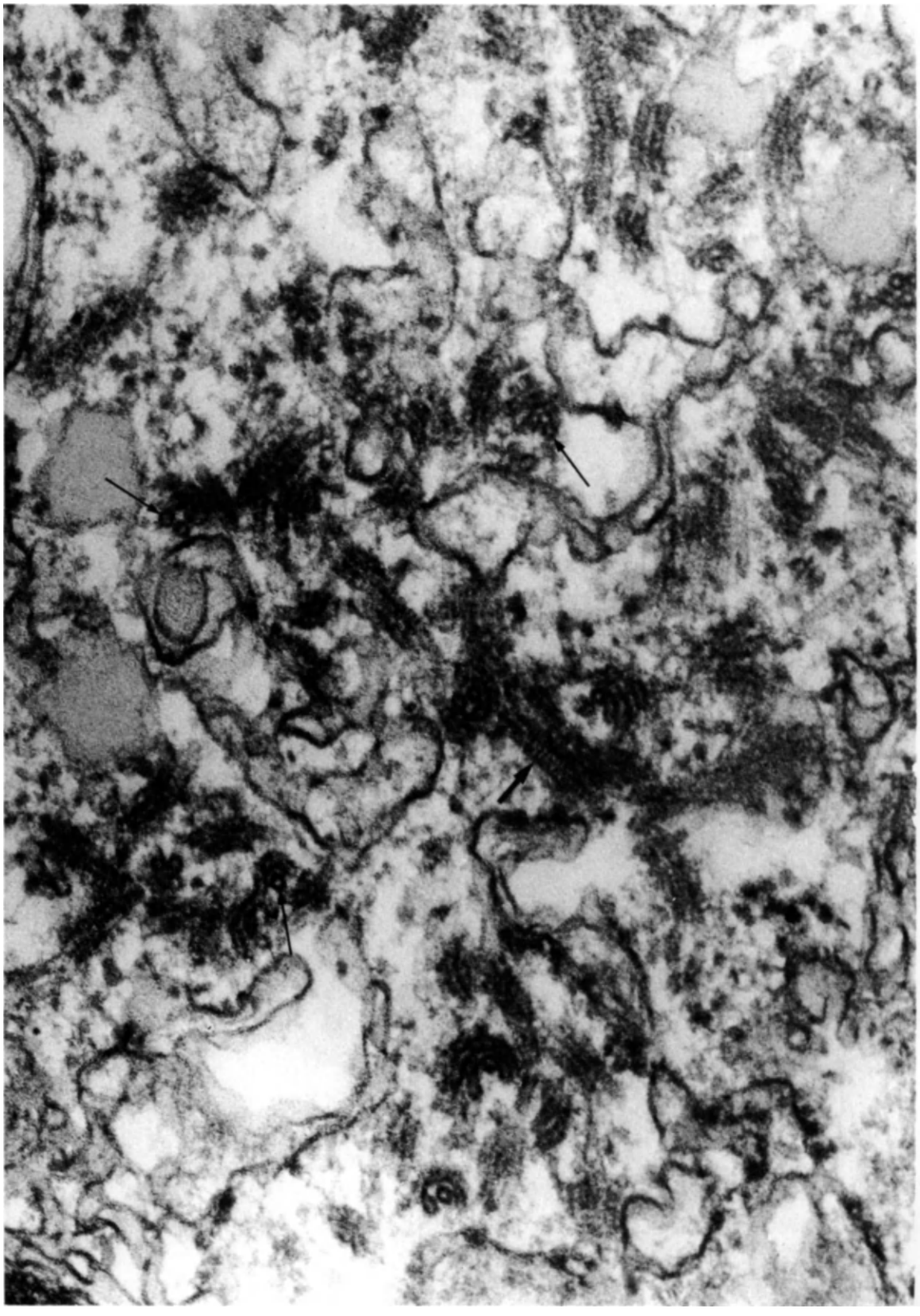
Microtubules are of rare occurrence in most tumours but they are common in ganglioneuroma and neuroblastoma (*Plate 399*) and this is of diagnostic import. The presence of microtubules, intracytoplasmic filaments and neurosecretory granules distinguishes these tumours from other tumours with which they may be confused such as Ewing's tumour and lymphoma (Ghadially, 1985).

In the liver of chimpanzees inoculated with sera from cases of Non-A, Non-B hepatitis, a variety of alterations are seen in the hepatocytes. They include the formation of: (1) microtubuloreticular structures (*Plates 224* and *225*); (2) membrane complexes (*Plate 229*); (3) tubular confronting cisternae (*Plate 205*); and (4) aggregates of microtubules scattered in the cytoplasm of the hepatocyte (*Plate 400*). The origin and significance of these microtubules is obscure. Whether they are somewhat modified microtubules composed of tubulin or

*This phenomenon has been repeatedly noted in several cell types and there have been arguments as to whether the increase in filaments is apparent or real. This matter has been discussed on pages 892, 896 and 910.

Plate 400

Hepatocyte from a chimpanzee inoculated with serum from a patient with Non-A, Non-B viral hepatitis. Aggregates of microtubules (thick arrow) showing a faintly striated pattern are present. The expected circular profiles representing transverse sections through microtubules are also evident (thin arrows). × 122000 (*Dienes, unpublished electron micrograph*)



microtubules of a different nature and composition is not known. The latter possibility seems more attractive because: (1) they are somewhat smaller in diameter (about 17 nm) than the usual microtubules, composed of tubulin which are about 24 nm in diameter; (2) they have thicker walls; and (3) in certain planes of sectioning they present a striated appearance*.

An interesting association between reovirus replication and microtubules has been demonstrated by many studies. Tournier and Plissier (1960) first demonstrated in tissue culture cells that cytoplasmic inclusions of reovirus formed along structures resembling microtubules of the mitotic apparatus and the studies of Dales and his associates (Dales, 1963; Dales *et al.*, 1965; Silverstein and Dales, 1968) have shown that, during virus development, the spindle microtubules become coated with a dense granular or fibrous material contiguous with aggregates of viral particles. A similar association of reovirus with neuronal microtubules and 5–6 nm kinky filaments was noted in the brains of suckling mice infected with reovirus (Margolis *et al.*, 1971; Gonatas *et al.*, 1971). Here also, microtubules coated with 'fuzzy' material were seen in association with virus particles (*Plate 401*). A similar association of virus and microtubules or filaments has been noted by Hassan *et al.* (1965) in reovirus myocarditis and by Jenson *et al.* (1965) in reovirus encephalitis. The significance of this unique relationship of reovirus to microtubules is not clear but Dales has pointed out that this association is not essential because destruction of the microtubules by colchicine alters the site but not the rate of virus formation.

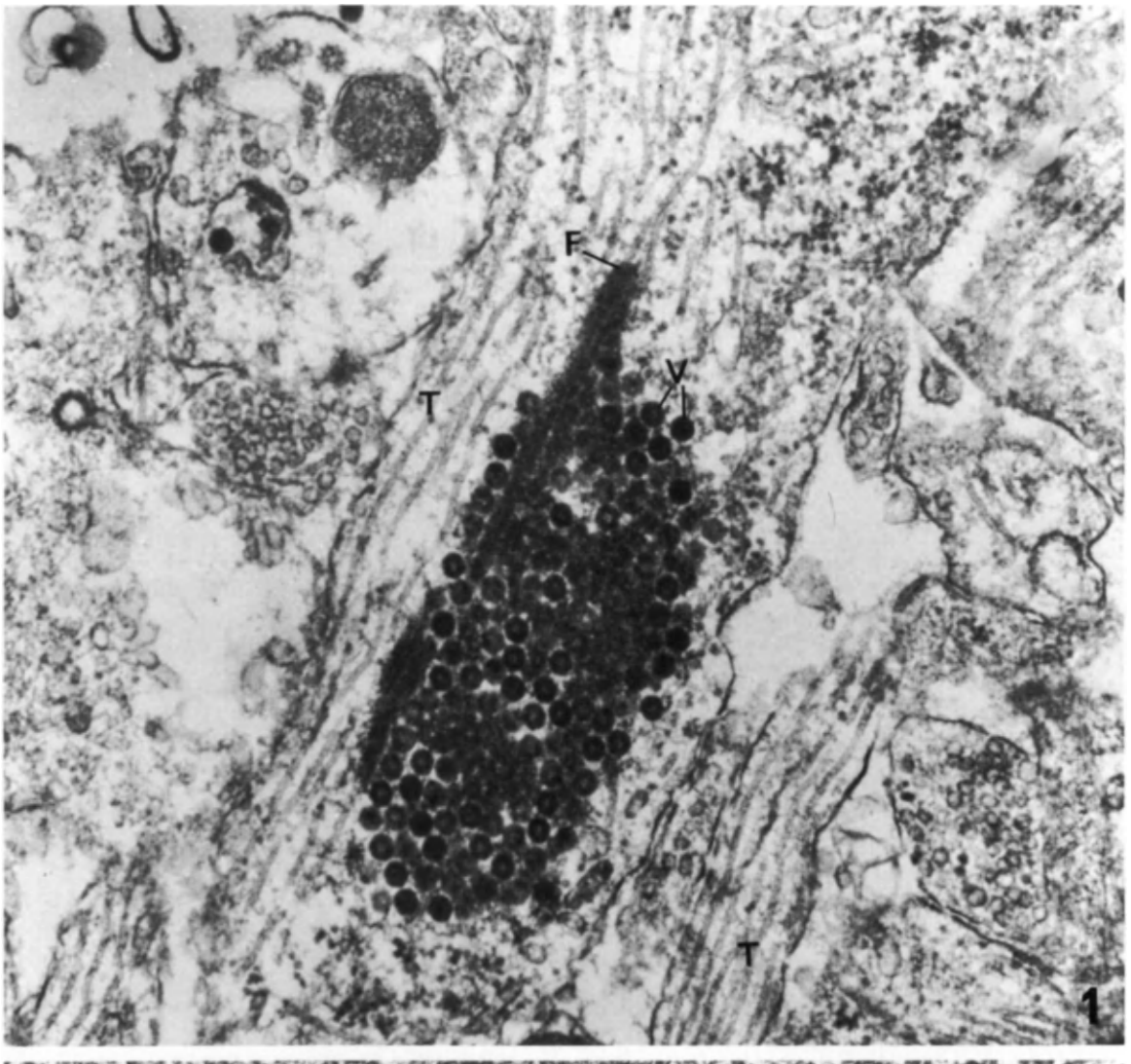
*This, however, is not a fundamental difference. True, in routine preparations the not quite so straight, slightly undulating cytoplasmic tubulin microtubules rarely if ever show a striated appearance, but the long straight microtubules of flagella and sperm tails sometimes do. This is not surprising when one considers the fact that tubulin microtubules are composed of highly ordered arrays (rows) of tubulin dimers. When the microtubule is straight and rigid and the plane of sectioning favourable, this geometric arrangement of dimers will create a striated appearance, but when not, the pattern of striations will be blurred or distorted and hence not recognizable as such.

Plate 401

Mouse brain with experimentally produced reovirus type III encephalitis. (*From Gonatas, Margolis and Kilham, 1971*)

Fig. 1. Electron micrograph showing numerous microtubules (T) and viral particles (V). Some of the microtubules are coated with 'fuzzy' material (F). × 48000

Fig. 2. Microtubules with 'fuzzy' coat (F) and associated viral particles (V) are seen here, as are unaltered microtubules (T). × 52000


F
V
T
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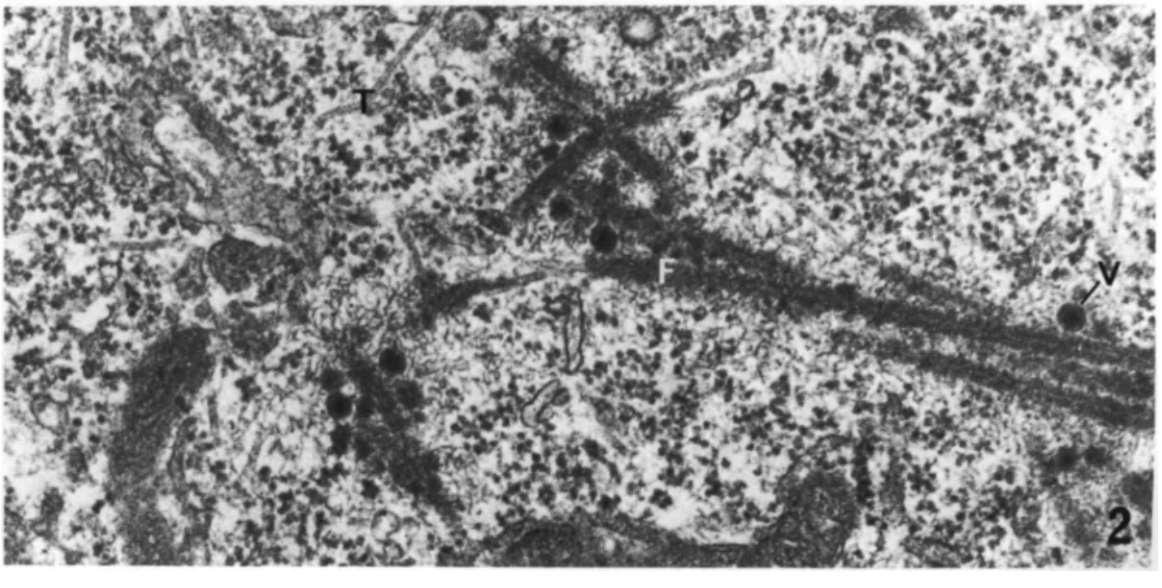
1


T
F
V
2

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13

Cytoplasmic matrix and its inclusions

Introduction

The living substance of cells (called 'protoplasm') is partitioned into two major compartments by the nuclear envelope. These are: (1) the nucleoplasm or nuclear matrix in which are suspended various structures such as the nucleolus and chromatin; and (2) the cytoplasm or cytoplasmic matrix* in which are suspended various organelles and inclusions. The term 'cytoplasmic inclusions' has long been used by light microscopists to distinguish certain intracellular structures or bodies (consisting of accumulations of metabolites or cell products) from organelles which were looked upon as miniature organs or specialized units performing specific functions within the cell. Such inclusions include secretory granules, pigment granules and various accumulations of protein (crystalline inclusions), fat (lipid droplets) or carbohydrates (glycogen) in the cytoplasm. To this group of inclusions pathologists have added various other 'bodies' found within the cell in diseased states, the best-known example being the inclusion bodies found in virus-infected cells.

With the advent of electron microscopy and the acquisition of more precise knowledge about many cell structures and the discovery of others whose function is still obscure, it has become increasingly difficult to classify the various cellular structures into one or other category. An example of this is the rod-shaped microtubulated body (Chapter 10), a membrane-bound structure which is morphologically acceptable as an organelle, but which one feels reluctant to classify as such until its function is known. Another example is the melanosome (Chapter 9), a well-characterized enzyme-containing (tyrosinase) membrane-bound structure which performs a specific function, and hence deserves to be classified as an organelle, but which ultimately becomes an inclusion: the inert melanin granule. At what stage of development one should cease considering a melanosome as an organelle and call it an inclusion is debatable, for the intermediate stages of development contain both tyrosinase and melanin. Similar difficulties are also encountered with lysosomes, which at one stage are clearly organelles in every sense of the word, but which end up as lipofuscin granules or siderosomes.

There seems little point now in pursuing with vigour the exercise of classifying all structures as either organelles or inclusions, yet such classic terms and concepts can hardly be totally ignored. In this chapter only some of the classic cytoplasmic inclusions are discussed. Pigment granules are not mentioned here, for melanin granules are dealt with under melanosomes (Chapter 9) and deposits of lipofuscin and haemosiderin are now best studied with lysosomes (Chapter 7). Similarly, both convenience and logic dictate that secretory granules are best dealt with when considering the Golgi complex (Chapter 4).

*The more precise term 'cytoplasmic matrix' should be used wherever possible because the term 'cytoplasm' has been used by some to include the cytoplasmic matrix and the organelles and inclusions, while others have used it as synonymous with the cytoplasmic matrix.

The dark cell-light cell phenomenon

Much has been written about this subject since Cohn (1892) and Clara (1932) described the dark and light cell phenomenon seen in histological sections. This phenomenon is also seen in plastic embedded semithin sections and in ultrathin sections examined with the electron microscope (*Plates 402–405*).

Briefly the dark cell–light cell phenomenon may be described as the occurrence, side by side, of dark and light variants of apparently the same cell type in a tissue preparation. Excluded from this are examples where the difference in overall density is due to differences in cell type. For example, in electron micrographs of breast tissue, fibroadenoma or carcinoma of the breast, the myoepithelial cells appear much darker than the epithelial cells. This is readily explained by the fact that the cytoplasm of the myoepithelial cell is packed with innumerable actin filaments. Such obvious easily explained differences in density between different cell types do not concern us here.

It is convenient to discuss this phenomenon in this chapter about the cytoplasmic matrix because the cytoplasmic matrix of the dark cell is markedly darker (i.e. more electron dense) than that in the light cell. However, it must be noted that the entire cell is dark; including the nucleus, which is denser, smaller and at times also crenated. This, combined with the fact that the dark cells are usually smaller in size, creates the impression that the dark cell is an inspissated, shrunken version of the light cell. Conversely, one may argue that the light cell is a swollen or hydropic version of the dark cell, but usually the appearance of the organelles does not support such an idea.

The dark cell–light cell phenomenon has been seen so often (*Plates 402–405*) and in so many normal and pathological tissues that it would be futile to try and list them all. It would be more fruitful to note but a few examples and studies which cast light on this controversial phenomenon.

No doubt the most extensively debated dark cell–light cell phenomenon is that seen in normal and experimentally damaged livers. This has been interpreted as: (1) pathological change engendered by hepatotoxic agents (Steiner and Baglio, 1963; Herdson *et al.*, 1964; Wood, 1965); (2) a non-specific or agonal change (Takaki, 1964; Papadimitriou, 1965; Theron, 1965; Ghidoni, 1967); (3) differences in hydration or functional state of the hepatocytes (Herdson *et al.*, 1964; Witzleben, 1966); or (4) an artefact of liver fixation (Ganote and Moses, 1968).

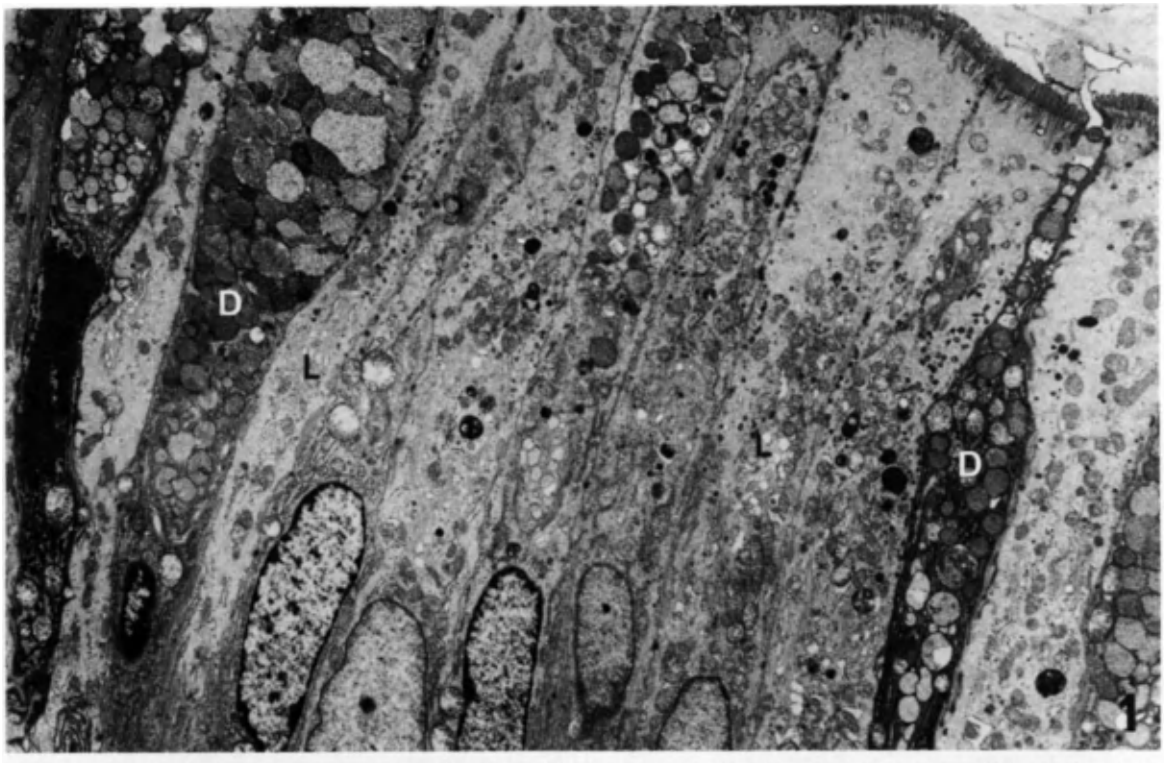
There is little doubt that conditions of fixation are a factor in the production of this phenomenon in the liver. Ganote and Moses (1968) fixed the livers of rats: (1) fed *ad libitum*; (2) fasted for one day; and (3) treated with carbon tetrachloride. They found no difference in the dark cell–light cell populations among the three groups of animals but 'variations in tissue block size, concentration of fixative reagents and fixation time all influenced the occurrence of light and dark cells'. In this experiment, blocks fixed in osmium alone and fixed in varying concentrations of glutaraldehyde followed by osmium were studied. Even when small blocks (0.5 to 1 mm) of liver were used, a fixation time of 15 minutes produced light and dark cells, regardless of the fixative solution used.

Their experiments show that any situation which leads to inadequate tissue fixation, increases the frequency of dark cells. In keeping with this is their finding that liver fixed by *in situ* vascular perfusion under optimal conditions does not show the dark cell–light cell phenomenon.

Plate 402

Fig. 1. Dark (D) and light (L) human colonic epithelial cells. From a specimen resected from carcinoma of the colon. ×3500

Fig. 2. Dark (D) and light (L) human cutaneous epithelial cells. From a skin biopsy of a case of tuberous sclerosis. ×5500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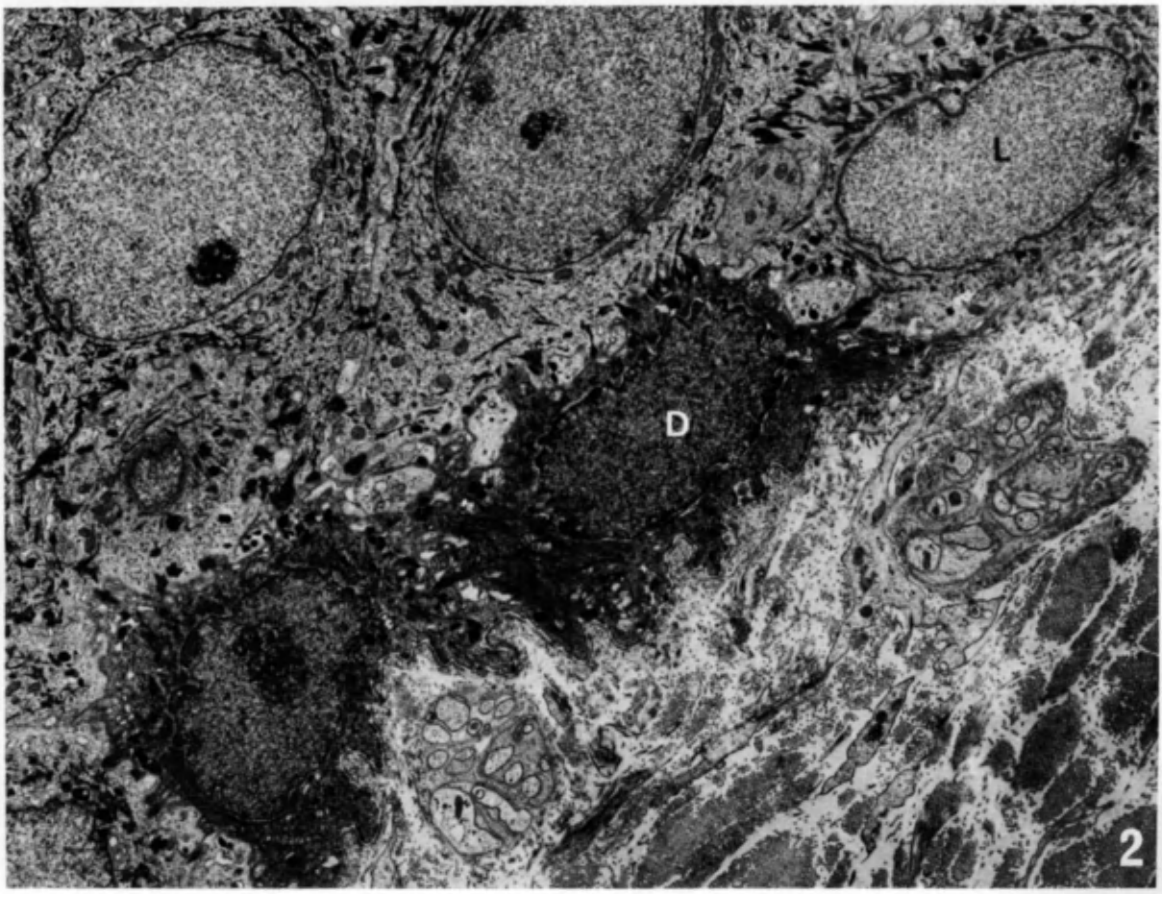
L
D
1


L
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2

However, Ganote and Moses (1968) quite correctly point out that 'The possible occurrence of dark cells in nature has not been excluded by the present study but it is evident that proper interpretation of variations in cellular density must take into account the conditions of fixation used in tissue preparation'.

The liver is not the only 'solid' organ in which light and dark cells are seen. For example, interstitial cells of the testis (de Kretser, 1967), cells of the exocrine pancreas (Ichikawa, 1967; Horký, 1971) (*Plate 403*) and the parafollicular cells of the thyroid gland (Ekholm and Ericson, 1968) also show this phenomenon. On the basis of unilateral castration experiments and review of the literature, von Sanfilippo (1970) concluded that Leydig-dark cells are functionally immature interstitial cells and that the Leydig-light cells are the mature functioning counterpart derived from the dark cell.

It is said that in the brain, light and dark neurons are seen after immersion fixation but not after fixation by vascular perfusion (Scharrer, 1938; Palay *et al.*, 1962). About the situation of neurons in sensory ganglia there is much dispute. Some believe it to be an artefact of fixation while others claim that the dark and light cells have different enzyme content and activities so they are two different types of cells (for a critique *see* Kalina and Wolman, 1970).

The dark cell–light cell phenomenon has been noted in various epithelia (*Plate 402*). *A priori* one can argue that here at least inadequate fixation due to poor or slow penetration of fixative is hardly likely to be a factor in producing this phenomenon because the superficially placed epithelial cells come in contact with the fixative immediately after immersion. Despite this, dark and light cells are seen in epithelia. The choroid plexus is a structure well suited for both immersion and perfusion fixation since it consists of a single layer of cells covering a microvascular plexus, yet Dohrmann (1970) has shown that dark and light cells are found in the epithelium of the mammalian choroid plexus (mouse, dog and human) fixed with glutaraldehyde and/or osmium by immersion or by perfusion. He therefore concludes that 'the dark and light cells of the choroidal epithelium, which appeared fine structurally similar to one another might represent varying states of cellular hydration at the time of fixation'. In this case then the dark cell–light cell phenomenon reflects an *in vivo* situation and not an artefact of fixation.

The secretory coils of mammalian eccrine sweat glands show light cells, dark cells and myoepithelial cells. It is generally thought that the light cells are responsible for the secretion of electrolytes and water while the dark cells probably secrete a mucoid substance (Sato, 1977). Doggett *et al.* (1971) found light and dark cells in labial salivary glands from normal subjects and patients with cystic fibrosis. No difference between the two groups regarding the occurrence of dark cells and light cells was noted but on the basis of histochemical studies they concluded that 'an acidic mucosubstance is being produced by dark cells and a neutral mucosubstance by light cells'.

Dark cells are often thought to be effete cells, metabolically inactive cells or dead or dying cells. This may be true in some but not all circumstances. For example, a few dark cells (called 'pencil cells', 'peg cells' or 'Stiftzellen', because of their slender compressed-looking profile) occur in the normal gall-bladder epithelium, but they are more abundant in pathological gall bladders (Fox, 1972). That such cells are not effete or metabolically inactive is evidenced by the work of Yamada (1962) who showed that they contain a high content of various enzymes. The

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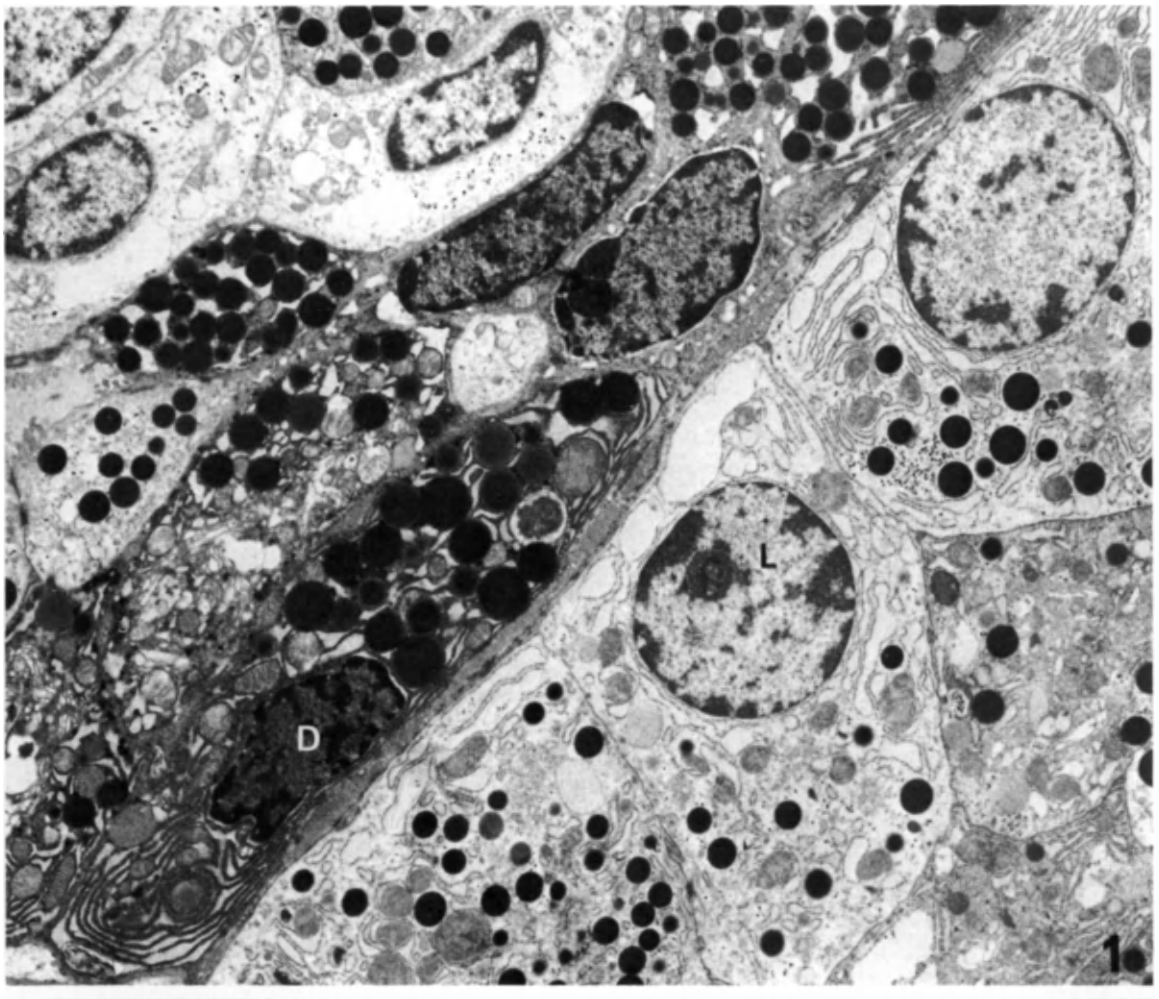
Plate 403

Fig. 1. Dark (D) and light (L) human pancreatic acinar cells. From a portion of the body of the pancreas resected with an insulinoma. ×5500

Fig. 2. Dark (D) and light (L) platelets from buffy coat of human blood. ×37 000


L
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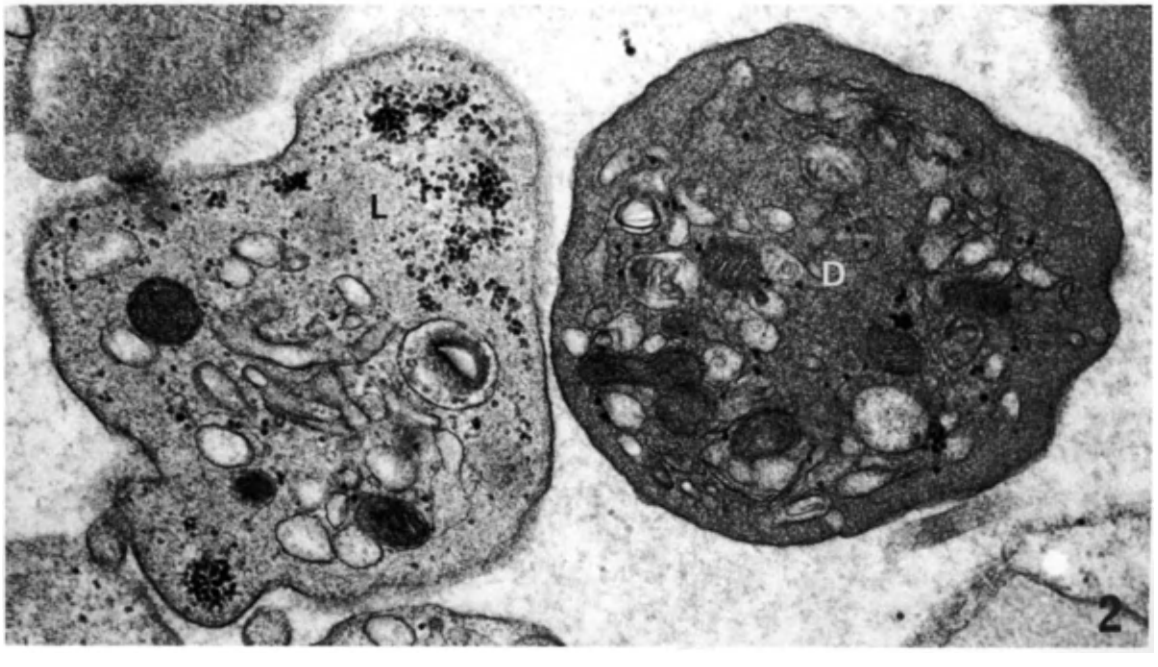
1


L
D
2

possibility that dark cells may be light cells that have suffered dehydration is attested by their abundance in some cases of chronic biliary disease where an abnormality of fluid transport across the gall-bladder epithelium is likely (Hayward, 1966, 1968) and by the studies of Kaye *et al.* (1966) who were able to increase the electron density of epithelial cells by reversing or suppressing water flow across the gall-bladder epithelium.

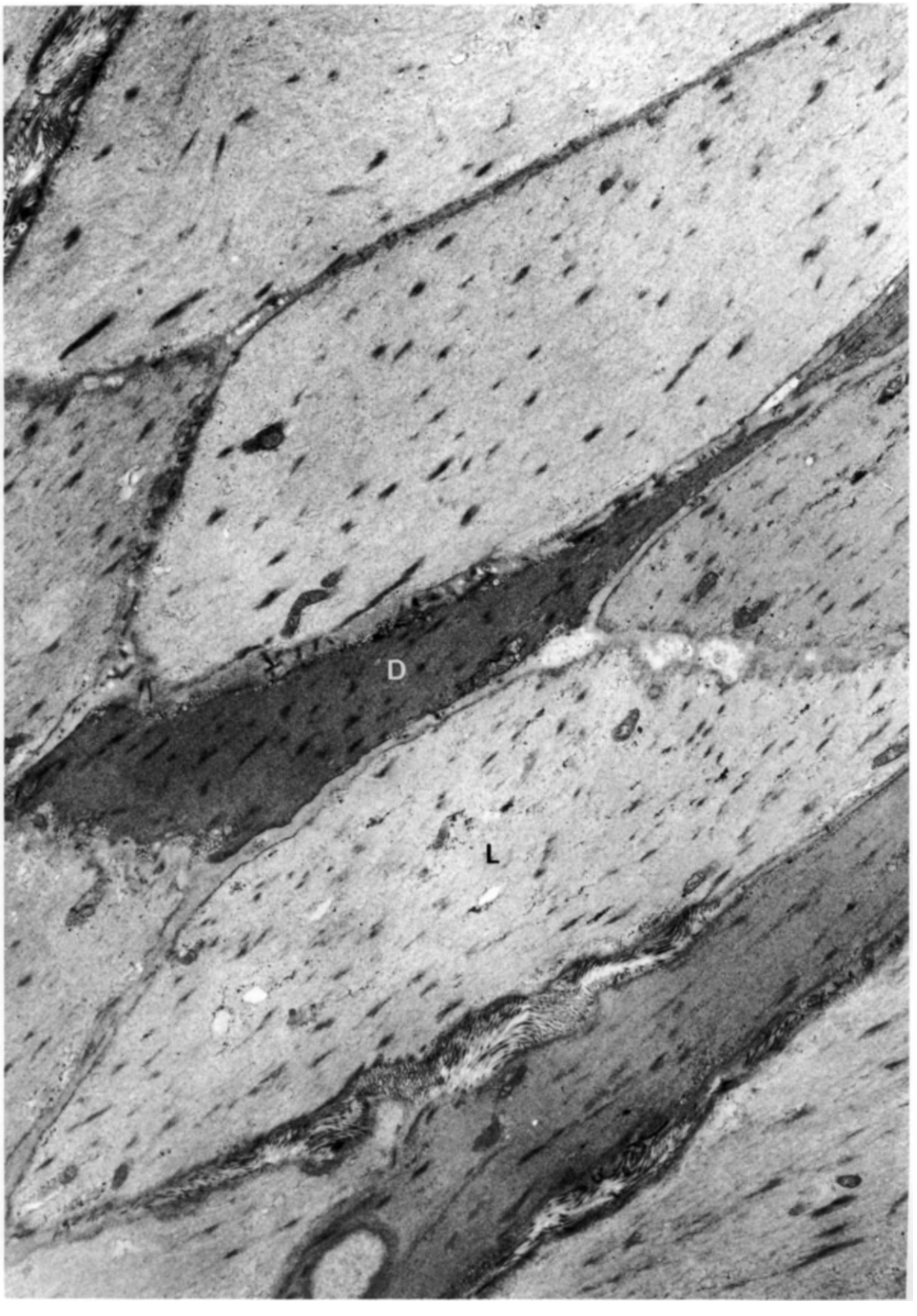
Dark cells were found in the squamous epithelium lining non-keratinizing odontogenic cysts (Hansen and Kobayasi, 1970) and it was concluded that dark cells were degenerating cells. Similarly, dark cells have been found in colonic mucosa and their tumours where they have been considered as dead or dying cells (Cooper *et al.*, 1975) showing a distinct mode of cell death for which the term 'shrinkage necrosis' has been coined (Kerr, 1971). Yet autoradiographic studies on carcinogen-induced rat colonic carcinoma (Barkla and Tutton, 1978) and carcinogen-induced rat dysplastic tracheal epithelium (Klein-Szanto *et al.*, 1980) has shown dark cells (and also of course light cells) are capable of actively incorporating ^{3}H-thymidine which argues against the idea that they are dead or effete cells. Such a contention is also supported by the observation that actinomycin D-induced nucleolar segregation is seen in both dark and light pancreatic acinar cells (Horký, 1971).

However, the cumulative evidence by no means negates the notion that at least in some instances dark cells are indeed dead or dying cells. An overall hypothesis explaining the dark cell–light cell phenomenon in all sites and situations is perhaps hard to formulate: yet one can commence by arguing that the common factor here is cellular dehydration and that excessive dehydration is what creates dark cells. One may then speculate that in some instances this 'dehydration' occurs *in vivo* engendered by physiological or pathological states and at times it can be a harbinger or even an indicator of cell death. On the other hand, this 'excessive' dehydration could at times be produced *in vitro* in perfectly normal cells, either by anoxia in the centre of the larger tissue blocks or by a complex interplay of the vagaries of penetration of fixatives and fixation rates which modify the permeability characteristics of cell membranes* to varying degrees. Subsequent treatment with increasing concentrations of alcohol could lead to varying degrees of loss of solutes and water, and this in turn could produce the dark cell–light cell phenomenon.

*Various fixatives including osmium and glutaraldehyde do not completely destroy the semipermeable properties of membranes but they do alter them to varying degrees (Jard *et al.*, 1966).

Plate 404

Dark (D) and light (L) smooth muscle cells from normal human vas deferens. ×8000


D
L

Dark and light cells in tumours

We have already noted (pages 954–959) that dark and light cells have been encountered in a variety of normal and pathological tissues. It only remains to point out that there is hardly a tumour type where dark and light cells are not sometimes seen. The very fact that this phenomenon is so ubiquitous warns us against evoking special separate theories and explanations each time this phenomenon is seen in a particular type of tumour.

Despite this, the literature on tumour ultrastructure abounds in reports where the light and dark cells in tumours have been construed to be: (1) different cell types of histogenetic significance (often called type I and II cells); (2) cells showing different degrees of differentiation; (3) a peculiar or characteristic feature of a particular tumour; or (4) a feature which suggests malignancy.

For example: (1) in Ewing's sarcoma, Hou-Jensen *et al.* (1972) interpreted dark cells as stromal cells different from the light tumour cells proper, but Povýsil and Matějovský (1977) and Llombart-Bosch *et al.* (1978) regarded them as regressive or degenerating cells; (2) it had been thought that dark and light cells in the Brenner tumour represented cellular components of differing histogenesis or functional states but Merkow *et al.* (1972) refute this idea and conclude that the dark cells are degenerating cells; (3) in a pineoblastoma, Markesbery *et al.* (1981) correlated dark cells with oligodendroglia or microglia but Hassoun *et al.* (1983) considered the dark cells and light cells found in pinealomas as 'two variants of tumour pinealocytes' and they believe that this is one of several 'specific markers for human tumour pinealocytes'. Such a statement is quite irreconcilable with reality, namely the occurrence of dark and light cells in virtually every type of tumour not just pineal tumours; and (4) in a primary leptomeningeal melanoma, Silbert *et al.* (1978) place great importance on 'a pattern of light and dark cells not previously noted' but revealed by their study. They conclude that 'The tumour cells appear to arise from these dark activated melanoblasts in the pia-arachnoid and proliferate into more differentiated light cells'. However, the dark cells depicted in their illustrations are shrunken cells with crenated nuclei and monoribosomes not polyribosomes in the cytoplasm. Such appearances suggest degenerate moribund or effete cells, not 'activated' cells.

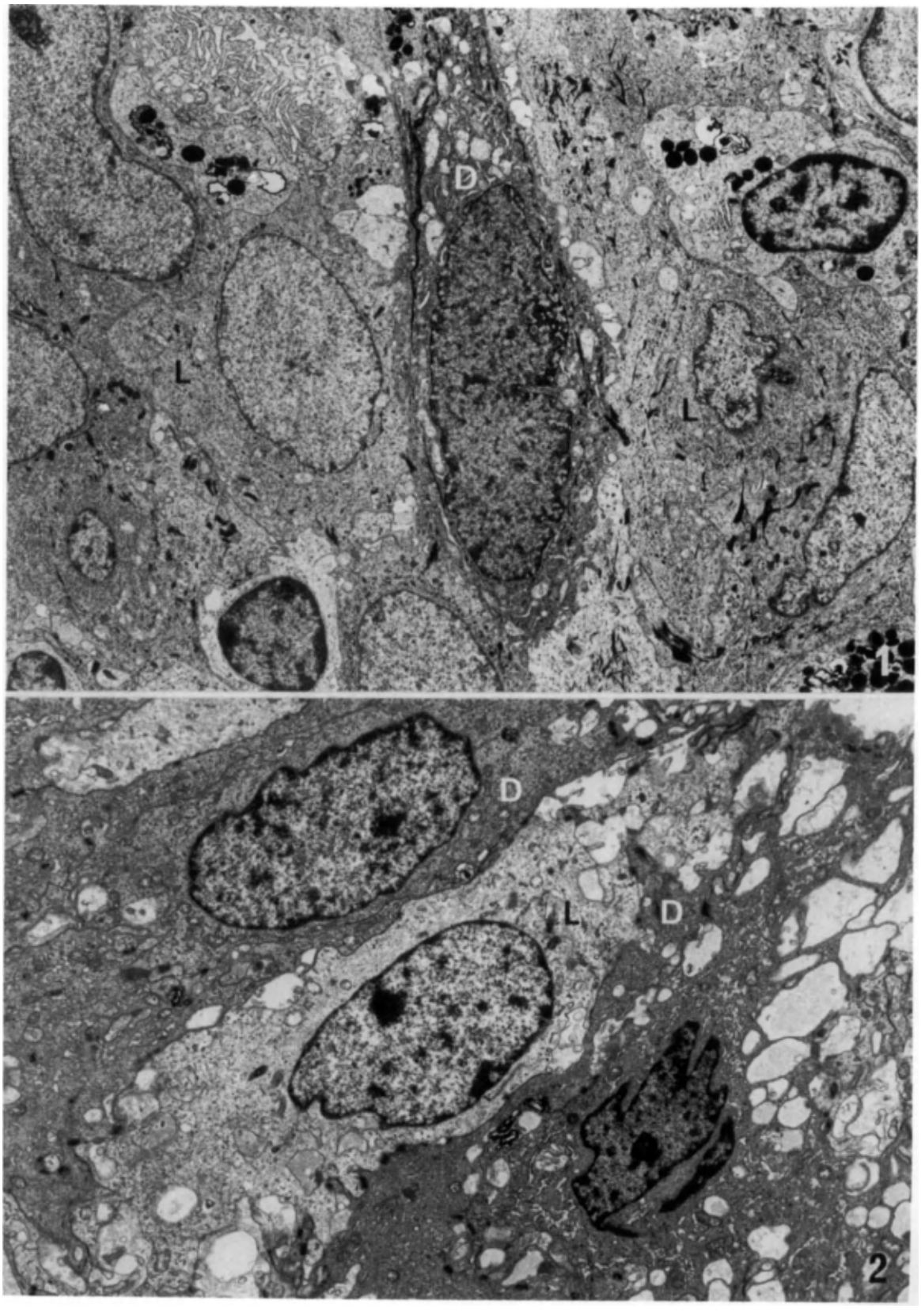
In my view, the dark cells seen in virtually all tumours are: (1) degenerate or dying cells; (2) cells damaged during tissue collection (dragging and drying); or (3) artefacts of improper fixation or processing.

From the above one may conclude that the occurrence of dark and light cells in tumours is neither of any diagnostic value nor of any histogenetic significance.

Plate 405

Fig. 1. Metastatic (in lymph node) squamous cell carcinoma of oral cavity. A dark neoplastic cell (D) is flanked by several light ones (L). ×5500

Fig. 2. Meningioma. A light cell (L) is flanked by two dark cells (D). ×5700 (*From a block of tissue supplied by Dr V.E. Gould*)


D
L
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L
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L
D
2

Glycogen

Carbohydrate is stored in animal cells as a polysaccharide called glycogen. Light microscopy and enzyme histochemistry have long demonstrated its presence in the cytoplasm of various cell types and shown it to be abundant in hepatocytes and skeletal muscle. Electron microscopy has confirmed this and also demonstrated that lesser amounts of glycogen occur in many more cell types than had been suspected before. In routine preparations, cells of the erythropoietic series from normal humans do not show glycogen*, but substantial deposits of glycogen are found in certain pathological situations (Skinnider and Ghadially, 1973) (*Plate 406*).

Glycogen may be found not only in the cytoplasm but occasionally in other cell compartments also. Its occurrence in the nucleus (*Plates 50* and *51*), mitochondrion (*Plate 132*), microbody (*Plate 333*), rough endoplasmic reticulum (*Plate 234*) and lysosome (*Plates 317* and *318*) has already been dealt with and will not be commented upon here. Suffice it to say that wherever it occurs, glycogen shows certain characteristic features which aid its identification.

In suitably stained ultrathin sections glycogen presents either as electron-dense granules approximately 15–30 nm in diameter (β particles or monoparticulate form) or as collections of such particles forming rosettes about 80–100 nm in diameter (α particles or rosette form). Atypical aggregations of monoparticulate glycogen generally larger than typical rosettes are sometimes seen. They are referred to as pseudorosettes. A faint but constant substructure is at times demonstrable in glycogen particles (β particles), and it is thought that monoparticulate glycogen may be composed of globular subunits about 3 nm in diameter. However, the possibility that this appearance might be due to a staining or focusing artefact is still debated.

It is now well established that solutions containing lead, such as lead citrate and lead hydroxide, stain glycogen intensely (Watson, 1958; Revel *et al.*, 1960; Vye and Fischman, 1970), while a staining of lesser intensity can be obtained with phosphotungstic and phosphomolybdic acid. Uranium stains glycogen very faintly or not at all.

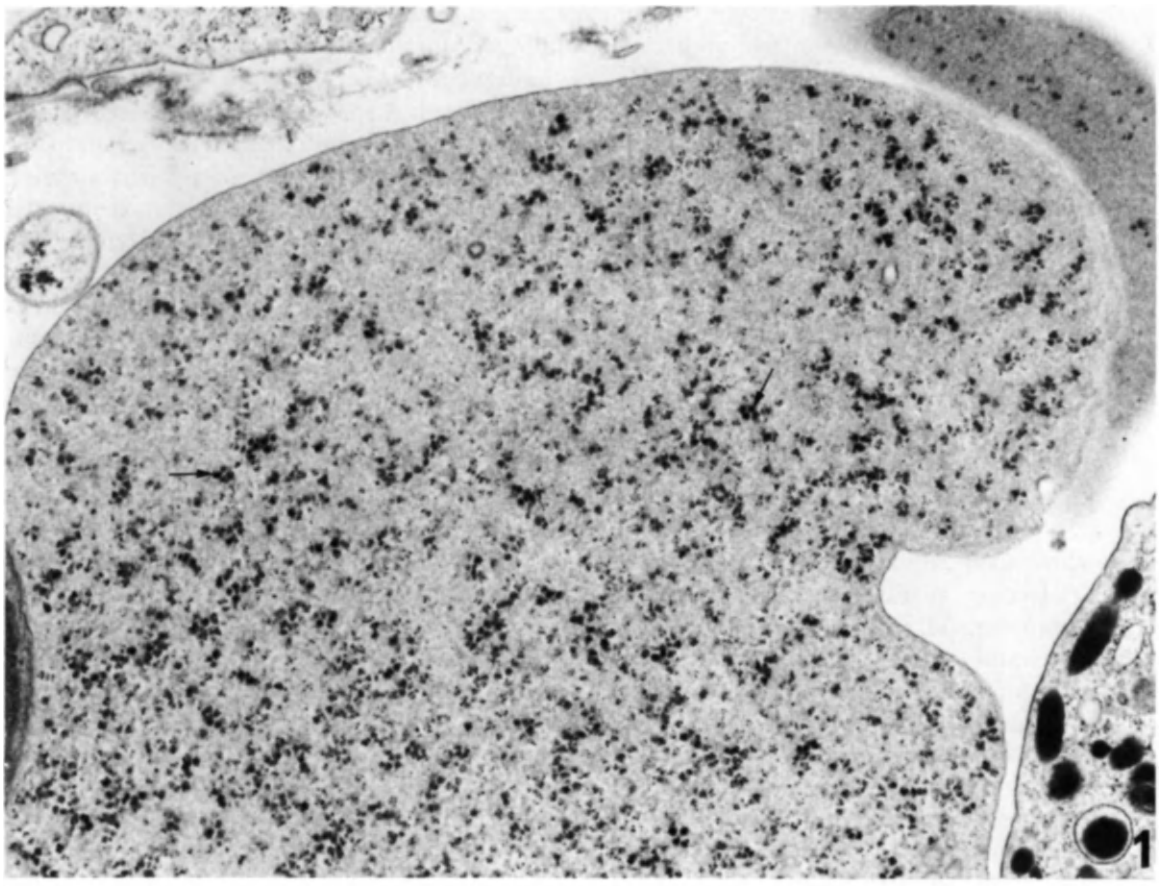
*It is worth noting, however, that a few particles of glycogen are demonstrable in normal erythroid cells by the periodic acid-thiosemicarbazide-silver proteinate reaction (Ackerman, 1973).

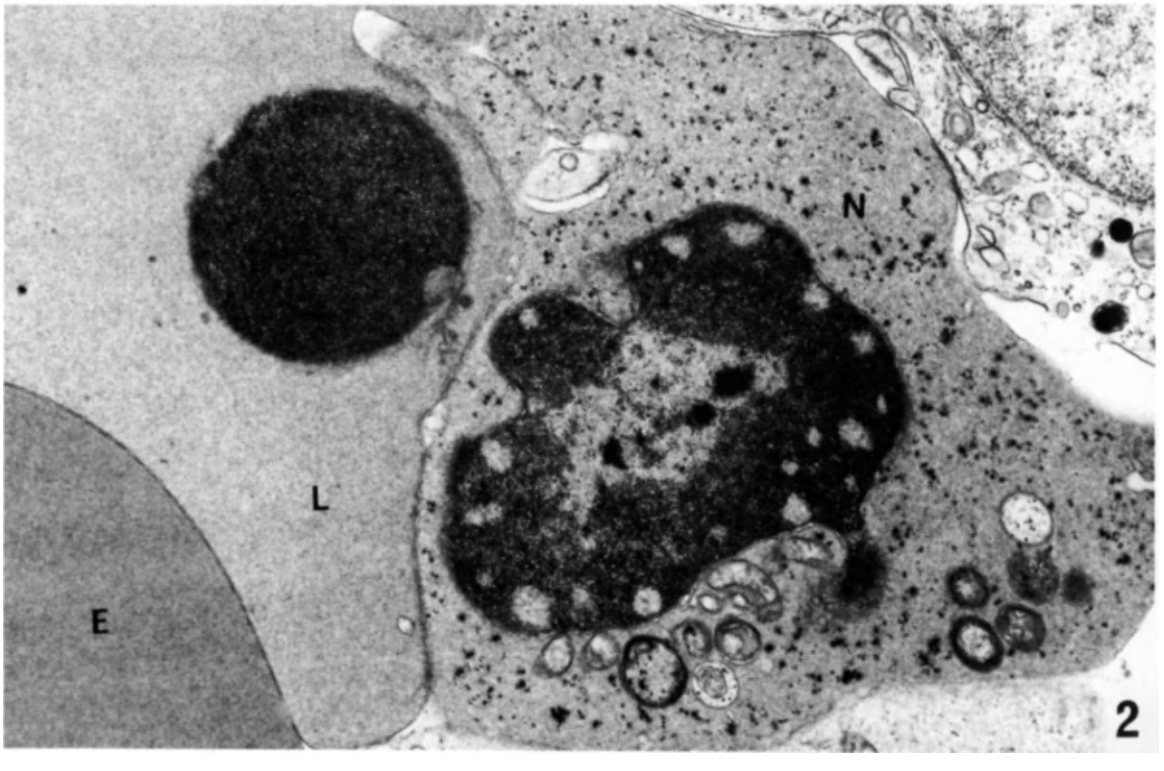
Plate 406

Glycogen-containing erythroid cells found in a case of erythroleukaemia.

Fig. 1. Mature erythrocyte with monoparticulate glycogen (arrows) in its cytoplasm. ×25 000 (*Skinnider and Ghadially, unpublished electron micrograph*)

Fig. 2. This illustration demonstrates the occurrence of a dual population of erythroid cells in erythroleukaemia. A glycogen-laden normoblast (N) is adjacent to a glycogen-free late normoblast (L) and erythrocyte (E) of normal morphology. ×19 000 (*From Skinnider and Ghadially, 1973*)


1


N
L
E
2

The appearance presented by glycogen particles and deposits depends to some extent on the method of tissue fixation and preparation employed, for this determines the proportion of glycogen preserved in the tissue and that lost during processing. The appearance seen is also dependent on the method by which the sections are stained. A spectrum of appearances is seen, but the two extremes of the scale are represented by lead staining where the glycogen particles appear highly electron-dense, and by uranium staining where solitary glycogen particles appear as 'holes' or very faintly stained particles, difficult to discern. In uranium-stained material collections of glycogen particles may present as lucent or faintly stained areas (glycogen lakes) within the cytoplasm (*Plate 407*).

Numerous other staining reactions for glycogen have been devised, some of which are said to stain glycogen in a more intense or selective manner than lead (*see* review by Vye and Fischman, 1971), yet unequivocal demonstration of glycogen still rests on evidence of diastase digestibility.

Unfortunately, this test cannot be executed on material routinely prepared for electron microscopy, since both osmium fixation and epoxy embedding render this impossible. The test for diastase digestibility has to be carried out on small blocks of aldehyde-fixed tissue, before post-fixation with osmium. However, such a method can only be operated with confidence when a fair amount of glycogen is present.

Glycogen particles of a small size (15–20 nm) are difficult to distinguish from ribosomes. Comparing adjacent sections – one stained with lead, the other with uranium – can help to resolve this difficulty, for ribosomes are visualized in preparations stained either with lead or with uranium but glycogen is evident only in the lead-stained sections.

A procedure which has gained some popularity is *en bloc* staining with unbuffered aqueous uranium acetate after fixation but prior to dehydration. This gives good visualization of general details and is particularly useful for the demonstration of the structure of cell membranes and cell junctions. The routine application of this technique is, however, hampered by the fact that much glycogen is extracted and what remains shows marked morphological alterations (Vye and Fischman, 1970).

It has been noted that two main forms of glycogen occur, namely the monoparticulate and the rosette form. Of these the monoparticulate form is by far the commoner and is the form usually encountered in most normal tissues. Rosettes are typical of glycogen deposits in the liver but occasional rosettes are also found in other normal tissues.

Rosettes sometimes occur in pathological tissues where one normally expects to see only monoparticulate glycogen. For example, the normal type of glycogen deposit in muscle tissue is monoparticulate glycogen, but in rhabdomyosarcoma collections of rosettes are seen besides

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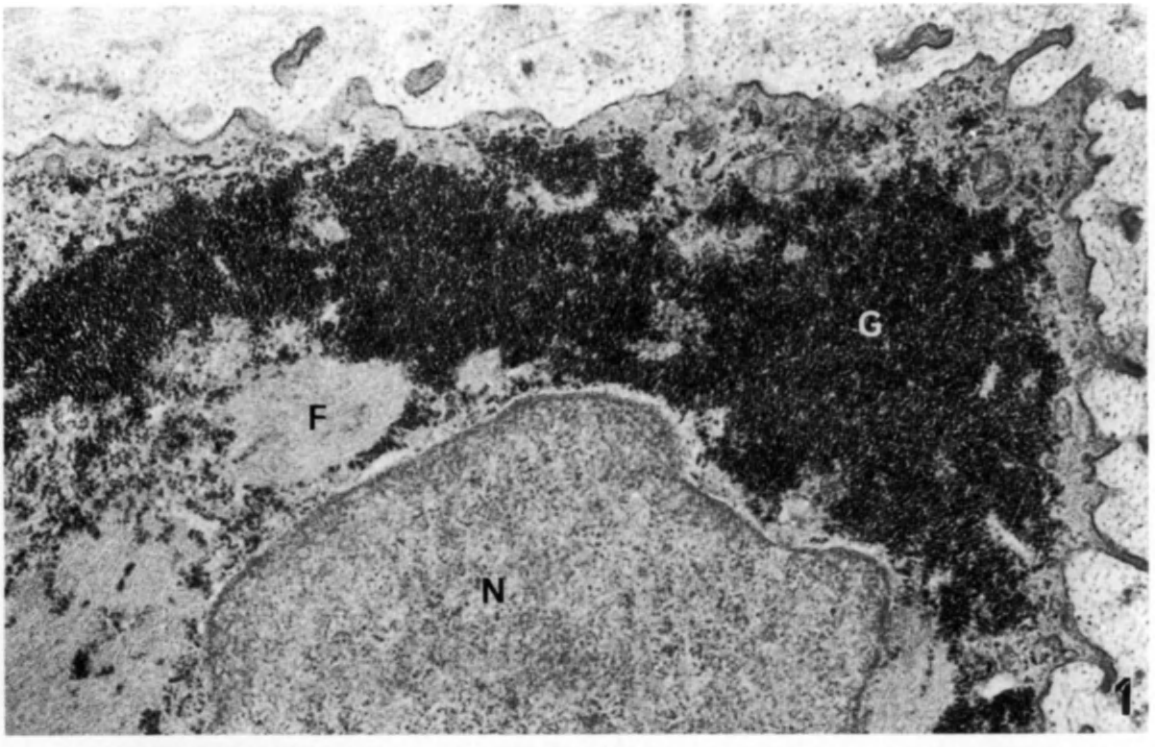
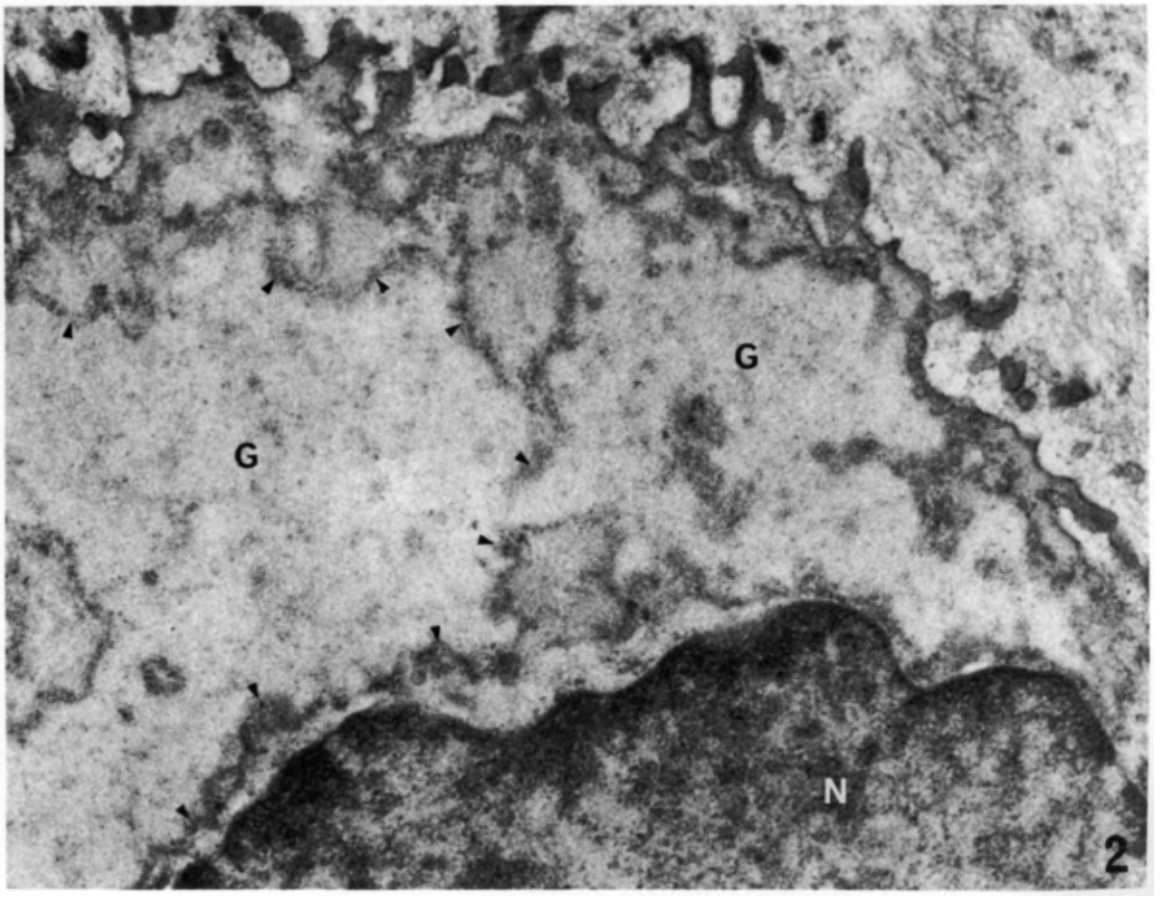
Plate 407

Osmium-fixed (cacodylate buffer) zone III chondrocytes from the articular cartilage of a two-year-old rabbit. Sections were cut from a single block of tissue. Some were stained with lead; others with uranium.

Fig. 1. In this lead-stained section large collections of intensely stained monoparticulate glycogen (G) are evident in the cytoplasm. Note also the poor visualization of the chromatin pattern in the nucleus (N) characteristic of lead staining and the abundance of cytoplasmic filaments (F). ×29 000

Fig. 2. In this uranium-stained chondrocyte, lakes of virtually unstained glycogen (G) are present. The boundary of one of these is indicated by arrowheads. Note also the good visualization of the chromatin pattern achieved in the nucleus (N) by uranium staining. ×29 000


G
F
N
1


G
G
N
2

the more substantial deposits of monoparticulate glycogen (Ghadially, unpublished observation) (*Plate 408*). A somewhat similar situation was noted by Biava *et al.* (1966) who found that while most of the kidney cells from normal human subjects contain monoparticulate glycogen, those in the collecting tubules contain a few glycogen rosettes also. However, in diabetic subjects* where there is a greater deposition of glycogen in the kidney, rosettes were found throughout the nephron, intermingled with larger deposits of monoparticulate glycogen. From observations made in various pathological states they conclude that 'in diseases associated with proteinuria, hypertrophic and hyperactive epithelial cells showed little or no glycogen. Conversely, glycogen tended to accumulate in atrophic cells and in cells, such as those of capsular epithelium in glomeruli, which are normally poorly differentiated and presumably less active'.

My experience is in keeping with this, for it is in degenerating chondrocytes (articular cartilage) rather than in active ones that the larger deposits of glycogen occur. Glycogen also seems to be more abundant in the chondrocytes of old as compared to young animals. However, I have never seen glycogen rosettes in normal or pathological chondrocytes. Similarly, accumulations of glycogen are known to occur in: (1) polymorphonuclear leucocytes as they get older (*Plate 283*); (2) cultured human fibroblasts as they approach senescence (Robbins *et al.*, 1970); and (3) glandular epithelial cells of guinea-pig seminal vesicles rendered atrophic by castration and administration of cyproterone acetate (Tam *et al.*, 1985).

In keeping with this are observations on the mammalian pineal gland whose secretory activity is under the influence of environmental lighting conditions and hence shows diurnal variations. Biochemical studies (Wurtman *et al.*, 1968) show that the metabolic activity of the pineal gland is depressed in light and enhanced in darkness, while histochemical studies (Kachi *et al.*, 1971) show that glycogen stores are maximum at the end of the light period and lowest at the end of the dark period. So, here also there is an inverse relationship between metabolic activity and glycogen deposits.

Glycogen is a metabolic fuel stored in cells; hence one might have imagined that its abundance would indicate a metabolically active cell. However, as we have seen, this is not so. In many instances in fact, an increase in the amount of glycogen in the cell cytoplasm is an indicator of diminished usage and accumulation rather than a sign of increased metabolic activity.

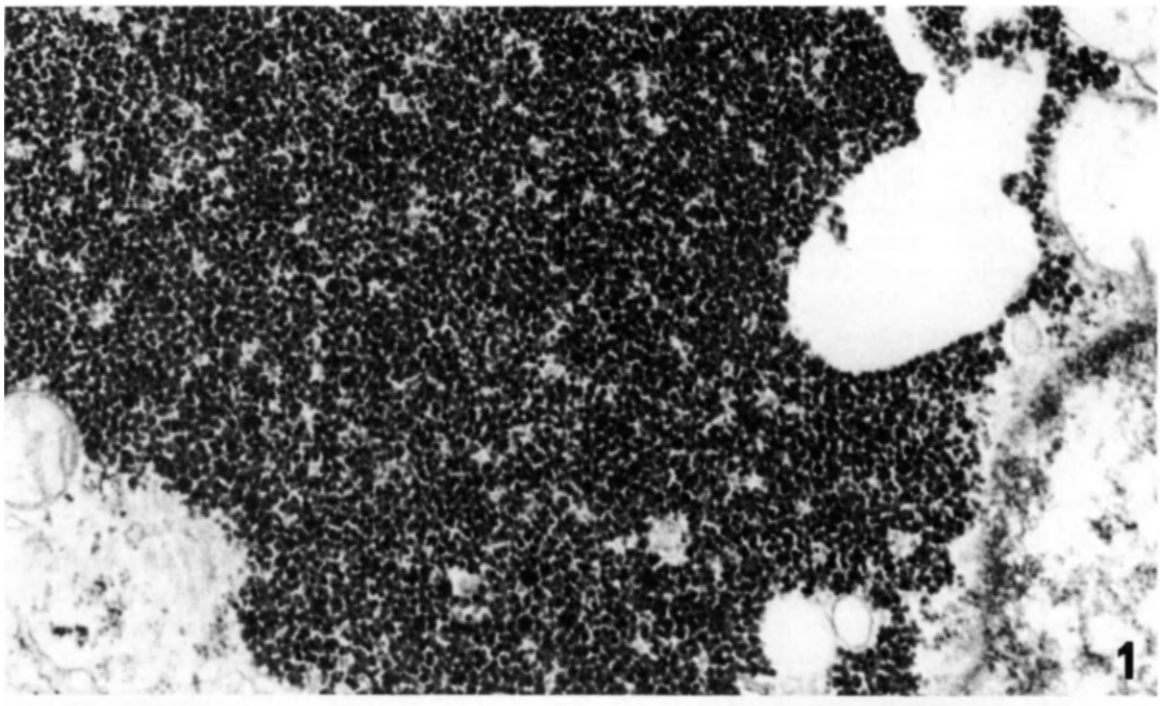
With the advent of diagnostic electron microscopy of tumours much attention has been focused on the abundance or paucity of glycogen in various tumours, because knowledge about such matters is at times of diagnostic importance. Therefore a brief review of this topic is presented here.

As is well known, Ewing's tumour is at times difficult to distinguish from neuroblastoma and lymphoma. There are several ultrastructural differences† between these tumours, one of them being the amount and distribution of glycogen. Glycogen occurs in Ewing's sarcoma principally as small and large focal deposits within the cytoplasmic matrix. At times a lipid droplet is seen within the focal glycogen deposit‡. Some glycogen is also scattered about in a

*In uncontrolled diabetes mellitus insulin-sensitive tissues such as muscle and liver become depleted of glycogen, while insulin-insensitive tissues like kidney and retina accumulate glycogen (for references *see* Sosula *et al.*, 1974).
†It is not possible to deal with all these here. For this, the reader should consult Ghadially (1985). Here we deal only with glycogen in these tumours.
‡Such a situation is by no means unique, for a lipid droplet (or droplets) surrounded by glycogen is seen in several sites and situations such as: normal chondrocytes of articular cartilage, and in cells of tumours such as chondrosarcoma, renal cell carcinoma and embryonal rhabdomyosarcoma. Even so, the glycogen deposit with a 'hole' in it, is quite characteristic of Ewing's tumour, and hence of some diagnostic value when taken in conjunction with other findings.

Plate 408

Rhabdomyosarcoma.
Fig. 1. A tumour cell containing monoparticulate glycogen. ×35 000
Fig. 2. A tumour cell containing glycogen rosettes. ×37 000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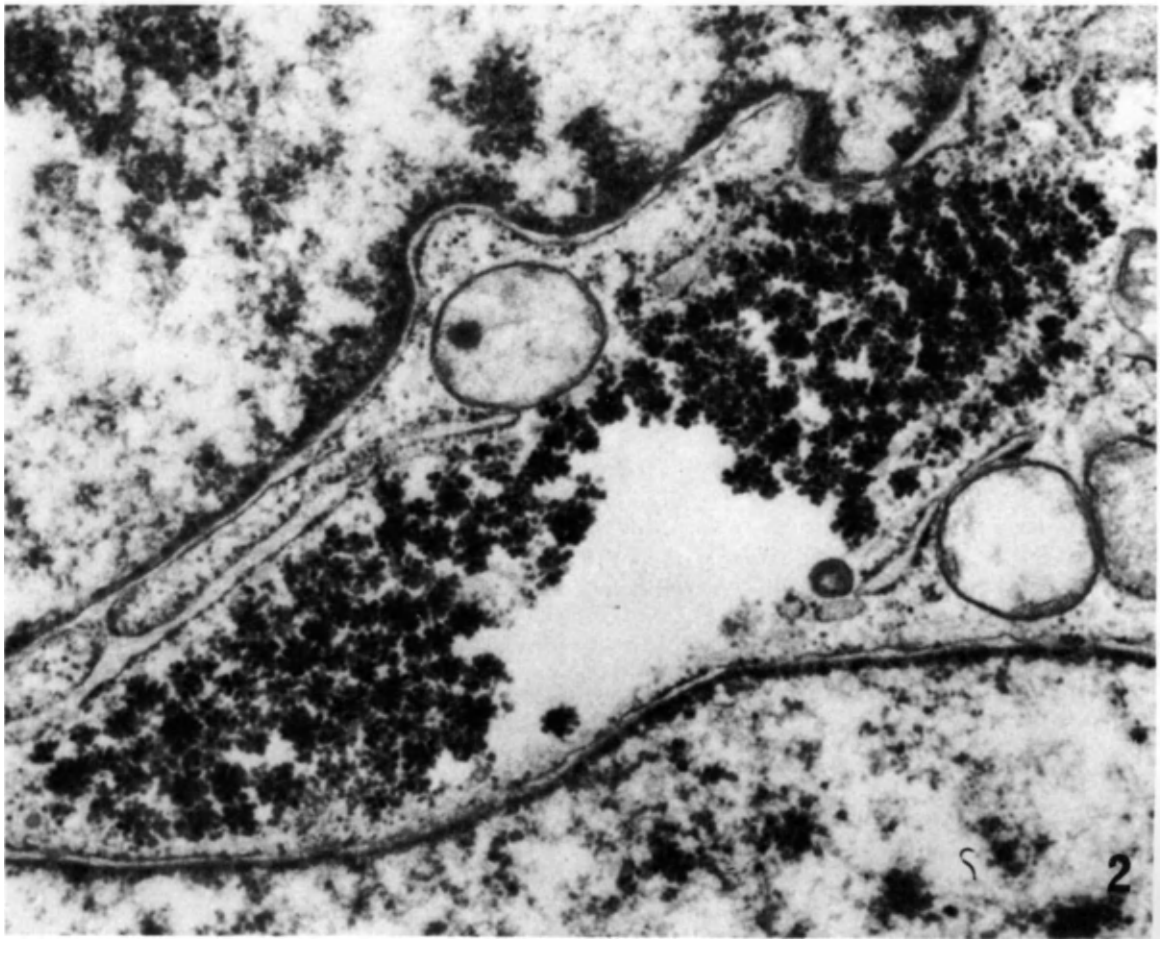
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diffuse fashion in the cytoplasmic matrix and some glycogen is at times seen between the tumour cells.

The positive finding of glycogen in tumour cells has become one of the ultrastructural criteria which must be met before one can diagnose Ewing's tumour (*Plate 409*). The possibility that there may be some examples of this tumour devoid of glycogen has at times been suggested (e.g. MacIntosh *et al.*, 1975). However, most authors hold the converse view. For example, Mahoney and Alexander (1978) who studied 21 Ewing's tumours state: 'Glycogen is present in all Ewing's sarcoma cells but may be sparse and rare in some tumours'. Certainly the amount of glycogen present varies from tumour to tumour and it is possible that in some rare instances one may fail to detect glycogen. In such a situation it would be virtually impossible for an electron microscopist to support the diagnosis of Ewing's tumour.

A feature of considerable diagnostic importance which helps to distinguish neuroblastoma from Ewing's tumour is the absence or paucity of glycogen; the little that is sometimes present does not, as a rule, form focal aggregates or large lakes. However, a detailed ultrastructural study (40 cases) on this point was carried out by Yunis *et al.* (1979) who found that in 10 per cent of the cases quite large focal deposits of glycogen do occur in neuroblastoma. In lymphomas there is almost invariably a virtual absence or marked paucity of glycogen.

As is well known there are several clear cell tumours* where the clear cell appearance is mainly or wholly due to glycogen deposits in tumour cells. This includes several epithelial tumours (e.g. glycogen-rich clear cell tumours of breast, thyroid, parathyroid, lung, salivary glands, ovary, urethra and urinary bladder) and also some sarcomas (e.g. glycogen-rich clear cell sarcoma of tendons and aponeurosis and clear cell chondrosarcoma). The significance of these large accumulations of glycogen in tumour cells is not known, but on the basis of what we know about accumulation of glycogen in normal cells (page 966) one may speculate that these are probably metabolically depressed cells and that tumour growth and spread occurs from cells which are not heavily laden with glycogen.

We noted that it is the cytoplasmic matrix of senescent chondrocytes which contains large glycogen deposits. In keeping with this is the observation: (1) that glycogen is prominent in chondroma and low grade chondrosarcoma but not in poorly differentiated chondrosarcoma, and (2) that the tumour called 'clear cell chondrosarcoma' (a variant which contains abundant glycogen) is virtually benign or of low grade malignancy (Le Charpentier *et al.*, 1979; Angervall and Kindblom, 1980).

Two other neoplasms ultrastructurally characterized by their glycogen content are erythroleukaemia and the sugar tumour of the lung. As already mentioned (page 960) erythroleukaemia seems to be the only condition in which quite substantial deposits of glycogen occur in erythrocytes and this is a point of some diagnostic value. The cells of the sugar tumour (also called 'clear cell tumour of the lung') contain fair amounts of monoparticulate and rosette forms of intracytoplasmic glycogen. However, the unusual feature (as far as tumours go) here is that much of the glycogen occurs in single-membrane-bound bodies which may or may not be lysosomal in nature. The significance of this phenomenon is obscure (*see* page 724 and Ghadially (1985) for more details).

*Accumulations of various substances such as glycogen, lipid, mucin and colloid or a combination of these can produce a clear cell appearance. A few examples of such clear cell carcinomas include: (1) clear cell carcinoma of kidney (glycogen and lipid); (2) papillary clear cell carcinoma of thyroid (colloid); (3) clear cell carcinoma of larynx (mucin and glycogen); and (4) some clear cell carcinomas of breast (lipid or mucin).

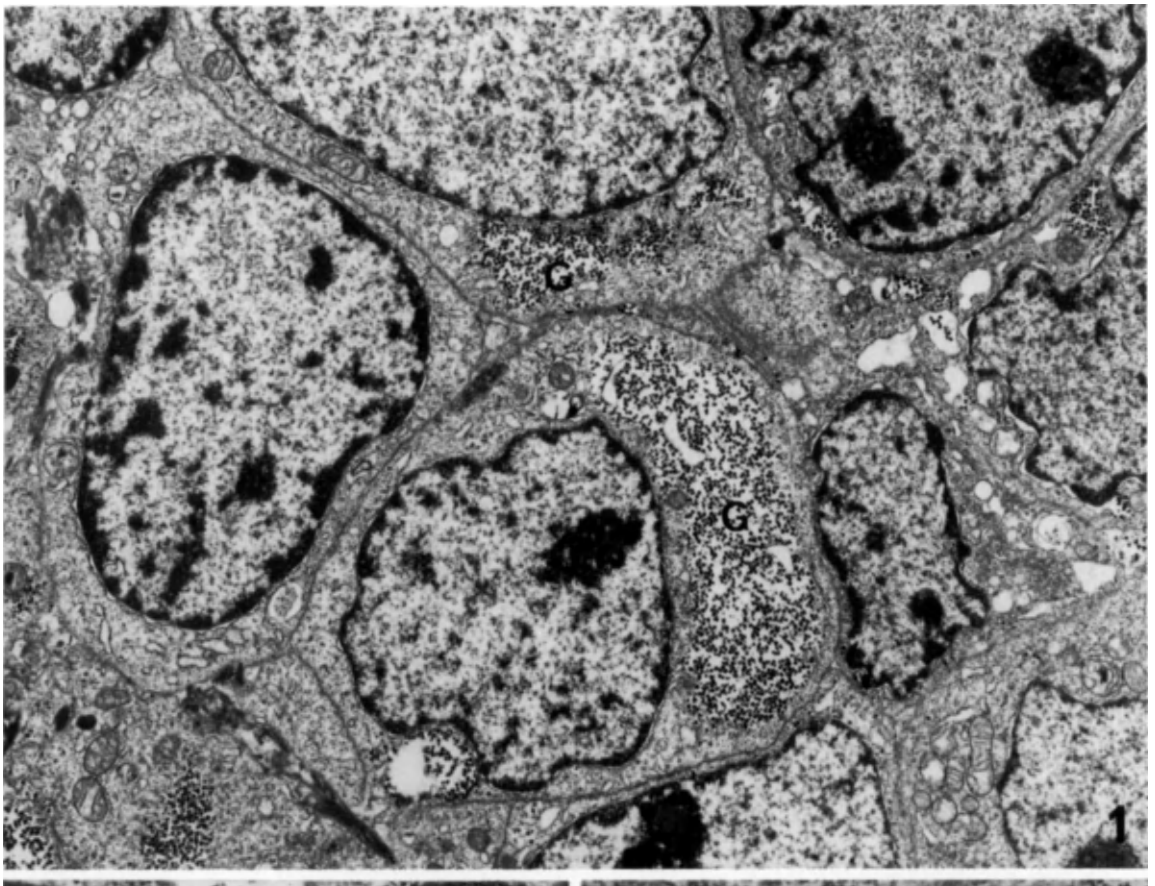
Plate 409

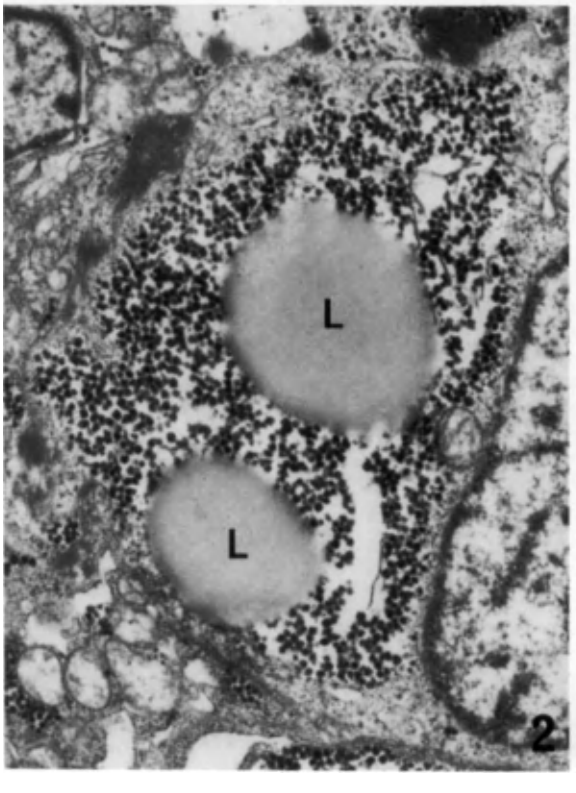
Ewing's tumour (*From a block of tissue supplied by Dr G. Mierau*)

Fig. 1. Tumour cells containing focal glycogen (G) deposits. ×8800

Fig. 2. Two lipid (L) droplets are seen lying in a focal glycogen deposit. ×15000

Fig. 3. Glycogen (arrowheads) is seen between the tumour cells. ×25000


G
G
1


L
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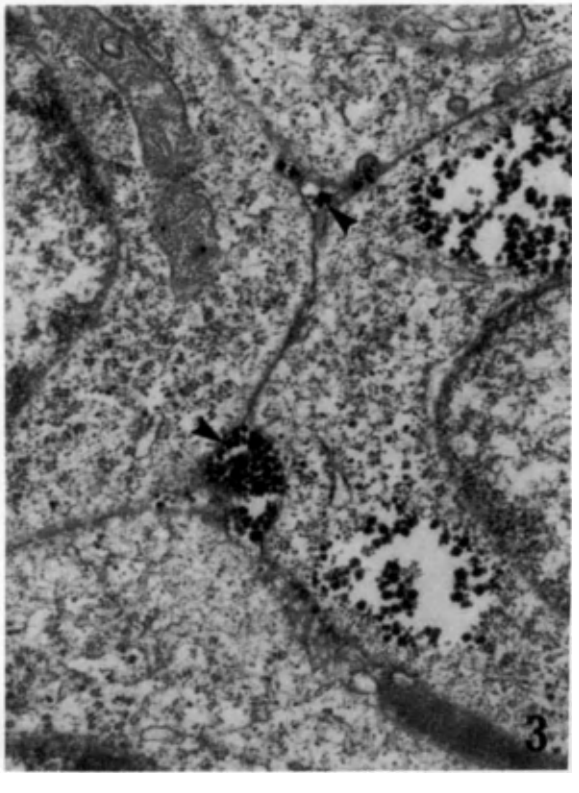
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Polyglucosan bodies (corpora amylacea, Lafora's bodies, Lafora-like bodies, Bielschowsky's bodies and amylopectin bodies)

The histological, histochemical and ultrastructural similarity if not identity of corpora amylacea*, Lafora's bodies, Lafora-like bodies, Bielschowsky's bodies and amylopectin bodies is now well recognized and all of them are now considered to be varieties of polyglucosan bodies†. The different names reflect little more than the various clinical settings in which these cytoplasmic inclusions were first seen.

Ultrastructurally, the polyglucosan body presents as a rounded, oval or elongated body composed of short filaments (about 8 nm thick), electron-dense particles and electron-dense amorphous material. These components are usually homogeneously distributed but at times the amorphous material forms an electron-dense core. The particles are often deployed along the filaments so that a 'beaded filament' appearance is produced. Some authors speak about 'branching filaments' but one cannot be certain of this by just looking at routine electron micrographs (as explained on page 840).

Virchow (1854) coined the term 'corpora amylacea' to describe certain inclusions in the brain which he thought resembled starch granules. It is now well known that corpora amylacea (i.e. polyglucosan bodies) accumulate in the central nervous system during ageing. They are rarely seen in individuals less than 40 years old. These bodies have been seen in axons and astrocytic processes in the central nervous system and axons of intramuscular nerve fibres, optic nerve and retina (Ramsey, 1965; Anzil *et al.*, 1974; Avendano *et al.*, 1980).

The term 'Lafora's bodies' is used to describe polyglucosan bodies seen in Lafora's disease (*Plates 410* and *411*). In the classic form of Lafora's disease large polyglucosan bodies, generally but not invariably with a central electron-dense core, are seen in cerebral cortex, thalamus, globus pallidus and substantia nigra (Odor *et al.*, 1967; Collins *et al.*, 1968; Gambetti *et al.*, 1971; Schnabel and Gootz, 1971; Vanderhaeghen, 1971; Cajal *et al.*, 1974). However, such bodies are outnumbered by small homogeneous (i.e. no central core) polyglucosan bodies in the neuropil of the cortex (Robitaille *et al.*, 1980). In the protracted or Lundborg form of Lafora's disease, polyglucosan bodies are much less numerous and elongated forms are seen in axons (Jakob, 1969; Kraus-Ruppert *et al.*, 1970). Aside from their occurrence in the central nervous system, Lafora's bodies have also been seen in the retina, nerves (intramuscular and optic), hepatocytes, striated muscle (cardiac and skeletal) (Harriman and Millar, 1955; Seitelberger *et al.*, 1964; Schwarz and Yanoff, 1965; Carpenter *et al.*, 1974; Coleman *et al.*, 1974; Neville *et al.*, 1974; Berard-Badier *et al.*, 1980; Nishimura *et al.*, 1980) and the epithelium of the duct of sweat glands (Cinti, unpublished observation).

The term 'Bielschowsky's bodies' is used to describe polyglucosan bodies seen in longstanding double choreoathetosis (Bielschowsky, 1912; de Leon, 1974; Ule and Volk, 1975;

*In this section of the text we are talking about the corpora amylacea found in the central nervous system. The term 'corpora amylacea' is also used to describe certain inclusions found in other organs and tissues such as prostate, lung and tooth pulp. These inclusions which have a different chemical composition and ultrastructure from the corpora amylacea in the central nervous system (Steele *et al.*, 1952), do not concern us here.

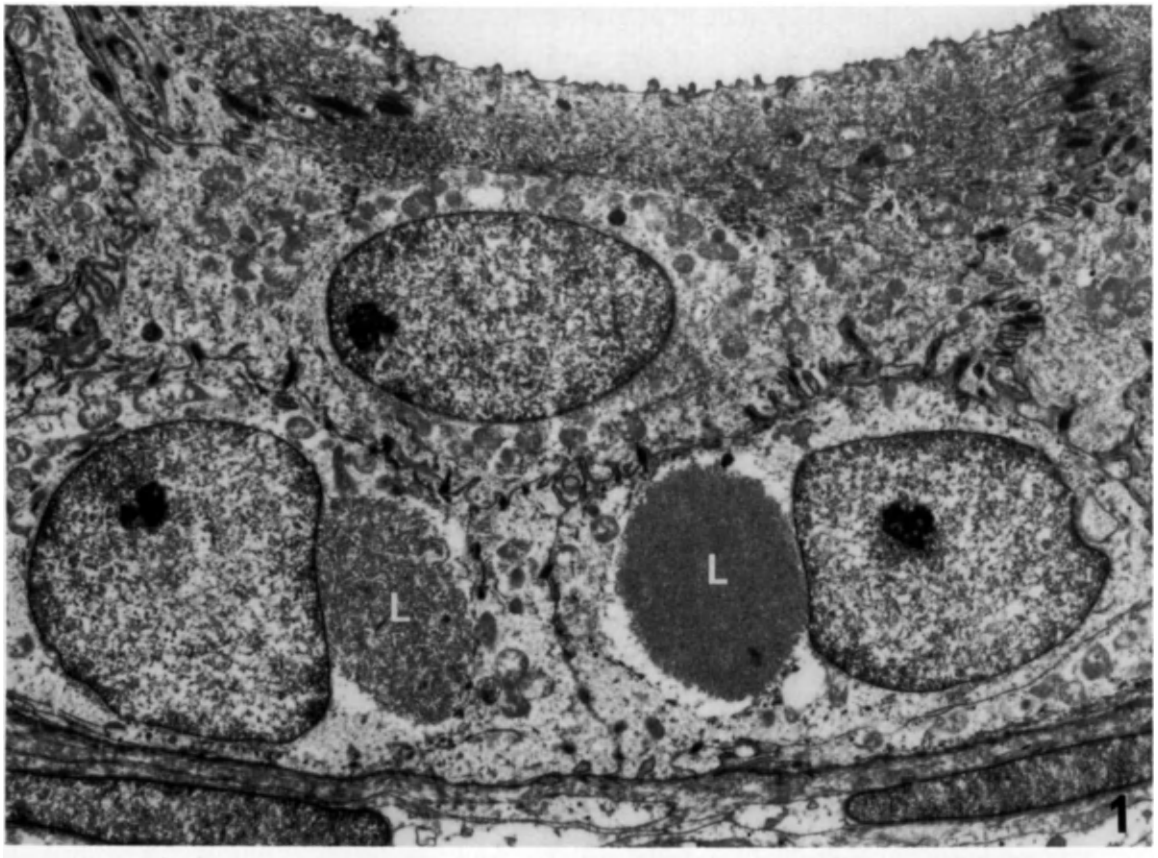
†The term 'polyglucosan bodies' was coined by Robitaille *et al.* (1980) to signify the common polysaccharide basis of all these inclusions. The components of polyglucosan bodies (i.e. filaments, particles and amorphous material) and glycogen particles are viewed as different supramolecular organizations of polysaccharides (Sakai *et al.*, 1970; Vanderhaeghen, 1971; Palmucci *et al.*, 1982a, b).

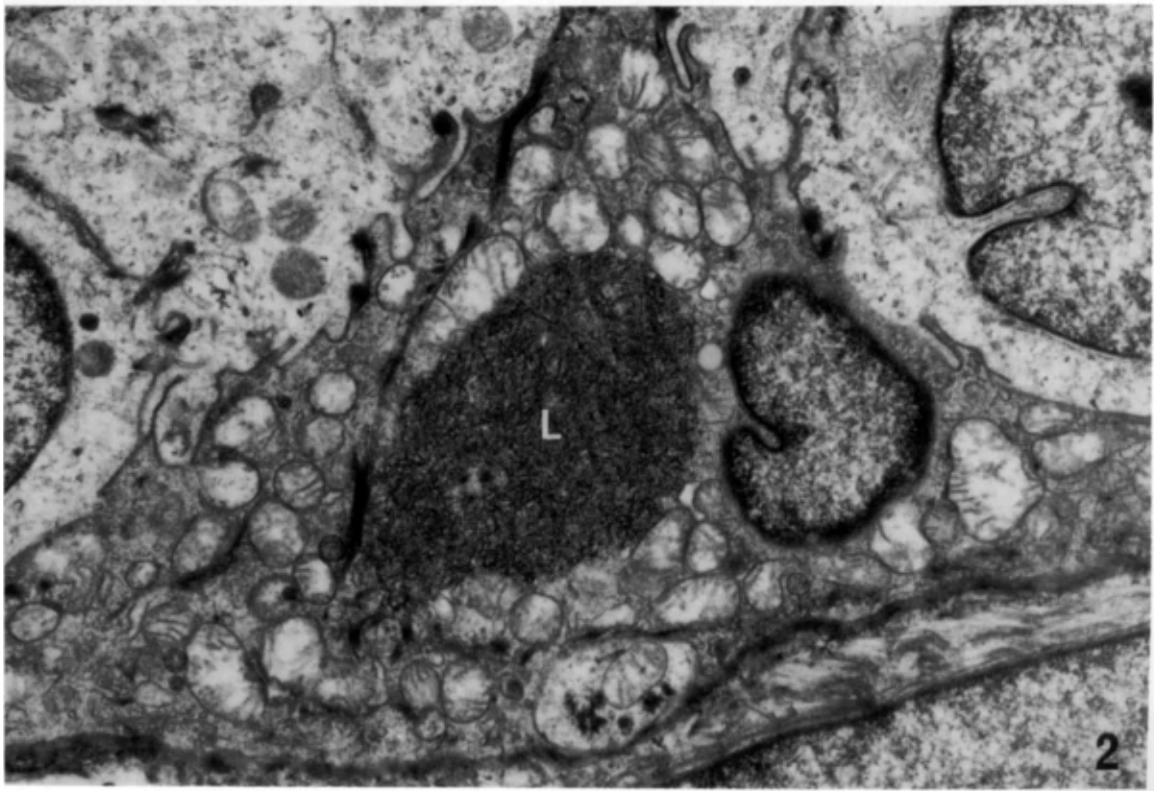
Plate 410

Skin from a case of Lafora's disease (*From a block of tissue supplied by Dr S. Cinti*)

Fig. 1. Two Lafora's bodies (L) are seen in the basal epithelial cells of a sweat gland duct. Both lie in a clear space (probably a shrinkage or extraction artefact) but not in a true membrane-bound vacuole. ×8000

Fig. 2. Sweat gland duct. Seen here is a dark basal cell showing oncocytic transformation (as evidenced by the numerous mitochondria and virtual absence of other organelles) and a Lafora's body (L) which lies in the cytoplasmic matrix and is clearly not membrane-bound. Note the filamentous texture of the Lafora's body. ×16 500


L
L
1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Adler *et al.*, 1982). In this condition prominent accumulations of polyglucosan bodies are seen in the lateral segment of the globus pallidus. Few bodies are seen in perikarya, many are seen in axons and some have an elongated shape.

The term 'Lafora-like bodies' has been employed to describe polyglucosan bodies seen in: (1) the central nervous system in amyotrophic lateral sclerosis, olivopontocerebellar atrophy and miscellaneous difficult to classify neurological disorders (Jakob, 1969; Orthner *et al.*, 1973; Petito *et al.*, 1973; Barz *et al.*, 1976; Yoshimura, 1977); and (2) the liver of alcoholics treated with disulfiram (Antabuse) (Vazquez and Pardo-Mindan, 1979). Polyglucosan bodies referred to as such or as 'Lafora-like bodies' have also been seen in certain cases of chronic neurological disease which Robitaille *et al.* (1980) describe as 'a distinct form of adult polyglucosan body disease with massive involvement of central and peripheral neuronal processes and astrocytes'★.

The term 'amylopectin bodies' describes polyglucosan bodies seen in the recessively inherited disease called 'type IV glucogenosis' or 'amylopectinosis'. In this condition there is a deficiency of the branching enzyme†, alpha-1, 4-glucan: alpha-1,4 glucan 6-glycosyltransferase which leads to an accumulation of abnormal polysaccharides in many organs including the liver which becomes markedly cirrhotic. Numerous small polyglucosan bodies are seen in bodies of astrocytes (Schochet *et al.*, 1970; McMaster *et al.*, 1979).

Polyglucosan bodies have been found in various animals besides humans. For example, polyglucosan bodies have been seen in: (1) the retina of healthy dogs, cats and monkeys (Antal, 1982); (2) the retina and sciatic nerve of diabetic rats (Yaniguchi, 1969; Powell *et al.*, 1977); and (3) the neuropil of the conus medullaris and smooth muscle cells of the GI tract of dogs. It is said that their number increases with age (Suzuki *et al.*, 1979; Kamiya *et al.*, 1983).

Except in type IV glycogenosis, the factors or causes which lead to the production of polyglucosan bodies are not known. In type IV glycogenosis there is a deficiency of the brancher enzyme but in Lafora's disease it appears to be normal (Gambetti *et al.*, 1971). Schwalbe and Quadbeck (1975) suggest that 'corpora amylacea of the brain are caused by the glucose requirement of the brain being reduced, while the supply of glucose for the brain is normal', while Suzuki *et al.* (1979) claim that polyglucosan bodies are 'a metabolic by-product (of glycogen) caused by decreased enzyme activity in the glycogenesis–glycogenolysis pathway'.

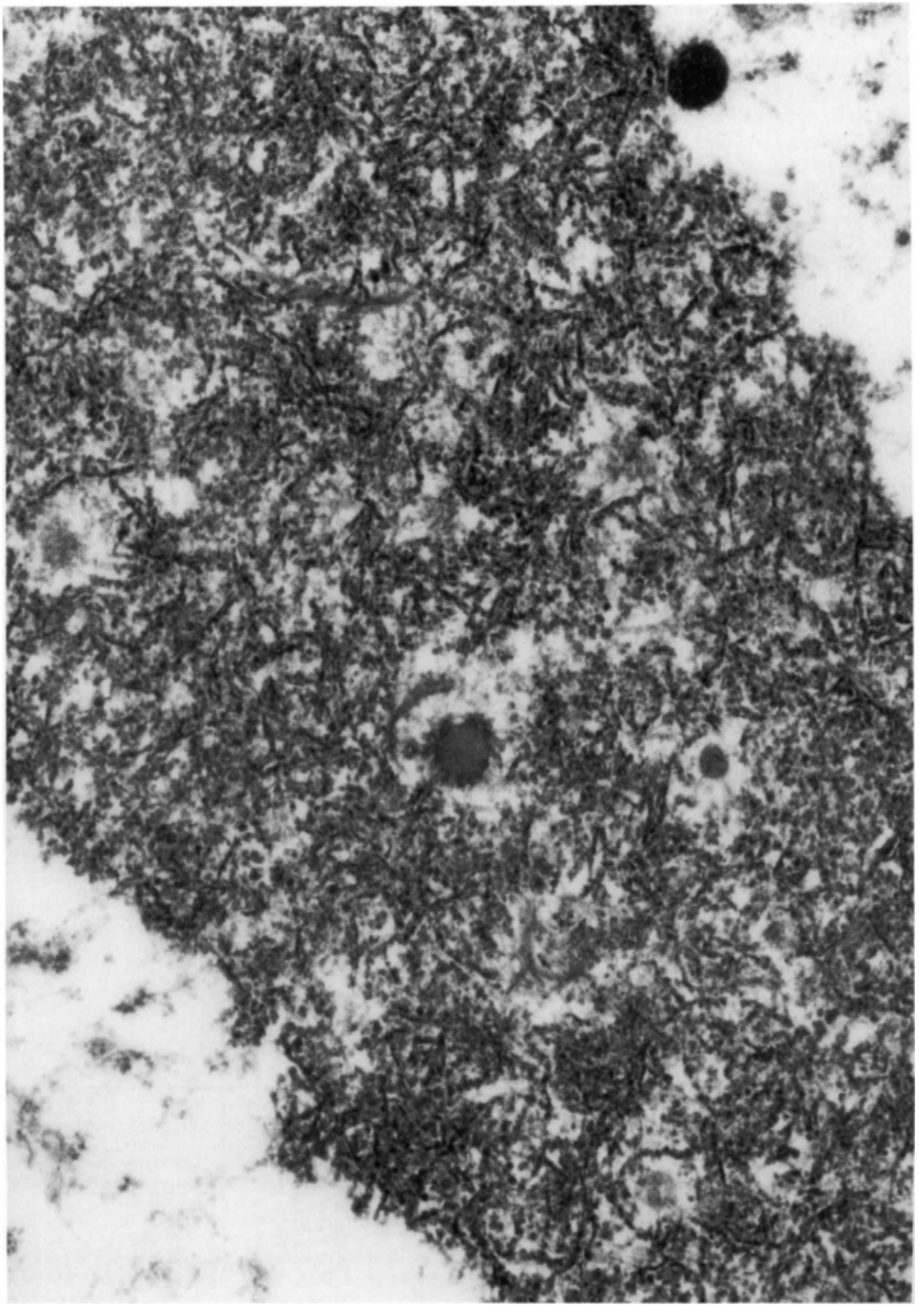
Innumerable published electron micrographs attest that polyglucosan bodies lie free in the cytoplasmic matrix. Hence one may surmise that like glycogen particles they are produced in the cytoplasmic matrix. However, it is said (e.g. Cajal *et al.*, 1974) that at times polyglucosan bodies lie in membrane-bound spaces, and it is claimed that they may develop in peroxisomes (Carpenter *et al.*, 1974) or mitochondria (Powell *et al.*, 1979). There is little or no evidence to support such contentions.

Robitaille *et al.* (1980) state that 'growth of a polyglucosan body in an individual axon would in time be deleterious to that axon – at the very least impeding or blocking the axonal flow.' They also note that an increase in neurofilaments is often seen around polyglucosan bodies in axons. This is in keeping with many observations (*see* pages 892–896) which show that an increase in intracytoplasmic filaments indicates degenerative changes in a cell.

★The occurrence of polyglucosan bodies in sural nerve biopsies is thought to be highly characteristic of this condition.
†In adult polysaccharide storage myopathy where there is probably a mild deficiency of the branching enzyme (also called 'brancher enzyme'), polyglucosan bodies and diffuse accumulations of polyglucosans are seen in sural nerve and muscle (Komure *et al.*, 1985)

Plate 411

Same specimen as *Plate 410* (*From a block of tissue supplied by Dr S. Cinti*)
A higher-power view showing the structure of a Lafora's body which lay in a clear space in the cytoplasmic matrix of a basal epithelial cell of a sweat gland duct. Note the filaments and the particles and how superimposition or juxtapositioning of the two produces the beaded filament appearance. ×54 000



Lipid

Triglycerides of fatty acids represent the commonest form of lipid inclusion found in the cells of animals. In the mature adipocyte of white fat* a large droplet of such lipid is found within the cell, but smaller lipid droplets are not uncommon in the cytoplasm of many other cell types. The size and number of such lipid droplets in cells of various tissues vary markedly in different physiological and pathological situations. A few lipid droplets are considered a normal inclusion of the cytoplasm and represent a potential source of energy and short carbon chains for the synthesis of cytomembranes and other lipid-containing material produced by cells. Gross deposits of lipid in parenchymatous cells (e.g. liver or myocardium) are pathological and constitute fatty degeneration.

Lipid droplets† occur not only in the cytoplasm but at times also in other cell compartments, such as the nucleus (*Plate 52*), mitochondrion (*Plate 134*), Golgi complex (*Plates 150* and *151*), endoplasmic reticulum (*Plates 235* and *236*) and lysosome (*Plates 261–263*). It is worth noting that lipid droplets in these sites show the same variations of morphology as do lipid droplets in the cytoplasm.

The appearance of intracytoplasmic lipid in ultrathin sections is dependent upon various factors, such as size of the droplet, the method of fixation and tissue preparation, the saturated and unsaturated fatty acid content of the lipid and the degree of unsaturation of the fatty acids present. The basic form of the lipid droplet is spherical, and small droplets usually present in this manner; larger droplets tend to have a somewhat distorted or irregular outline. At times, lipid droplets can be markedly crenated. Large droplets are difficult to fix because they are poorly penetrated by fixatives, hence they tend to be extracted during processing. This is particularly so in the case of lipids with a high content of saturated fatty acids for such lipid does not bind osmium avidly.

The density of the lipid droplet as seen in electron micrographs varies from electron lucent to markedly electron dense. Roughly, this may be correlated with the saturated and unsaturated fatty acid content of the triglyceride and the degree of unsaturation of fatty acids present (*Plate 412*).

*Brown fat and hibernoma are dealt with on pages 256–259.

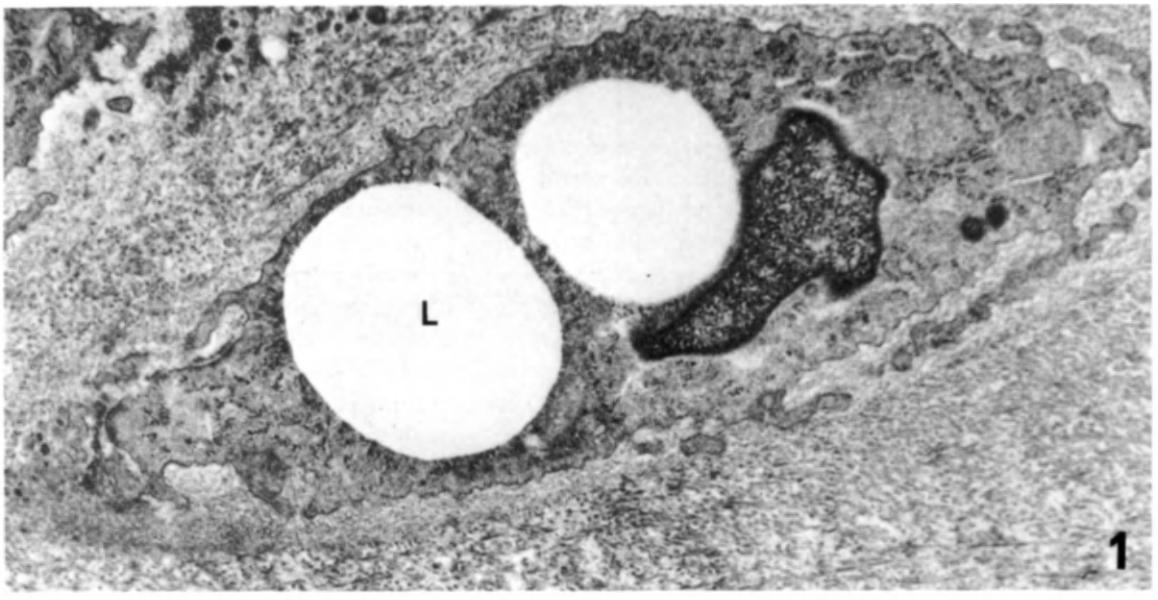
†The term 'lipid droplet' is preferable to many others such as 'lipid vacuoles', 'lipid granules', 'lipid bodies' and 'globular bodies' because *in vivo* intracellular lipid is fluid, not solid. In the living cell, because of surface tension, the 'lipid droplet' or 'oil droplet' is always spherical. The variety of appearances seen in electron micrographs stem from the interplay of variations in the chemical composition of the lipid, tissue processing and artefacts that cannot always be controlled.

Plate 412

This plate demonstrates that one of the factors which determines the electron density of the lipid droplet is the saturated and unsaturated fatty acid content of the lipid. When lipid is injected into a joint (in this case, rabbit knee joint) some of it is taken up by the chondrocytes. If a lipid rich in saturated fatty acid is used (e.g. autologous depot fat) the chondrocytes come to contain electron-lucent lipid droplets. If corn oil (which is very rich in unsaturated fatty acid) is injected, the chondrocytes show numerous electron-dense lipid droplets.

Fig. 1. Chondrocytes from an autologous fat-injected joint, showing electron-lucent lipid droplets (L). ×8000 (*From Mehta and Ghadially, 1973*)

Figs. 2 and 3. Chondrocytes from joints injected with corn oil, showing moderately to markedly electron-dense lipid droplets (L). ×18 000; ×21 000 (*Fig. 2, from Mehta and Ghadially, 1973; Fig. 3, Mehta and Ghadially, unpublished electron micrograph*)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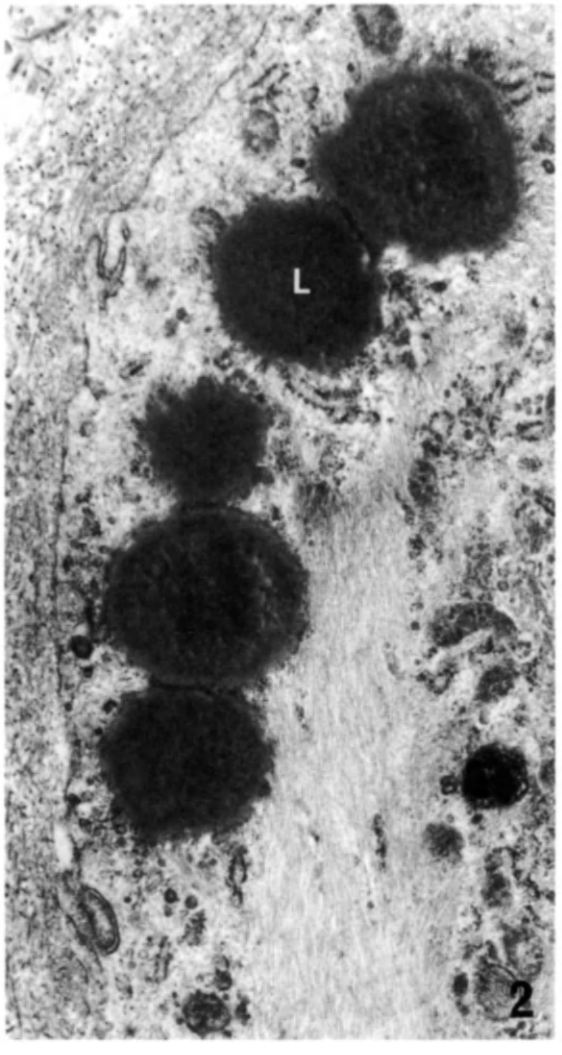
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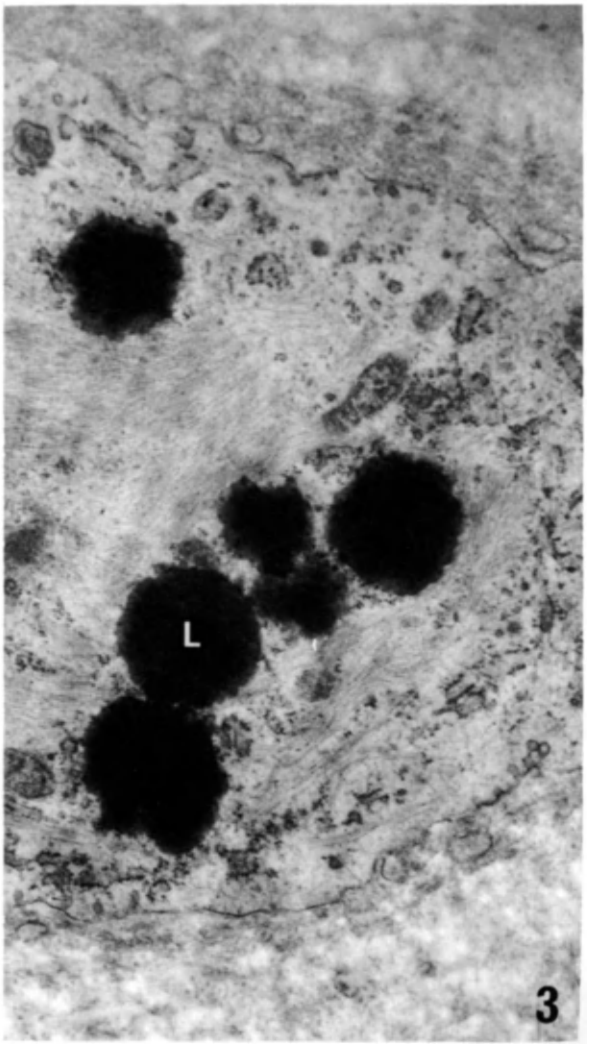
L
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The greater the unsaturated fatty acid content and degree of unsaturation, the more electron dense the lipid is likely to appear, for it will tend to bind more osmium*. The density of lipid droplets tends to be lower in glutaraldehyde-fixed material as compared to that fixed primarily with osmium.

Characteristically, intracytoplasmic lipid inclusions are not bounded by a true trilaminar membrane, but an osmiophilic 'ring' or a membrane-like structure is sometimes seen around a lipid droplet. This has at times been confused with a true limiting membrane. However, as already noted (pages 526–529), membrane-bound lipid droplets called 'liposomes' are known to occur in the cytoplasm, but these represent lipid lying in vesiculated endoplasmic reticulum and Golgi complex, and hence are not, strictly speaking, a true example of intracytoplasmic lipid.

Lipid droplets at times appear peppered with electron-dense particles, and occasionally accumulations of such particles are also seen on the periphery of the lipid droplet, forming granular irregular focal deposits (*Plate 413*). The nature of these particles and the significance of this phenomenon are not known.

Other interesting variations of lipid morphology occasionally encountered are droplets with a pale or clear centre (annular lipid inclusions) and droplets with a pale or clear halo (*Plate 413*). Such appearances are consistent with the idea that some material (presumably lipid) is removed from the centre or the periphery of the lipid droplet during tissue preparation, but why this should be so is not easy to understand. In the case of the annular lipid inclusion one may suggest that this is due to poor penetration of the fixative and a subsequent removal of the central unfixed lipid during tissue dehydration and clearing. Yet such a hypothesis obviously does not explain why lipid droplets sometimes have a clear halo.

A theory that could explain the occurrence of both types of partially extracted lipid droplets would be that such droplets are not homogeneous, and that there may be physical (e.g. state of hydration or emulsification) or chemical (e.g. saturated and unsaturated fatty acid content) differences within a lipid droplet that determine which part is fixed or not fixed and which part is retained or lost during tissue preparation (Parry and Ghadially, 1966). This, however, is pure speculation and, as mentioned earlier, the basis of this and some other variations of lipid morphology (e.g. the nature of the dense particles which pepper lipid droplets) has not as yet been satisfactorily explained.

*The primary site of reaction of osmium is with the C=C double bonds of unsaturated fatty acids; one molecule of OsO_4 reacts with each double bond. Although at times the amount of osmium bound is less than that expected from the degree of unsaturation of the lipid, the general validity of the thesis that unsaturated lipids appear dense because of the osmium they bind cannot be doubted (Stoeckenius and Mahr, 1965).

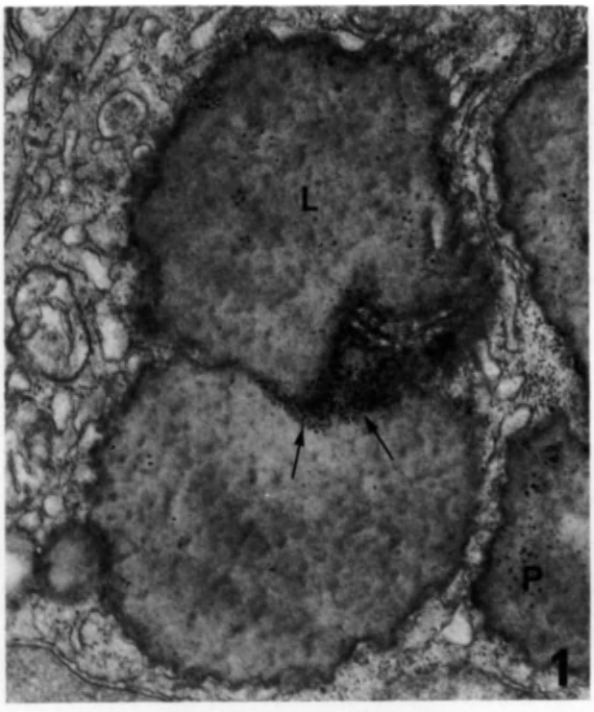
Plate 413

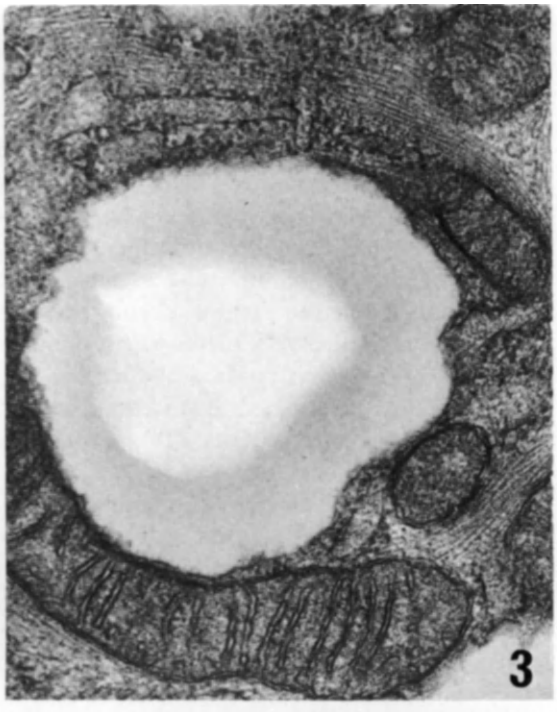
Fig. 1. The medium-density lipid droplets (L) seen in this electron micrograph have a dense rim which in some places creates a false impression of a limiting membrane. The droplets are peppered with fine electron-dense particles (P) and there are also focal accumulations of such particles (arrows) in one area. From the liver of a rat bearing a carcinogen-induced sarcoma in its flank. ×26 000

Fig. 2. A lucent halo is seen around these lipid droplets found in a human hepatocyte. That this is no shrinkage artefact is evidenced by the well preserved mitochondria and other structures in the surrounding cytoplasm. ×18 000

Fig. 3. An annular lipid inclusion (i.e. a lipid droplet with a lucent centre) is seen in a chondrocyte. From the articular cartilage of a normal dog. ×42 000

Fig. 4. This cell, found in a cirrhotic human liver, shows lipid droplets of varying morphology. One of the droplets is lucent (L), some others of medium density (M) and there is one which has a clear centre (C). ×17 500


L
P
1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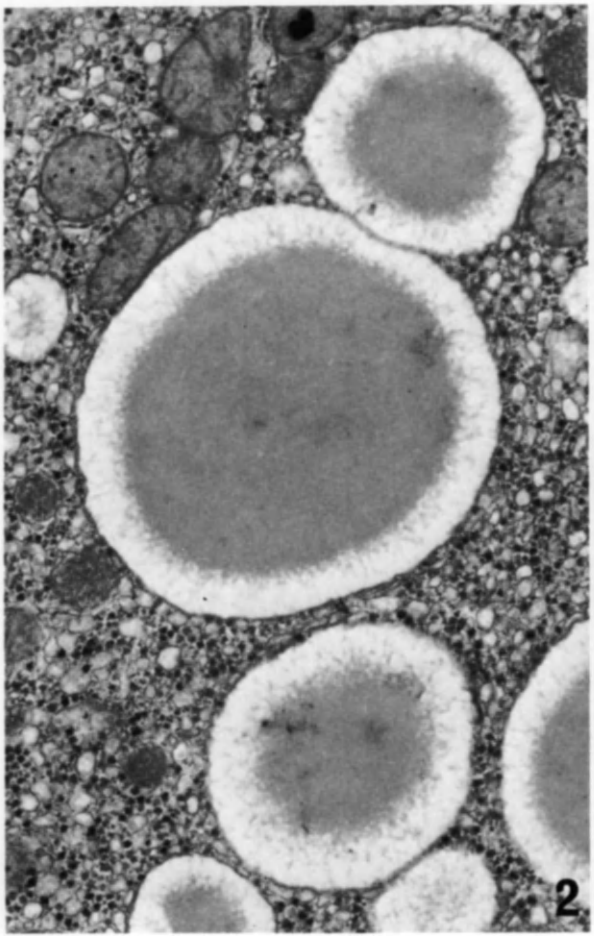
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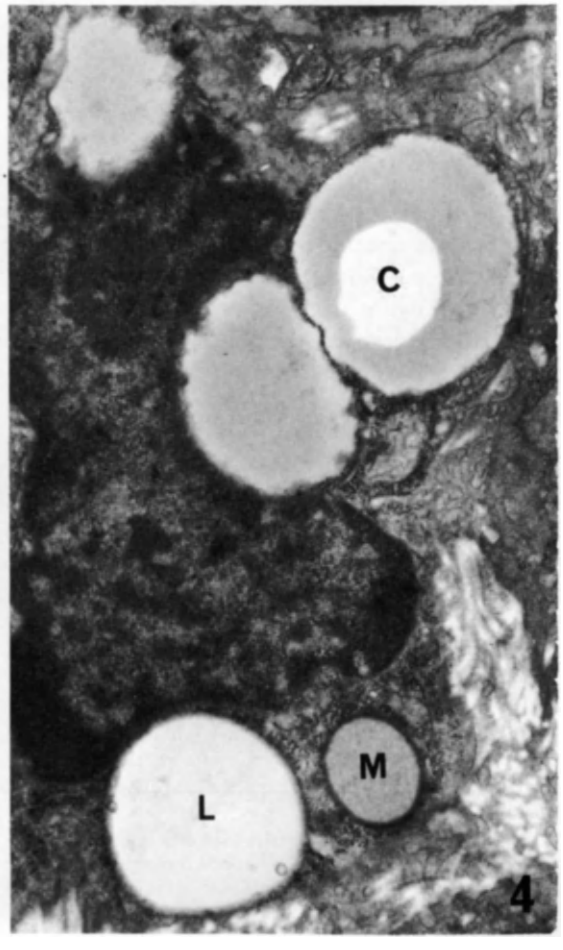

C
M
L
4

Crystalline inclusions

Crystalline inclusions thought to be protein or mainly protein in nature have been found in various cell compartments, such as the nucleus (*Plates 55, 56, 58* and *59*), mitochondrion (*Plates 136* and *137*) endoplasmic reticulum (*Plate 240–242*) and cytoplasmic matrix. However, translocation of a crystal from its site of genesis (e.g. mitochondrion, endoplasmic reticulum or Golgi complex) may occur, so that at times it is difficult to label the crystal as belonging to one or other specific cell compartment.

An example of this is seen during yolk platelet formation in amphibian oocytes, where crystals formed in the mitochondrion are extruded and come to lie in single-membrane-bound structures called yolk platelets (for references *see* Karasaki, 1963; Massover, 1971). Similarly, in an alveolar soft part sarcoma Shipkey *et al.* (1964) have illustrated numerous small crystals in single-membrane-bound structures in the Golgi region and also larger crystals lying free in the cytoplasmic matrix (*Plate 414*). That the latter are derived from the former seems a reasonable assumption.

Particularly difficult to classify are the crystalline inclusions seen on rare occasions in endothelial cells (*see below*). Both membrane-bound and non-membrane-bound versions of these crystals occur, but there is no clue as to where the membrane comes from or in which cell compartment this crystal is born. In view of this I am not at all sure in which chapter of this book they belong. They are dealt with here simply because at least sometimes they seem to be non-membrane-bound.

In contrast to this, the crystal of Reinke found in the interstitial cells (Leydig's cells) of the human testis seems to evolve in the cytoplasmic matrix and is not membrane-bound at any stage of development (*Plate 415*). Reinke's crystal has, however, also at times been found in the nucleus (page 118). Reinke's crystals (intracytoplasmic) have now been found in: (1) Leydig's cells of human testis after puberty (references given later); (2) cells resembling Leydig's cells in the antebranchial organ of the ring-tailed lemur (*Lemur catta*) (Montagna, 1962; Sisson and Fahrenbach, 1967); (3) Leydig's cells of Australian bush rat (Rattus fuscipes) (Kerr *et al.*, 1986); (4) Leydig's cells from an arrhenoblastoma (Berendsen *et al.*, 1969); (5) ovarian hilar cells (which resemble Leydig's cells) (Sternberg, 1949; Gardner *et al.*, 1957); (6) numerous cases of hilar cell ovarian tumours (for references, *see* Merkow *et al.*, 1971); and (7) testicular interstitial cell (Leydig's cell) tumours (Savard *et al.*, 1960; Silverberg *et al.*, 1966).

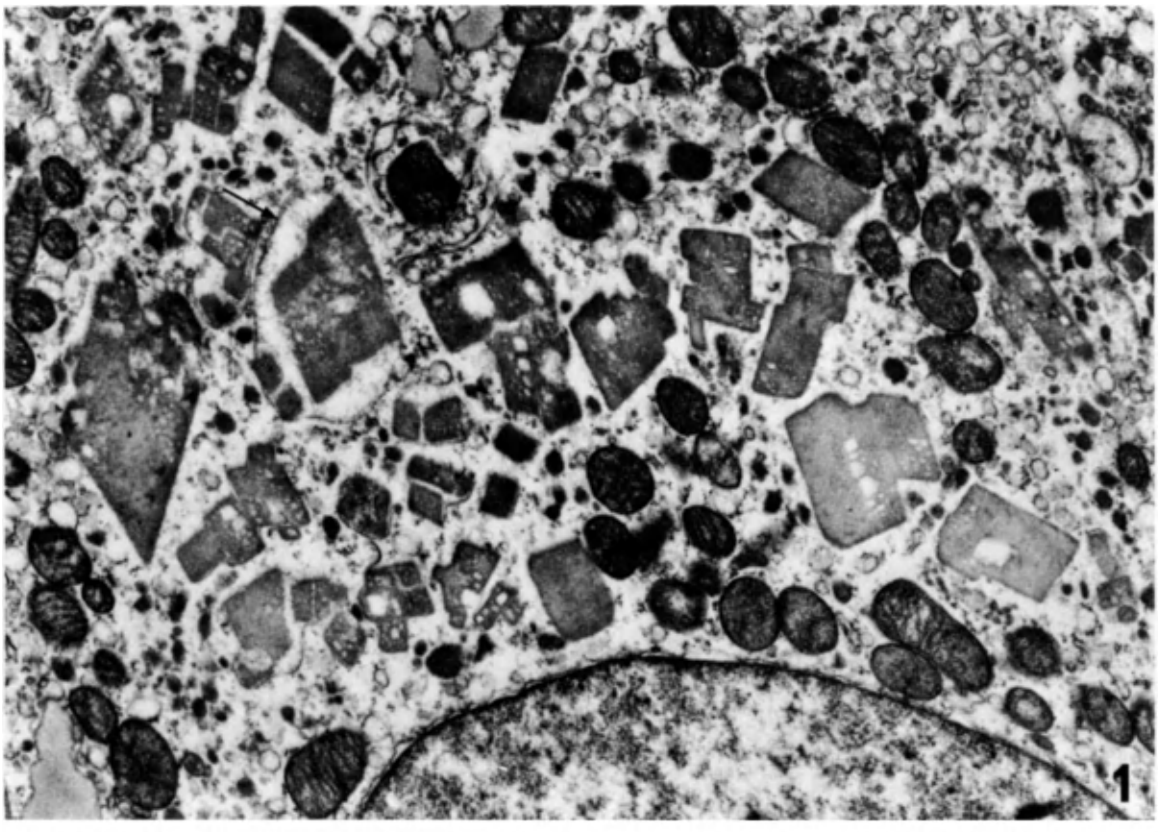
The ultrastructural morphology of Reinke's crystal in the interstitial cell of the human testis has been extensively investigated (Fawcett and Burgos, 1960; Yamada, 1962, 1965; Fawcett, 1966; De Kretser, 1967, 1968; Nagano and Ohtsuki, 1971). The structure of such crystals in some of the other sites mentioned above has also been described. Depending upon the plane of sectioning the crystal shows various patterns of dots, parallel lines or a prismatic or hexagonal lattice. From such images the structure of the crystal has been interpreted by various workers as composed of globular macromolecules 15 nm in diameter (Fawcett and Burgos, 1956, 1960), or 5 nm thick filaments (Sisson and Fahrenbach, 1967; Nagano and Ohtsuki, 1971) or 20–30 nm hexagonal microtubules (Yamada, 1962, 1965; Merkow *et al.*, 1971). Despite the numerous studies the significance of Reinke's crystal remains obscure.

Plate 414

From an alveolar soft part sarcoma.

Fig. 1. Portion of a tumour cell showing several crystals lying free in the cytoplasmic matrix. However, some crystals are seen in a membrane-bound (arrow) space. ×20 000 (*Shipkey, unpublished electron micrograph*)

Fig. 2. High-power view of a crystal demonstrating a periodic pattern of about 10 nm. ×167 000 (*From Shipkey, Lieberman, Foote and Stewart, 1964*)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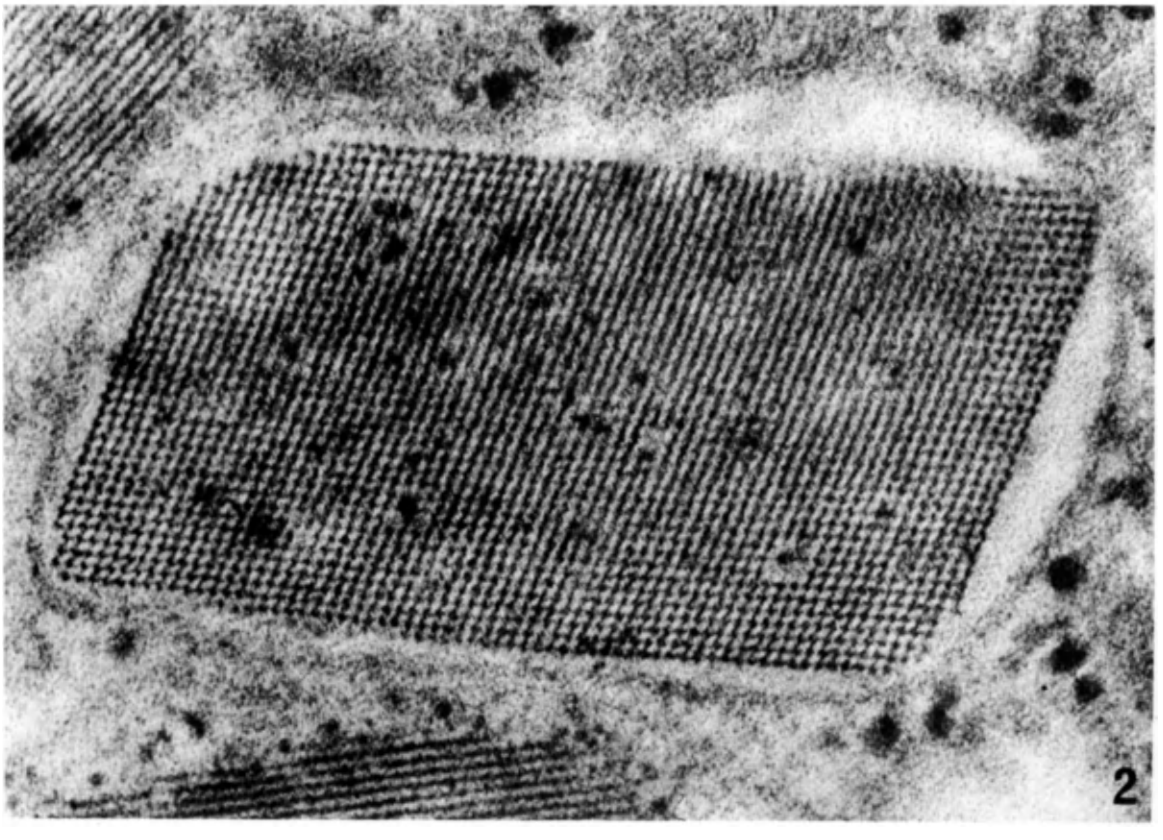
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Besides the above mentioned examples intracytoplasmic crystals have been found in: (1) fibroblasts in recurring digital tumour of childhood (Reye's tumour) (Burry *et al.*, 1970); (2) fibroblastic cells in a soft tissue neoplasm (Ekfors and Autio-Harmainen, 1986); (3) tumour cells in an islet cell tumour of pancreas (Mori *et al.*, 1978; Ordóñez *et al.*, 1985); (4) normal Schwann cells and cells of a gastric schwannoma (dissimilar crystals) (Sun *et al.*, 1973; Marcus *et al.*, 1981); (5) tumour cells of a ductal carcinoma of breast (Bockus *et al.*, 1985); (6) neurons of thalamus and substantia nigra (for references *see* Peña, 1980); (7) murine pancreatic acinar cells (Papadimitriou *et al.*, 1969); (8) renal proximal tubule cells in rats with experimentally produced bile fistulae (Hruban and Palmer, 1969); (9) hepatocytes of alcoholic and non-alcoholic patients (for references *see* Ishihara *et al.*, 1973); and (10) hepatocytes of mongrel dogs (Djaldetti and Feller, 1978). The idea that these crystals in hepatocytes (items 9 and 10) may be the common intramitochondrial crystals (*see Plates 120, 121, 136* and *137*) which have been liberated into the cytoplasmic matrix is not too attractive because there are substantial morphological differences between the intramitochondrial and intracytoplasmic crystals.

Intracytoplasmic crystals have also been found in: (1) the lymphocytes from peripheral blood of patients with Down's syndrome (Smith *et al.*, 1967); (2) lymphocytes infiltrating the stroma of a basal cell carcinoma (Friedmann *et al.*, 1971); (3) L-strain fibroblasts and human leucocytes treated with vinca alkaloids (Bensch and Malawista, 1969; Krishan and Hsu, 1969); (4) neurons of human frontal cortex incubated for one hour with vincristine (Dustin *et al.*, 1980). According to Bensch and Malawista (1969), the crystals formed after vinca alkaloid treatment consist mainly of microtubules, and this is accompanied by a disappearance of normal microtubules from the cell. The fact that such crystals (called 'tubulin crystals') form within polymorphonuclear neutrophils (a cell which in its adult form can synthesize little protein) and the speed (within 30 minutes) at which the tubulin crystals form in L-strain fibroblasts suggest that the crystals are formed from pre-existing microtubular protein and not from protein newly produced in the cell after exposure to vinca alkaloids.

In neurons incubated with vincristine (Dustin *et al.*, 1980) tubulin crystals developed not only in the cytoplasm but also in the nucleus★. This confirms what has been suspected from biochemical studies for some time now; namely that some normal nuclei of mammalian cells may contain tubulin even though microtubules are not normally found in nuclei†.

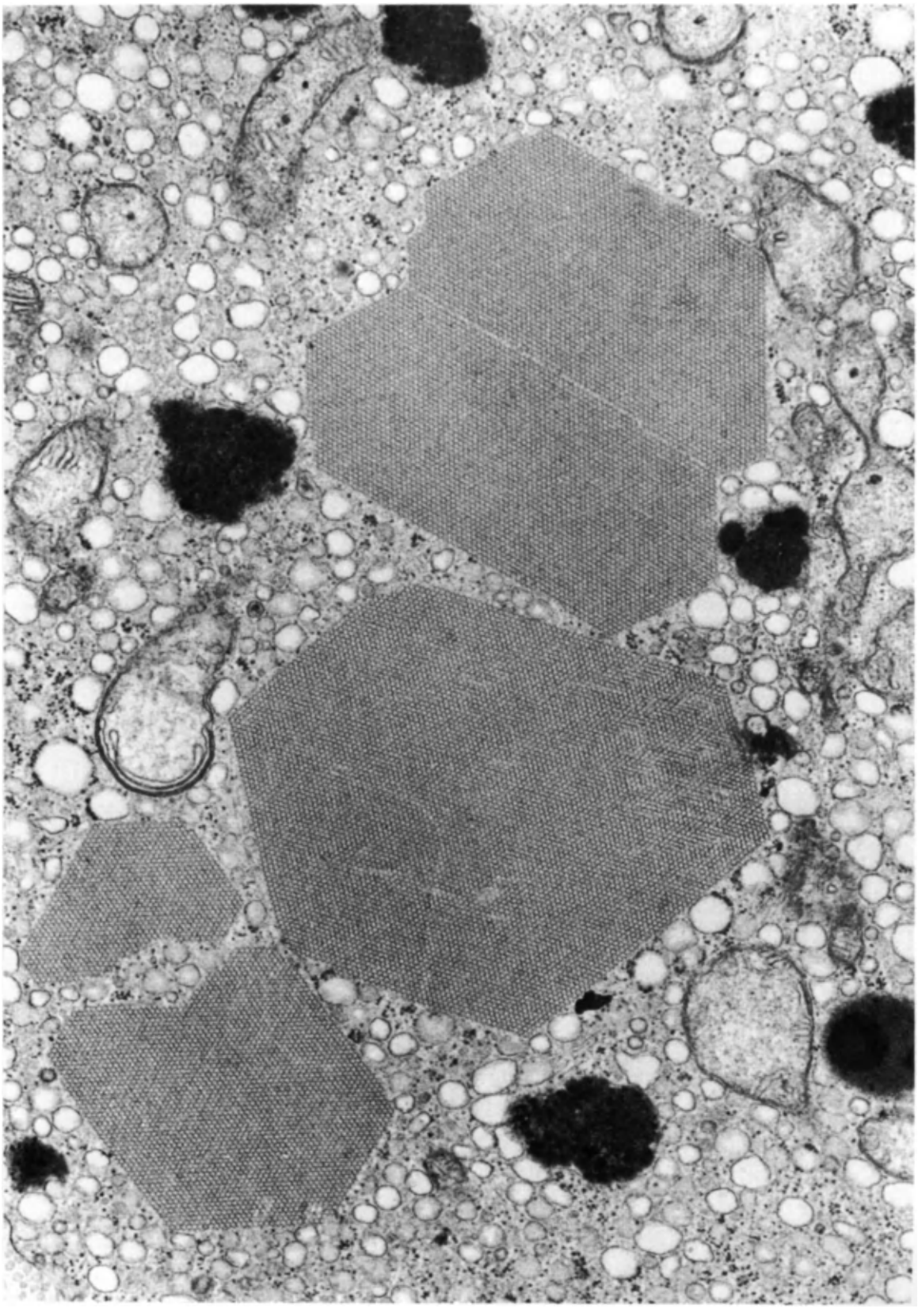
Certain intracytoplasmic crystals appear to be composed of filaments rather than microtubules. Perhaps the best known example of this is the Charcot–Böttcher crystal of Sertoli cells. Sertoli cells often contain quite prominent collections of intracytoplasmic filaments. It is thought that small aggregates of these filaments form what is known as the 'Spangaro's crystal' while larger aggregates form the spindle-shaped 'Charcot–Böttcher crystal' (*see* page 912 for references). Similarly, the Lubarsch's crystal or crystalloid found in the spermatogonia of the human testis is also composed of filaments (Sohval *et al.*, 1971). Indeed, it would appear that various intracytoplasmic filaments (intermediate filaments and actin filaments) can at times form intracytoplasmic crystals (*Plate 391*) or more complex structures called 'crystalline filamentous cylinders' (*Plates 392–394*).

★According to Dustin *et al.* (1980) these crystals bear a resemblance to the rodlets or 'tubulo-fibrillar' inclusions found in neurons (*Plate 55*).

†Reported sightings of microtubules in nuclei of normal cells of vertebrates are few and probably none have been described in mammals (Dustin *et al.*, 1980). However, microtubules appear in mast cell nuclei (De Brabander and Borgers, 1975; Bergstrom-Porter and Shelton, 1979), when these cells are incubated with 'uncouplers' of oxidative phosphorylation such as sodium azide, 2,4, di-nitrophenol and oligomycin.

Plate 415

Interstitial cell from human testis, showing several crystals of Reinke. These crystals have a polygonal shape. Note that each side of the crystal is parallel to the corresponding opposite side. ×23 000 (*From Nagano and Ohtsuki, 1971*)



Yet another interesting group of crystals found in lymphocytes contain immunoglobulins. Crystalline inclusions of this type were first observed by Bessis (1951) with the light microscope in a case of chronic lymphocytic leukaemia and by Bernard *et al.* (1959) with the electron microscope. Since then various workers (Goldberg, 1960; De Man and Meiners, 1962; Flandrin *et al.*, 1971) have reported that these crystals are PAS-negative but contain immunoglobulins. Hurez *et al.* (1972) detected IgM lambda in these crystals while Cawley *et al.* (1973) found IgA lambda in similar crystals.

In three patients originally diagnosed as cases of chronic lymphocytic leukaemia but subsequently reclassified as cases of immunocytoma, Feremans *et al.* (1978) found crystals (*Plate 416*) which were shown to contain IgM lambda by immunofluorescence and peroxidase-labelled antibody methods. They too found that the crystals were PAS negative and they suggest that 'the immunoglobulin was not bound to a carbohydrate group. A defect in glucosyltransferase activity with failure to modify the immunoglobins could explain the absence of the PAS reaction and the accumulation of immunoglobulin in crystalline form before reaching the Golgi region'.

The collective evidence now indicates that immunoglobulin crystals formed in immunocytes (plasmacytoid cells) and plasma cells are first formed in the rough endoplasmic reticulum (*Plates 240* and *241*) and later extruded in the cytoplasmic matrix. Indeed, at times, small or quite large collections of these crystals can be found in an extracellular location* (Schvartz *et al.*, 1985). Whether the crystals are actually extruded from the cell or whether they are liberated after cell necrosis is debatable, but the latter concept is easier to envisage and accept.

As mentioned before (page 978) crystalline inclusions have been seen on rare occasions in endothelial cells. Such instances include vascular endothelial cells in: (1) post-capillary venules of mouse lymph nodes (Sugimura *et al.*, 1967); (2) fetal blood vessels (Spear, 1974; Spear *et al.*, 1975); (3) Duchenne type muscular dystrophy (Mair and Tomé, 1972); (4) capillary haemangiomas and cellular haemangiomas (benign haemangioendothelioma) (Pasyk *et al.*, 1983; Luzi *et al.*, 1987); (5) cerebellar capillary haemangioblastoma (Ho, 1985); and (5) giant cell tumour of tendon sheath (Carstens, 1987).

The crystalline inclusion in human endothelial cells presents (*Plate 417*) as a solitary crystal or an aggregate of crystals cut in various planes. The shape varies from round or oval to polygonal, the size from about 0.4–1 μm, and they may or may not be membrane-bound. A common profile seen is that of an ordered array of parallel electron-dense 'lines' with a periodicity of 14–22 nm and a thickness (of each line) of about 6 or 7 nm. Between these periodic dense lines one can discern a thin faint interperiod line.

*An interesting example of extracellular crystals are the intraluminal crystals that have been seen within the acini of prostatic adenocarcinomas (about 10–23 per cent of the cases), but not in the tumour cells themselves. Holmes (1977) states that 'histochemically and immunohistochemically they appear closely related, if not identical to Bence Jones crystals'. Ro *et al.* (1986), however, were unable to confirm this and state that 'the nature of the prostate crystalloids and their biochemical composition and mode of formation remains uncertain'.

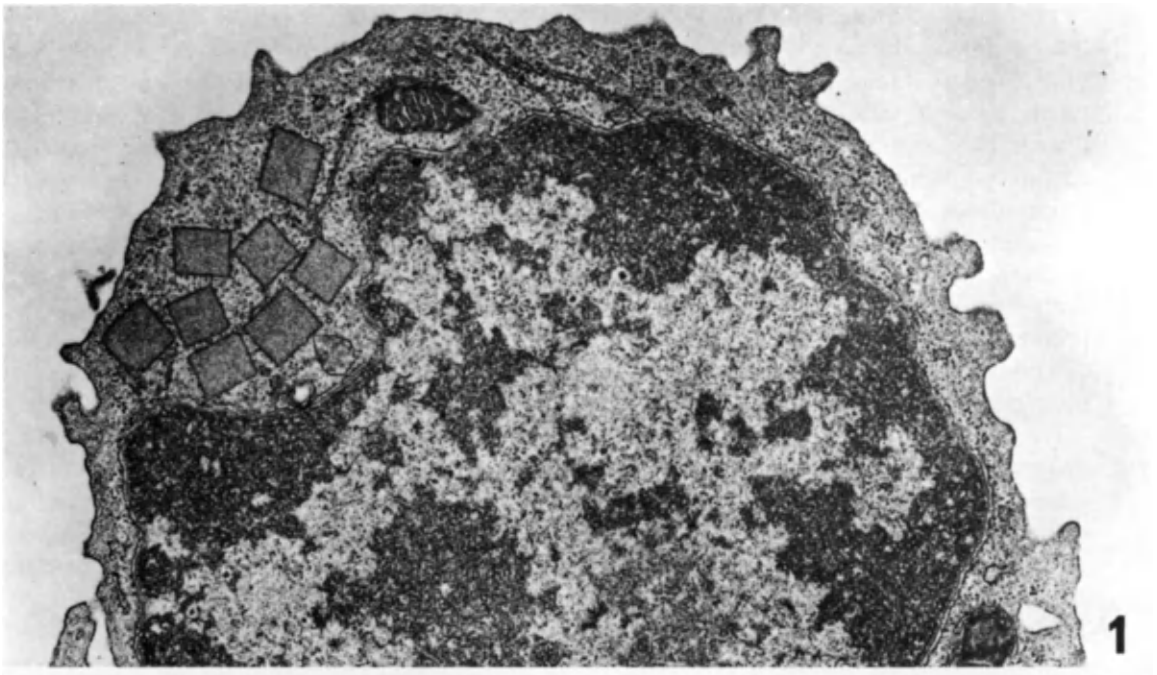
Plate 416

From Feremans, Neve and Caudron, 1978.

Fig. 1. A lymphocyte from a case of immunocytoma. Eight crystals are seen in the cytoplasm. The association (arrow) of one of these with a strand of endoplasmic reticulum is evident. ×26 000

Fig. 2. Appearance (arrow) seen here suggests that the crystal probably develops in the endoplasmic reticulum. ×72 000

Fig. 3. High-power view of crystals. A faint periodicity may just be discerned. ×85 000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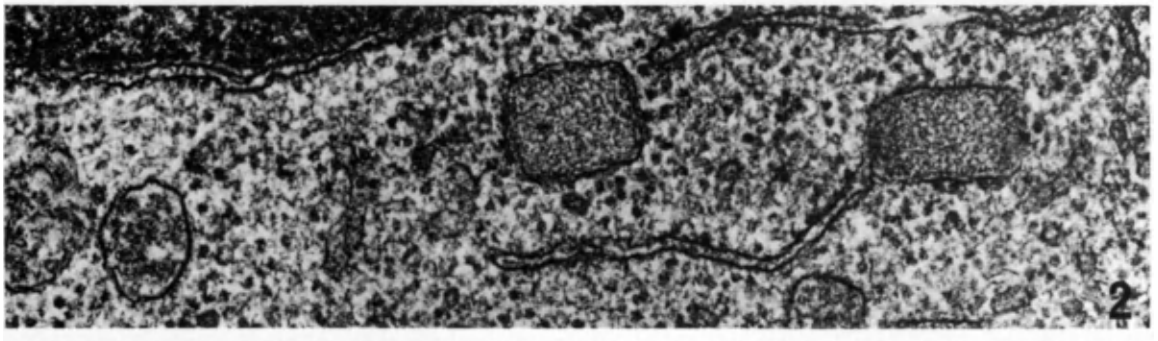
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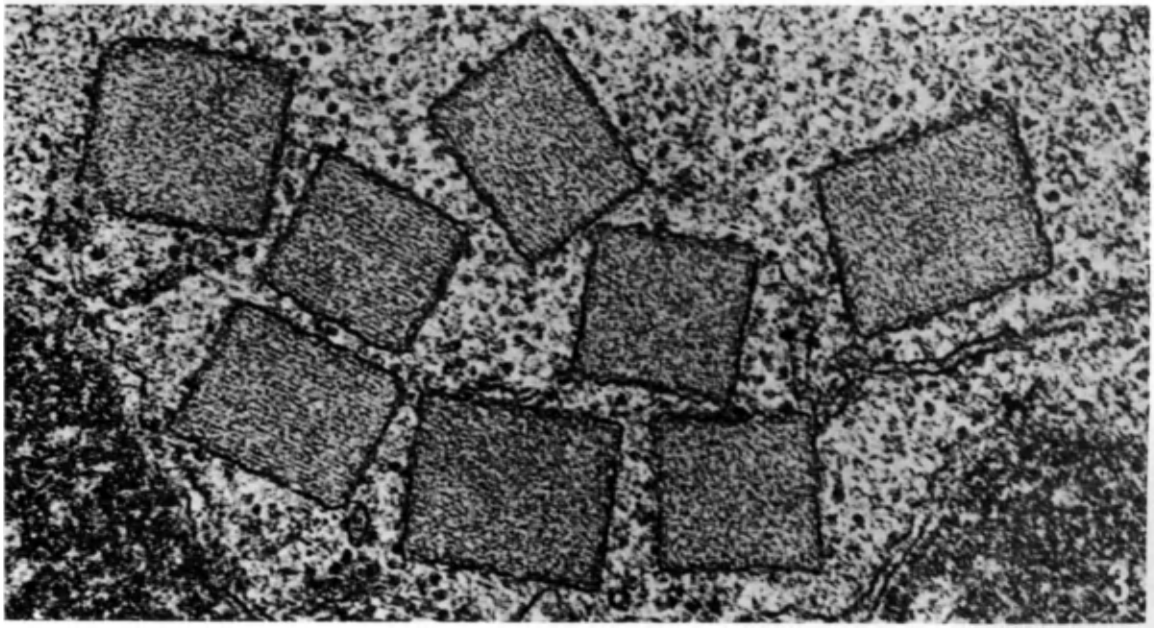


The mode of formation of these crystals is an enigma, for despite careful searching an early stage of formation has not been found in the rough endoplasmic reticulum or Golgi complex or mitochondria. Quite large rod-shaped microtubulated bodies are at times seen in endothelia where these crystals are found, and Carstens (1987) suggests that the latter derive from the former because intermediate forms between the two occur. However, Ho (1985) does not support this hypothesis because he did not find transitional forms between rod-shaped microtubulated bodies and crystals, and because the rod-shaped microtubulated body contains microtubules, but the crystalline inclusion does not. Ho (1985) suggests that these crystals may be derived from material endocytosed in pinocytotic vacuoles, and that they may be 'associated with lysosomes'. Since lysosomes contain acid hydrolases (including those that break down proteins), one does not expect to find proteinaceous crystals developing in this site. Indeed, the crystals one finds in lysosomes are composed essentially of indigestible materials, examples of these being the crystals of haemosiderin and crystals of metals and minerals (*Plate 310*).

However, the fact that some of these inclusions contain lipid droplets and electron-dense material (which taken together remind us of lipofuscin) suggests that they could be lysosomal in nature. Thus a plausible explanation would be that these crystals formed either in the cytoplasmic matrix, or in pinocytotic vacuoles, or in rod-shaped microtubulated bodies end up as lysosomal bodies (by fusion with primary lysosomes) which incorporate within themselves also cytoplasmic lipid droplets.

The significance of these crystalline inclusions is also obscure. Since neoplastic cells more resemble embryonic cells than adult cells, and since these crystals occur in vessels in fetal tissue, we* (Spear and Ghadially) thought that these crystals might well one day be found in vasoformative tumours. This has proven to be so for these crystals have now been seen in haemangiomas and haemangioblastomas. However, they have also been seen in the endothelium of vessels in a giant cell tumour of bone. Therefore, one could modify the hypothesis by suggesting that these crystals probably develop when there is active angiogenesis, whether normal or neoplastic. This, however, cannot be the only factor or even the main factor, otherwise these crystals would be quite common, while as a matter of fact they are extremely rare.

*This was at a meeting on Current Concepts in Ultrastructural Pathology (Diagnosis of Human Tumors) in Aspen, Colorado, 2–6 August 1982.

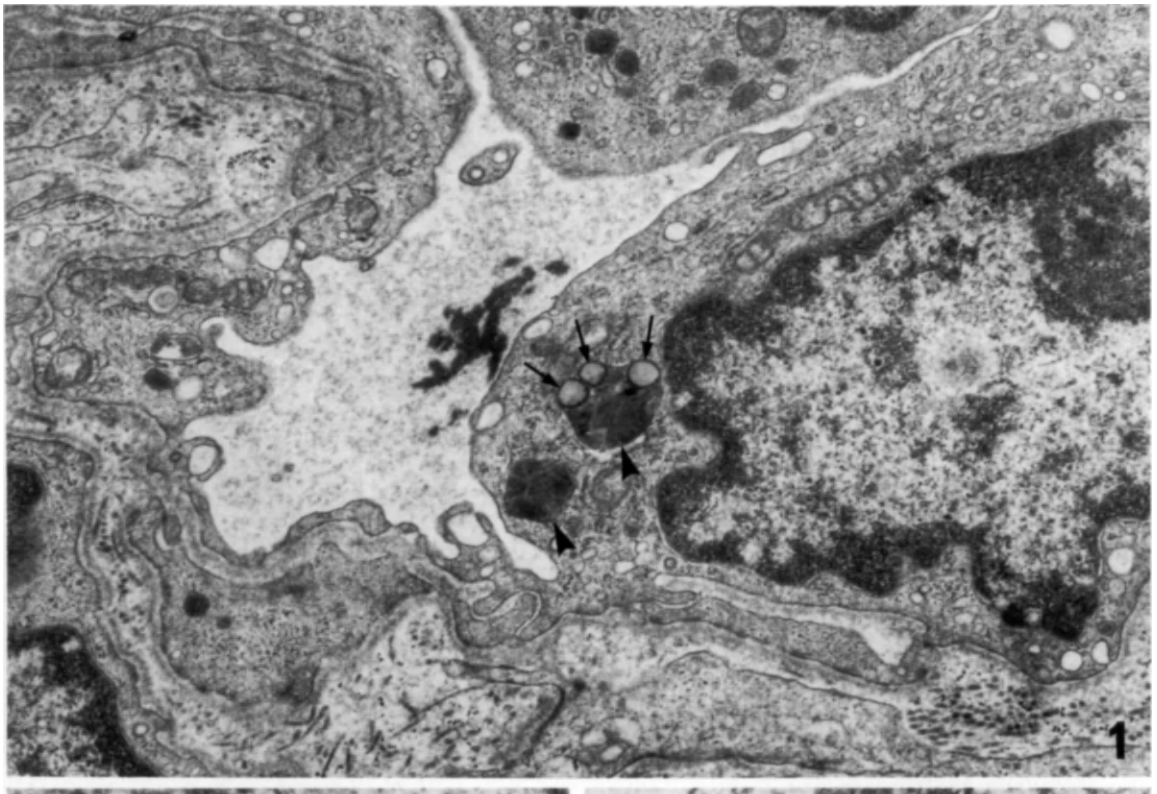
Plate 417

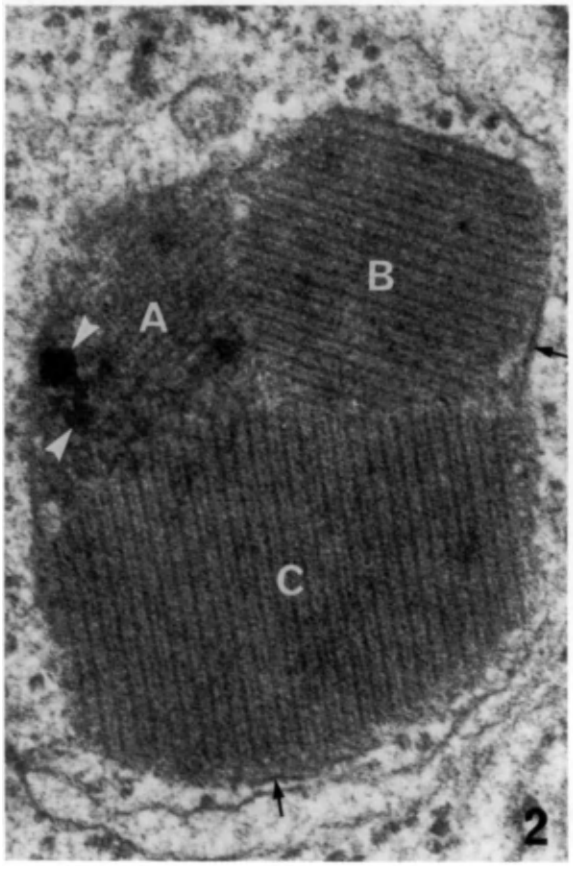
Capillary haemangioblastoma (*From Ho, 1985*)

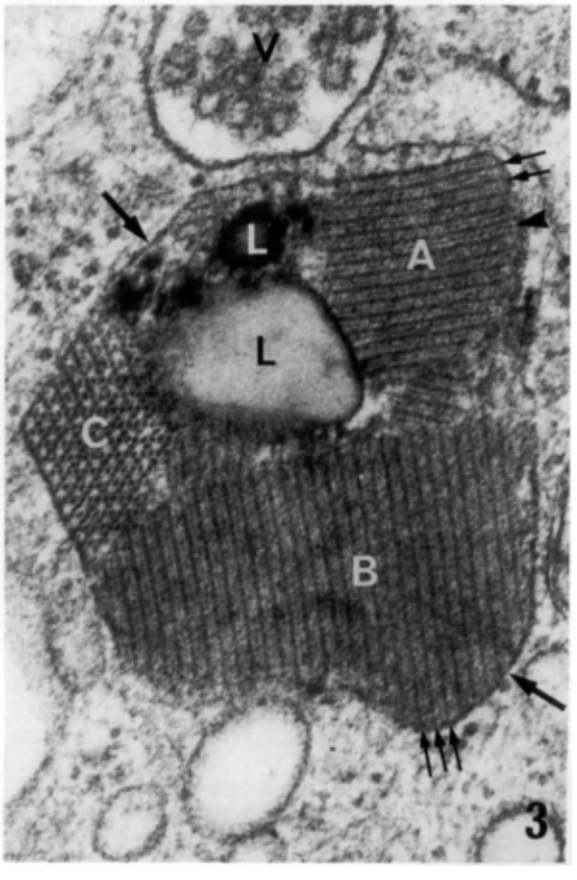
Fig. 1. Two crystalline inclusions (arrowheads) are seen in an endothelial cell. One of them also contains lipid droplets (arrows). ×18 500

Fig. 2. A crystalline inclusion composed of a poorly formed crystal (A), two well-formed crystals (B and C) and some electron-dense material (arrowheads). A clear-cut continuous limiting membrane is not seen but what could be fragments of a limiting membrane are evident (arrows). ×114 000

Fig. 3. A crystalline inclusion composed of three or more crystals, lipid droplets (L) and electron-dense material. Two of the crystals (A and B) show a highly ordered array of dense lines (thin arrows) with a periodicity of about 19 nm. Between these major periodic lines one can just discern a very thin interperiod line (arrowhead). The plane of section through the third crystal reveals a lattice composed of 'dots' which could represent sections through globular units or filaments. This crystalline inclusion is clearly membrane-bound (thick arrows). The structure at the top of the picture is a multivesicular body (V) of the type commonly seen in endothelial cells. ×100 000




A
B
C
2


V
L
L
A
C
B
3

Fibrin

Fibrin is not a normal intracytoplasmic inclusion but it has been seen in an intracytoplasmic location in certain experimental and pathological states. This fibrous (filamentous) protein can occur as irregular shaped aggregates or as fibrils or fibres. Unequivocal morphological identification of this fibrous protein is often possible because fibrin fibres show a striated or banded pattern with a periodicity* of 19–35 nm (Ruska and Wolpers, 1940; Hawn and Porter, 1947; Hall, 1949, 1963; Doolittle, 1984).

Although the spacings of the bands along a given fibre are remarkably constant, they may vary (19–35 nm) from fibre to fibre in the same specimen (Hall, 1949). Not all fibrin in a given sample appears banded, and at times known fibrin or fibrinoid deposits in pathological tissues or purified fibrin produced *in vitro* may fail to show striations (Haust *et al.*, 1965; Ghadially and Roy, 1969).

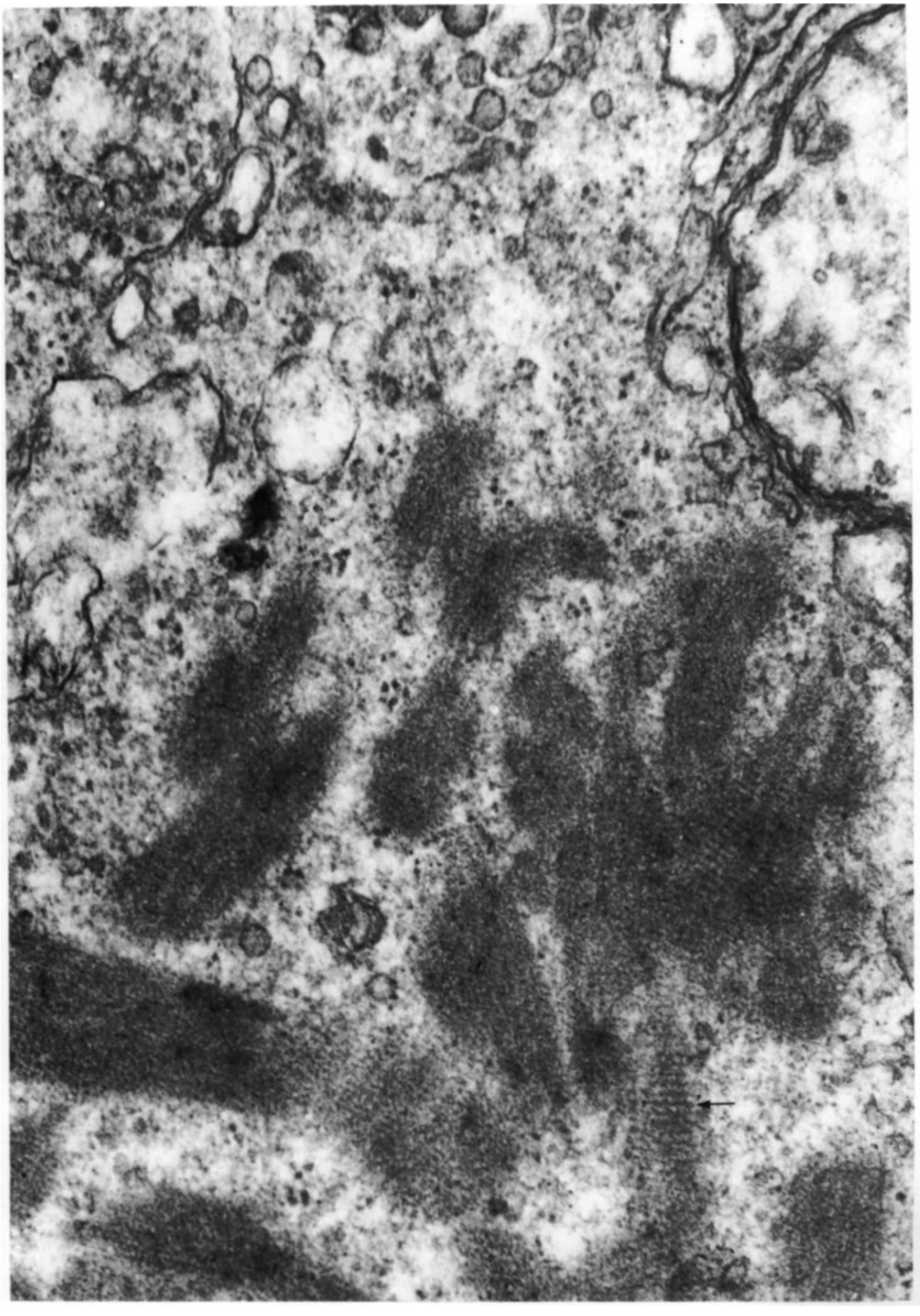
The reason for this is not clear but there is evidence that banding is better defined in clots formed at lower pH values (Hawn and Porter, 1947). The rate of clotting also seems to be important because clots formed very slowly in the presence of little or no thrombin show virtually no striations (Hall, 1949).

The occurrence of intracytoplasmic fibrin was noted by us (Parry and Ghadially, 1967) in the hepatocytes of regenerating post-hepatectomy liver in normal and tumour-bearing rats (*Plates 418* and *419*). Similar deposits lying free in the cytoplasm and also in membrane-bound vacuoles were seen by Ruebner *et al.* (1970) in hepatitis produced by lasiocarpine in mice. In our material, in some cells, striated and non-striated fibrin (non-membrane-bound) was found in

*The fibrin molecule (also called 'fibrin monomer') derived from the fibrinogen molecule after cleavage or excision of the terminal extensions (fibrinopeptides) is a trinodular rod (one nodule at each end and one in the middle) about 45–47 nm long (see diagram on *Plate 537*). Aggregation (polymerization) of these molecules in an approximately half-staggered fashion gives fibrin its characteristic periodicity of banding which is usually about 23 nm (i.e. about half the length of the molecule). Fibrin seen in tissues (i.e. *in vivo*) and fibrin formed under physiological conditions *in vitro* (i.e. by action of thrombin on fibrinogen) shows only this type of periodicity of banding (19–35 nm). However, the quite complex crystals produced *in vitro* after treating fibrinogen with proteases can show a periodicity of up to about 90 nm (Weisel *et al.*, 1983) but nothing resembling such crystals has ever been seen *in vivo*.

Plate 418

Partial hepatectomy was performed on a rat bearing a carcinogen-induced subcutaneous sarcoma. A specimen of liver was collected for electron microscopy six hours after operation. This electron micrograph shows fibrin lying in the cytoplasm of a hepatocyte; some of the fibrin shows characteristic banding (arrow). ×70 000 (*Parry and Ghadially, unpublished electron micrograph*)



the cytoplasmic matrix lying intimately mingled with the cell organelles while in other instances focal accumulations of fibrin were seen set in a light granular matrix. Some of these focal accumulations were at times partially delineated from the cell cytoplasm by a membrane, but completely sequestrated (membrane-bound) inclusions were not seen.

The situation here is reminiscent of that noted in studies on experimental malignant hypertension, where 'insudation' of plasma proteins into the vascular wall and fibrinoid or fibrin deposition is known to occur (Wiener *et al.*, 1965; Ooneda *et al.*, 1965; Hatt *et al.*, 1968; Still, 1968; Hüttner *et al.*, 1968, 1969; Gardner and Matthews, 1969; Kerényi and Jellinek, 1972). In such instances, however, the fibrin deposits are extracellular, although at times some fibrin is seen mingled with (or perhaps lying within) necrotic muscle fibres.

A phenomenon similar to the one reported by us (Parry and Ghadially, 1967) in hepatocytes has, however, been seen in cardiac muscle cells of the rat after experimentally produced malignant hypertension (Hüttner *et al.*, 1971). The illustrations in this paper show most convincingly fibrin fibres intimately mingled with myofilaments in cells that do not appear severely damaged. These authors also found membrane-bound focal accumulations of fibrin and plasma in the cell cytoplasm (thought to be derived by invagination of cell membrane). Regarding the non-membrane-bound fibrin they state: 'the fine structural identification of fibrin in a true intracytoplasmic location can be considered as direct morphological evidence for plasma protein entrance into the injured cells, where fibrinogen polymerized intracellularly to fibrin'.

We (Parry and Ghadially, 1967) postulate that a similar mechanism probably operates in hepatocytes, after hepatectomy. The sequence of events seems to be: (1) an escape of plasma proteins from vessels; (2) entry of plasma proteins into cell cytoplasm (a phenomenon referred to as insudation); and (3) release of factors from injured cells which leads to the conversion of fibrinogen to fibrin.

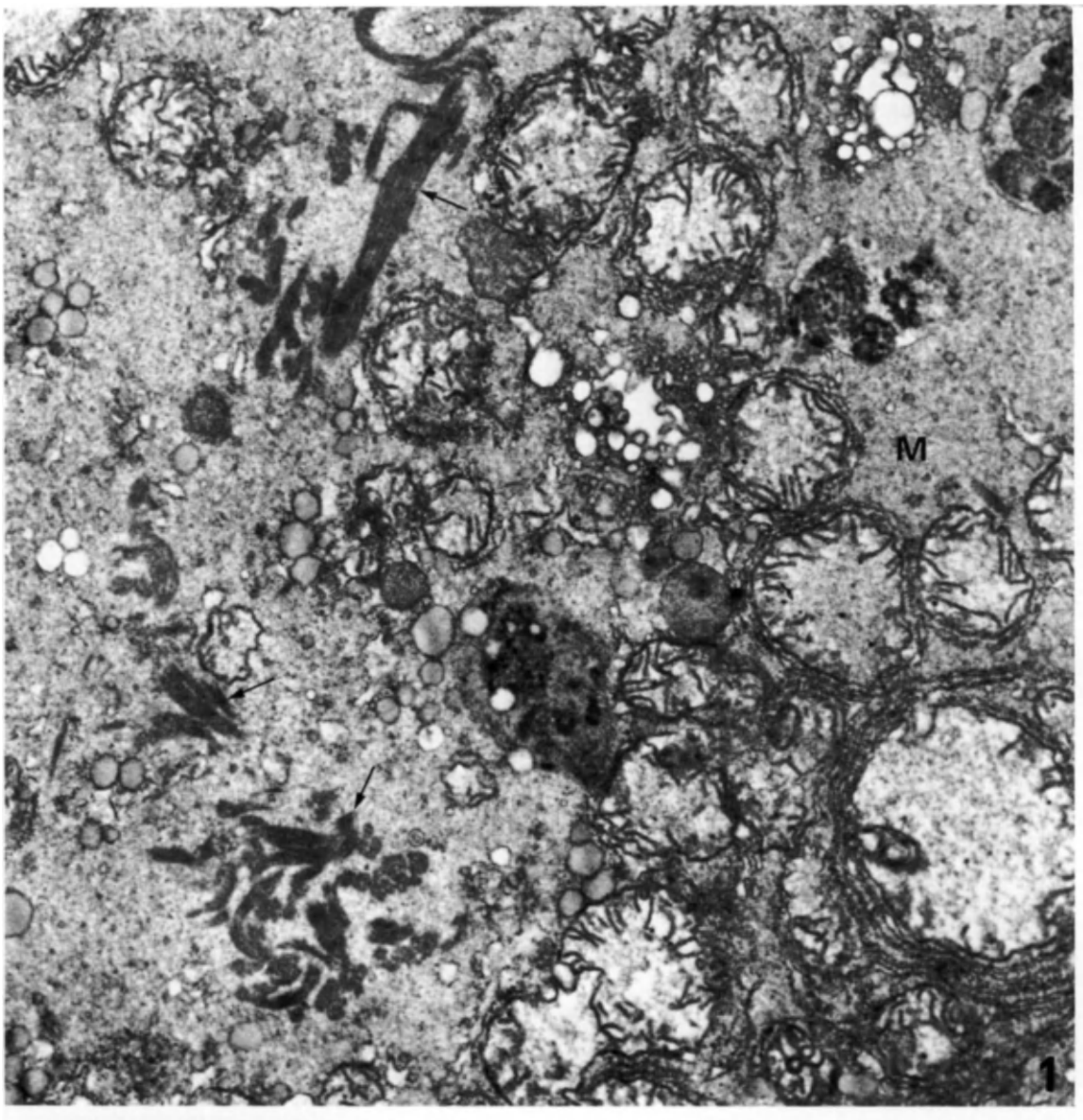
In our experience (Parry and Ghadially, 1967), the fibrin in most instances appears to lie in pools of finely granular material which probably represents a mixture of cytoplasmic matrix and plasma proteins (*Plate 419*). It is at the edge of such areas that the fibrin appears to lie in the cytoplasmic matrix in close association with cell organelles.

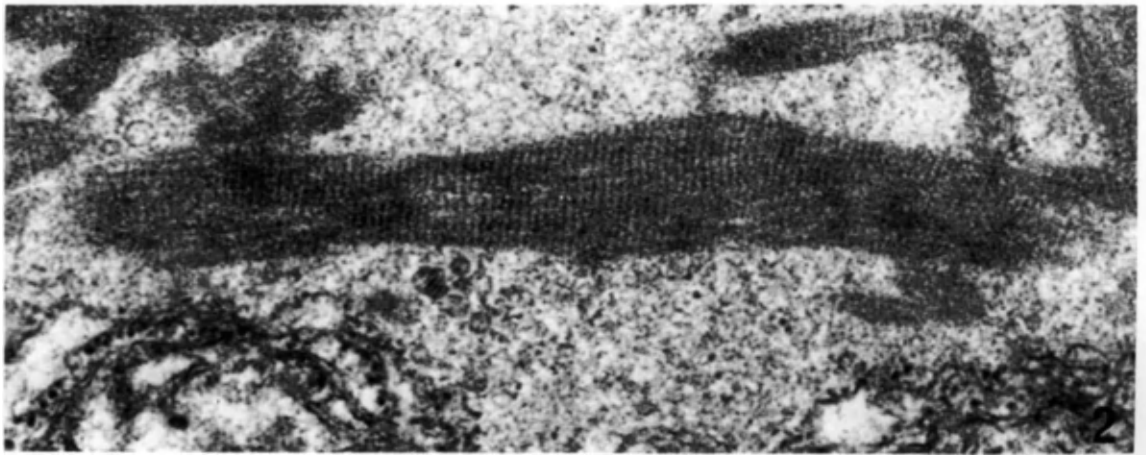
Plate 419

From the liver of a rat bearing a carcinogen-induced subcutaneous sarcoma. The specimen was collected six hours after partial hepatectomy. *(Parry and Ghadially, unpublished electron micrograph)*

Fig. 1. Collections of fibrin (arrows) and cell organelles are seen lying in a finely granular matrix (M) thought to represent a mixture of cytoplasm and insudated plasma proteins. ×18 500

Fig. 2. High-power view of fibrin shown in *Fig. 1.* Note banding with a periodicity of approximately 24.5 nm. ×60 000


M
1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Heinz bodies

Light microscopically, Heinz bodies (also called 'Ehrlich–Heinz bodies') present as rounded, oval or angular inclusions (0.3–2 μm in diameter) in erythrocytes (sometimes also in reticulocytes) in certain pathological states. They can occur as free-floating inclusions in the cytoplasm as originally described by Heinz (1890), or as marginated inclusions abutting or attached to the membrane of the erythrocyte (Rifkind and Danon, 1965; Hollan *et al.*, 1968).

Heinz bodies are rarely visualized* in blood smears stained by Romanowsky stains. They are refractile in fresh blood preparations. Supravital staining with crystal violet is particularly useful in demonstrating them. In such preparations they appear as small purplish inclusions in erythrocytes. Heinz bodies are resistant to haemolysis and are in fact better visualized in erythrocyte ghosts where they often have a granular appearance (i.e. each body presents as a compact or loose conglomerate of granules).

Ultrastructurally, Heinz bodies usually present as irregular-shaped or granular electron-dense bodies (*Plate 420*) of the free-floating type. The marginated type of inclusion where the Heinz body abuts, or is fused to the erythrocyte membrane is infrequently encountered†.

Heinz bodies stem from oxidative injury‡ leading to denaturation and precipitation of haemoglobins, particularly abnormal or unstable haemoglobins and haemoglobin in erythrocytes with glucose-6-phosphate dehydrogenase deficiency. Heinz bodies can be produced *in vitro* by treating blood with phenylhydrazine or sulphonamides. Several drugs (e.g. antimalarials, sulphonamides, nitrofurans, antipyretics, analgesics and sulphones), fava beans and other factors (e.g. infections) produce Heinz bodies and haemolysis of erythrocytes of patients with glucose-6-phosphate dehydrogenase deficiency (Bessis, 1973; Williams *et al.*, 1983).

Heinz bodies have been found in other animals besides man. These include: (1) sheep with chronic copper poisoning (Soli and Nafstad, 1976); (2) cattle with selenium deficiency (Morris *et al.*, 1984); (3) hens fed n-butyl mercaptan and n-butyl disulphide (Maxwell, 1981; Abdo *et al.*, 1983); (4) dogs, calves and turkeys after administration of phenylhydrazine (Simpson, 1971; Taylor *et al.*, 1973; Lynch *et al.*, 1978); (5) herring gulls (*Larus argentatus*) and Atlantic puffins (*Fratercula artica*) after ingestion of crude oil (Leighton, 1985); (6) apparently normal and ill cats (Jain and Keeton, 1975); and (7) cattle, sheep, horses, cats and dogs after dietary or experimental ingestion of onions (for references *see* Harvey and Rackear, 1985).

There is an increase in the number of Heinz bodies after splenectomy. This is because the spleen plays an important role in the removal of Heinz bodies and other 'solid particles' like siderosomes, Howell–Jolly bodies and autolysosomes from circulating erythrocytes (*see* page 648 for a description of the pitting action of the spleen).

*This is because there is as a rule little tinctorial difference between the denatured haemoglobin in the Heinz body and the erythrocyte haemoglobin denatured by air-drying of the blood smear.

†This is easy to comprehend when one realizes that margination will only be demonstrated when the plane of sectioning passes through the zone of attachment. To a lesser extent such a situation also prevails in smears of blood examined with the light microscope, for margination will only be evident in erythrocytes flattened in a plane at right angles to the plane of attachment.

‡Heinz bodies appear to be the final product of a series of peroxidation reactions. The first step appears to be oxidation of haemoglobin to methaemoglobin. Subsequent oxidation products are thought to include hemichromes and haem. According to Eisinger *et al.* (1985) the haem moiety may lose its iron atom and be converted into dipyrrolic compounds which are excreted in the urine.

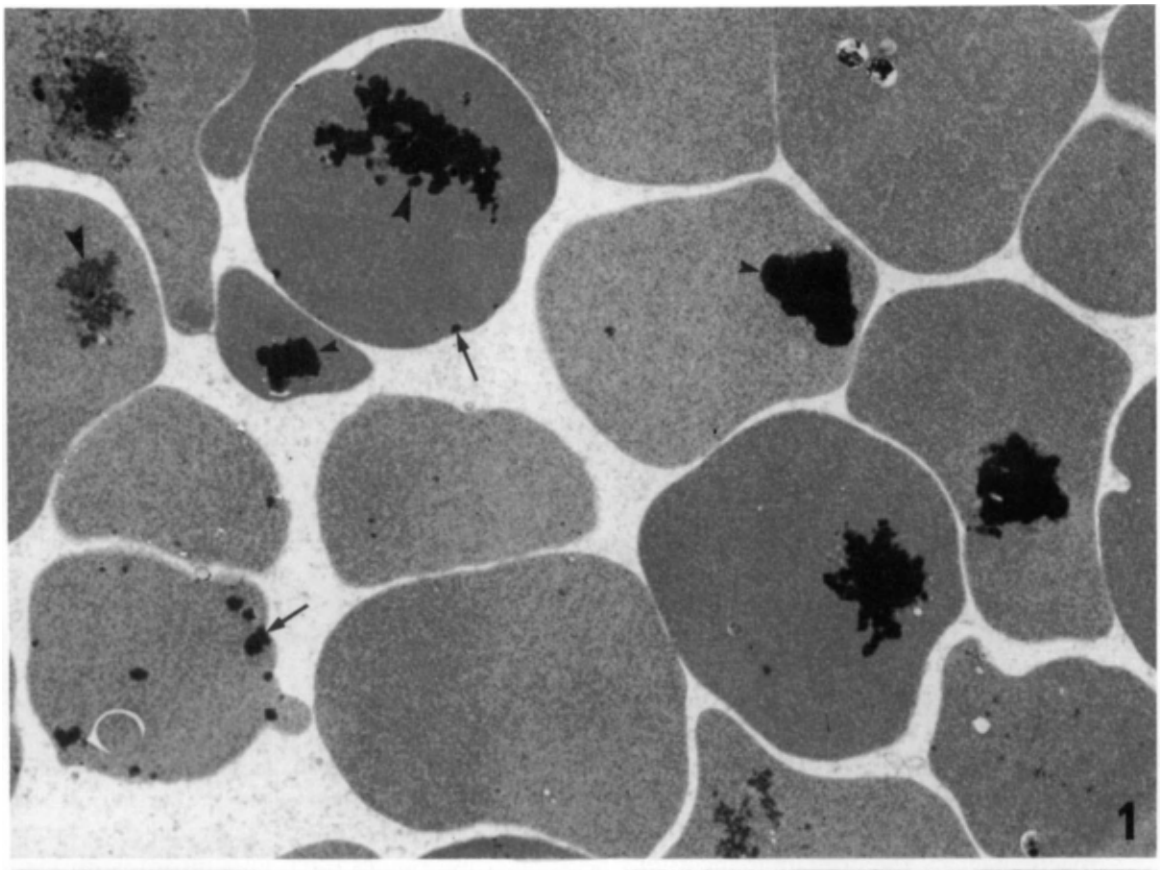
Plate 420

Peripheral blood from a patient with abnormal haemoglobin (type not determined)

Fig. 1. Compact irregular shaped Heinz bodies (small arrowheads) and granular Heinz bodies (large arrowheads) are present in the erythrocytes. Most of the Heinz body profiles appear free-floating only a few are marginated (arrows). ×18000

Fig. 2. Erythrocyte containing marginated Heinz bodies. ×7600

Fig. 3. Erythrocyte ghosts containing Heinz bodies. ×6000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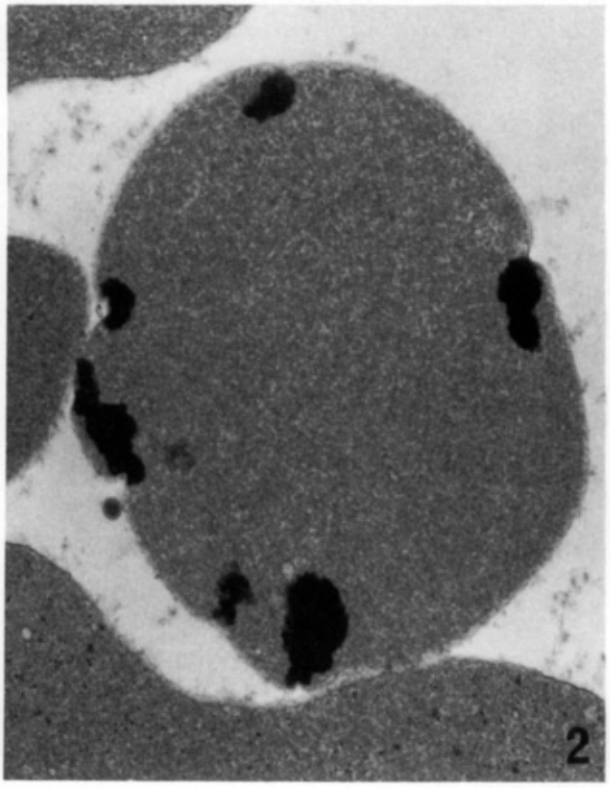
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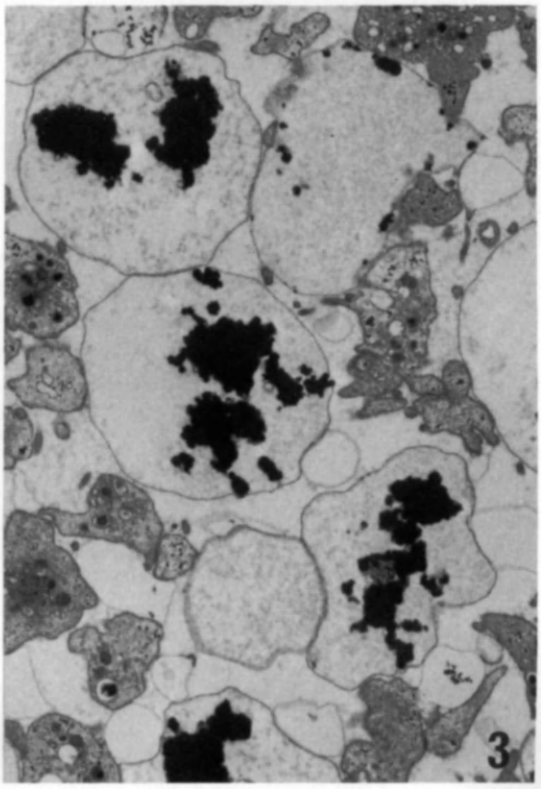
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Porphyrin inclusions

Porphyrin inclusions are found in the livers of cases of erythropoietic porphyria (more fully referred to as 'erythropoietic protoporphyria') and porphyria cutanea tarda. In the former instance the inclusions contain mainly protoporphyrin, in the latter mainly uroporphyrin and heptacarboxyporphyrin.

An important early histopathological feature of protoporphyrin liver disease is the occurrence within hepatocytes, bile canaliculae and Kupffer cells of brown granular protoporphyrin deposits which exhibit birefringence on polarization microscopy and red fluorescence when submitted to ultraviolet radiation.

Electron microscopy reveals (*Plate 421*) that these 'granules' are in fact aggregates of straight or curved needle-like electron-dense profiles (0.4–0.5 μm long and 7–13 nm wide). These profiles are at times demarcated by two electron-dense parallel 'lines' with less dense material and faint parallel lines between them. This appearance plus their birefringence with polarized light makes them acceptable as crystals or crystalloids. One surmises that the needle-like profiles result from sections through plate-like crystals because circular profiles acceptable as sections through needles are not seen. Aggregates of these crystalline profiles often deployed in a sunburst (i.e. rosette or radial) or curly hair pattern are seen to lie in a matrix which varies from electron-lucent to quite dense.

In the hepatocytes, aggregates of these crystals form fairly compact well demarcated inclusions which may be seen: (1) lying free in the cytoplasmic matrix; (2) lying within a clear space or non-membrane-bound 'vacuole'; and (3) rarely perhaps also in a true membrane-bound vacuole*.

Crystalline aggregates of identical morphology are seen in bile canaliculi and one presumes that they are derived from extrusion of hepatocellular inclusions or by concentration and precipitation of protoporphyrin excreted in the bile. In Kupffer cells and infiltrating macrophages†, the crystalline inclusions lie in heterolysosomes often in company with lipid droplets and ingested cell debris. These heterolysosomes are usually angulated (*Plate 422*), and hence they look like the angulate lysosomes or Gaucher-like lysosomes seen in various conditions described on pages 698–707.

Protoporphyrin inclusions have been seen in the liver of: (1) patients with erythropoietic protoporphyria (Klatskin and Bloomer, 1974; Bloomer *et al.*, 1975; Wolff *et al.*, 1975; Bruguera *et al.*, 1976; MacDonald *et al.*, 1981); (2) mice with 1,4-dihydro-3,5-dicarbethoxycollidine-induced porphyria (Waterfield *et al.*, 1969); (3) mice with griseofulvin-induced porphyria (Matilla and Molland, 1974); (4) dogs with 2-ethyl-2 phenyl butyramide-induced porphyria (Zaki *et al.*, 1973); (5) mice, rats and dogs with 3-[2-(2,4,6-trimethylphenyl)-thioethyl]-4-methylsydnone-induced porphyria (Stejskal *et al.*, 1975).

The livers of patients with erythropoietic protoporphyria contain massive amounts of protoporphyrin, at least some of which is of hepatic origin. The crystalline inclusions (*Plate 421*) no doubt reflect the overloading of hepatic cells with protoporphyrin. Biochemical

*A membrane-bound appearance can be created by displaced endoplasmic reticulum at the periphery of the inclusion. When crystals are seen in a true membrane-bound vacuole the structure also as a rule contains lipid droplets and cell debris. These have to be regarded as autolysosomes.

†Focal inflammatory infiltrates containing macrophages are seen in the porphyric liver, presumably as a response to the presence of necrotic hepatocytes.

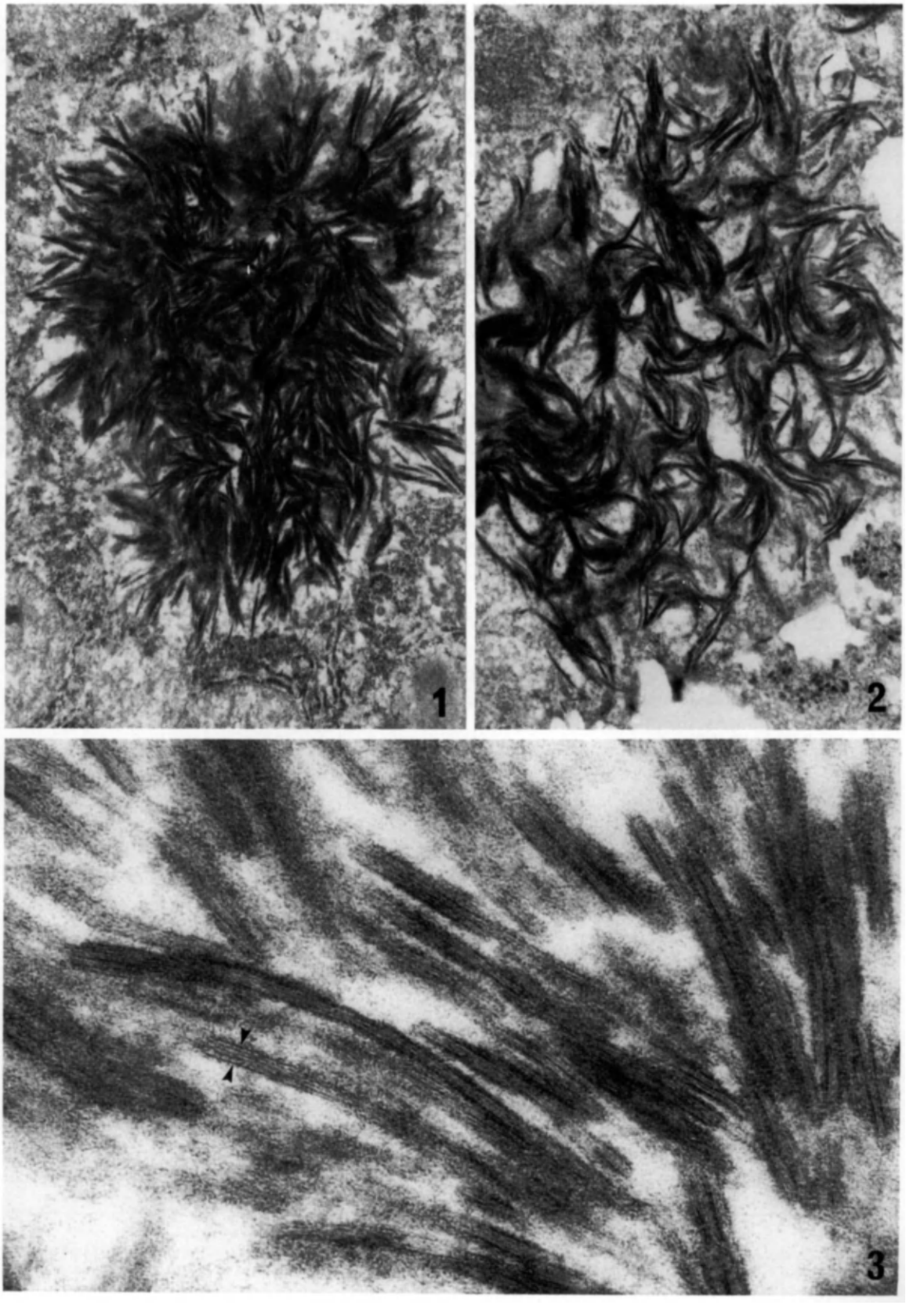
Plate 421

Hepatocytes from a case of erythropoietic protoporphyria (*From a block of tissue supplied by Dr S. Bhuta*)

Fig. 1. A protoporphyrin deposit composed of radiating needle-shaped profiles. ×23 000

Fig. 2. A protoporphyrin deposit composed of curved electron-dense profiles resembling curly hair. ×26 000

Fig. 3. High-power view of needle-shaped profiles (similar to those shown in *Fig. 1*). A longitudinally striated pattern can just be discerned in some places (between arrowheads). ×204 000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examination of cell fractions obtained from livers of mice with experimentally produced protoporphyrin liver disease have shown that these crystals contain protoporphyrin. Moreover, *in vitro* recrystallization of protoporphyrin extracted from such livers produces crystals identical to those found *in vivo*. (For references supporting these statements *see* Wolff *et al.*, 1975, 1982.)

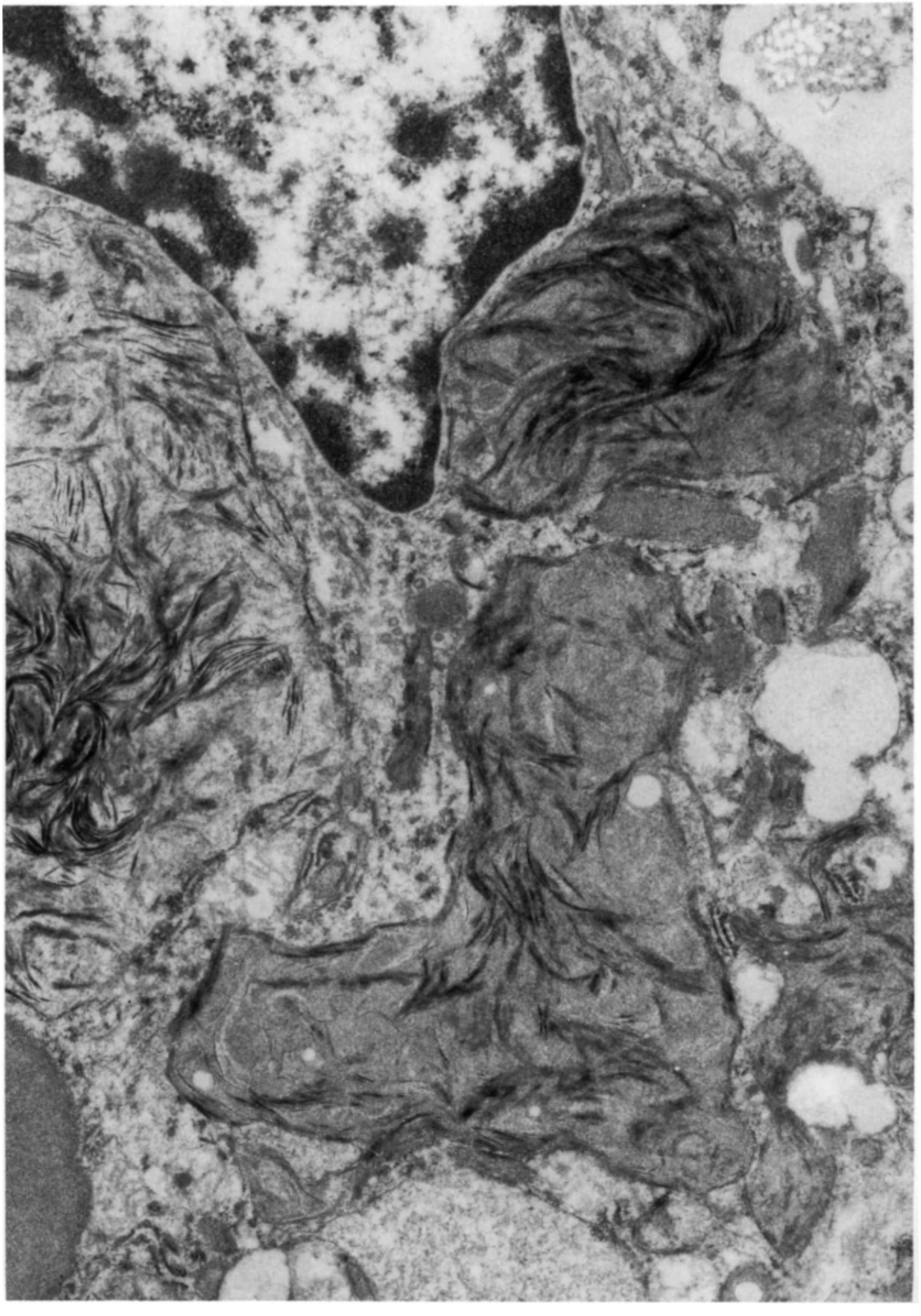
In porphyria cutanea tarda (a bulious dermatosis associated with liver damage) the liver contains an excess of uroporphyrin and heptacarboxyporphyrins. These porphyrins produce long (up to several μm) slender crystals (which being birefringent are better visualized with polarized light) in the cytoplasm of hepatocytes. However, James *et al.* (1980) have shown that these crystals are totally removed if tissue sections are left floating in water for 10 minutes. Little wonder then that in several light microscopic reports and some electron microscopic reports as well (e.g. Biempica *et al.*, 1974), there is no mention of crystals. However, there are two reports (Timme, 1971; Waldo and Tobias, 1973) where long rod-like electron-lucent clefts, no doubt derived from dissolved crystals have been demonstrated in the liver of cases of porphyria cutanea tarda. Thus solubility differences of porphyrins produce quite distinct and different porphyrin inclusions in porphyria cutanea tarda and erythropoietic protoporphyria.

Since mitochondria are known to be involved in the production of porphyrins (*see* page 310), electron microscopists have quite rightly tried to find porphyrin crystals in hepatocyte mitochondria in various types of porphyria in man and experimentally produced porphyrias in animals. All have failed to demonstrate crystals in mitochondria, some frankly confess their failure to do so, but other make quite curious statements divorced from reality. Thus for example, MacDonald *et al.* (1981) state 'Despite lack of definitive evidence, our study supports the view that crystals arise initially in mitochondria', while Waterfield *et al.* (1969) fail to show crystals in mitochondria (their *Figure 3* is unconvincing and the legend is ambiguous) but state that protoporphyrin crystals 'formed within the mitochondria though they were also seen free in the cytoplasm'. Surely if facts do not fit a theory one should modify the theory and not the facts. I think that the biochemical and morphological findings can be reconciled by hypothesizing that protoporphyrin formed in mitochondria diffuses out or is transported out into the cytoplasm and that it crystallizes when a high concentration is reached.

Although porphyrin crystals have not been demonstrated in mitochondria, normal-sized mitochondria and giant mitochondria containing crystals of the type shown in *Plates 120* and *137* are frequently seen in porphyric hepatocytes. Such crystals have been seen in a large number of diseases and disorders, and even perhaps at times in normal hepatocytes. Hypertrophy of smooth endoplasmic reticulum, fatty change, haemosiderin deposition and ultimately frank necrosis of hepatocytes are other changes seen in the porphyric liver.

Plate 422

Macrophage from the liver of a case of erythropoietic porphyria (same case as *Plate 421*) showing rounded and angulate lysosomes containing straight and curved electron-dense profiles (similar to those shown in *Plate 421, Figs. 1 and 2*) acceptable as protoporphyrin crystals. ×22000 (*From a block of tissue supplied by Dr S. Bhuta*)



Intracellular and intracytoplasmic collagen

The term 'intracellular collagen' is used to describe collagen fibrils lying within membrane-bound tubular or vesicular profiles in the cell* (*Plate 423, Fig. 1*). The term 'intracytoplasmic collagen' is used to describe collagen fibrils presumably lying free in the cytoplasmic matrix.

Interpretations of images suggesting that collagen is lying in an intracellular site and the significance of such phenomena are singularly difficult to evaluate. Various possibilities have been discussed in the literature, including: (1) polymerization of collagen fibrils from procollagen within the cell after removal of the tissue from the body; (2) phagocytosis of matrical collagen fibrils by the cell; (3) collagen fibrils lying in a bay, invagination or deep infolding of the cell membrane (*Plate 423, Fig. 2*); (4) an overlaying of collagen fibrils on the cell within the section thickness; (5) rapid synthesis of procollagen followed by its polymerization into collagen fibrils (i.e. synthesis of collagen fibrils) within the cell, presumably because the rate of procollagen synthesis exceeds the rate of transport into the extracellular space; and (6) trapping of extracellular collagen fibrils by cells fusing to form a giant cell. Before evaluating these possibilities it is essential to classify the situations in which intracellular and intracytoplasmic collagen fibrils have been sighted and the interpretations of various workers on this subject.

Intracellular collagen which has been interpreted as synthesis of collagen fibrils in the cell have been seen in: (1) epithelial cells of chick embryo cornea (*in vivo* and *in vitro*) (Hay and Dodson, 1973; Trelstad, 1971); (2) epithelial cells of notochord of chick embryo (Carlson, 1972); (3) fibroblasts from regenerating forelimb of the newt (Norman and Schmidt, 1966, 1967); (4) human fibroblasts from benign and malignant tumours and tumour-like conditions (desmoid fibroma, angiofibroma, non-ossifying fibroma, fibrosarcoma, liposarcoma and osteogenic sarcoma), chronic osteomyelitis and keloids (Welsh, 1966; Welsh and Meyer, 1967; Tannenbaum, 1971†; Allegra and Broderick, 1973); (5) granular cells in cardiac valves from Marfan's syndrome and Hurler's syndrome (Rentería and Ferrans, 1976; Rentería *et al.*, 1976); (6) chondrocytes of normal rabbit semilunar cartilages (Ghadially *et al.*, 1978) (*Plates 423* and *424*); (7) chondrocytes of ear cartilage of rabbits treated with papain (Sheldon and Kimball, 1962); (8) chondrocytes of human chondrosarcoma (Mandalenakis, 1974†); (9) cells from a Müllerian adenosarcoma of the uterus (Katzenstein *et al.*, 1977); (10) osteoblasts, odontoblasts and fibroblasts from alveolar bone, teeth and peridontal ligament of rat (Weinstock, 1972); and (11) cultured osteoblasts and fibroblasts exposed to antitubulin agents such as colchicine or

*One of the instances in which membrane-bound collagen occurs is when collagen is phagocytosed and degraded in lysosomes. This is briefly touched upon here but references and details are not given. It is recommended that this section be read in conjunction with the section on 'collagen in lysosomes' pages 716–719.

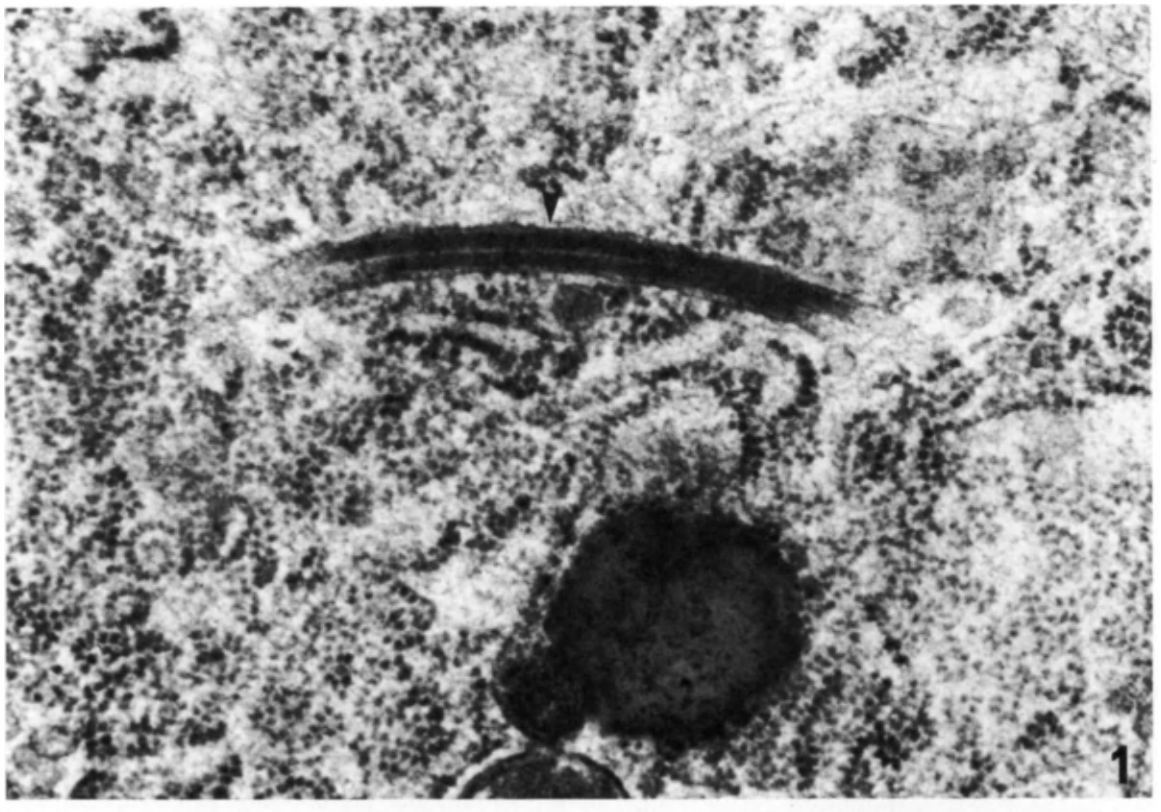
†In the infantile fibroma described by Tannenbaum (1971) the periodicity of the intracellular fibrils was 21 nm while in the chondrosarcoma described by Mandalenakis (1974) the periodicity of the intracellular fibrils was 21.7 nm. Tannenbaum states that 'Fetal and reconstituted collagen are also known to have a periodicity of approximately 210 Å' but gives no reference. In both these studies these fibrils were in the cisternae of the rough endoplasmic reticulum.

Plate 423

Rabbit semilunar cartilage (*From Ghadially, Thomas, Yong and Lalonde, 1978*)

Fig. 1. A tubular structure containing two collagen fibrils found deep in the cytoplasm of a chondrocyte (arrowhead). Faint striations reminiscent of collagen-type banding are discernible in the original electron micrograph but they are unlikely to be evident in this reproduction. ×68 000

Fig. 2. Close association of collagen fibrils with this chondrocyte has resulted in an overlaying of collagen fibrils on the cell (between arrowheads) within the narrow confines of the section thickness. An appearance is also created as if the collagen fibrils were sprouting from the cell surface (arrows). The appearance of an island of collagen (C) lying within the cell is due to a section through a depression on the cell surface. The expected membrane surrounding the collagen fibrils is not visualized because of tangential sectioning but its presence can be sensed by the moderately electron-dense rim round the fibrils. ×29 000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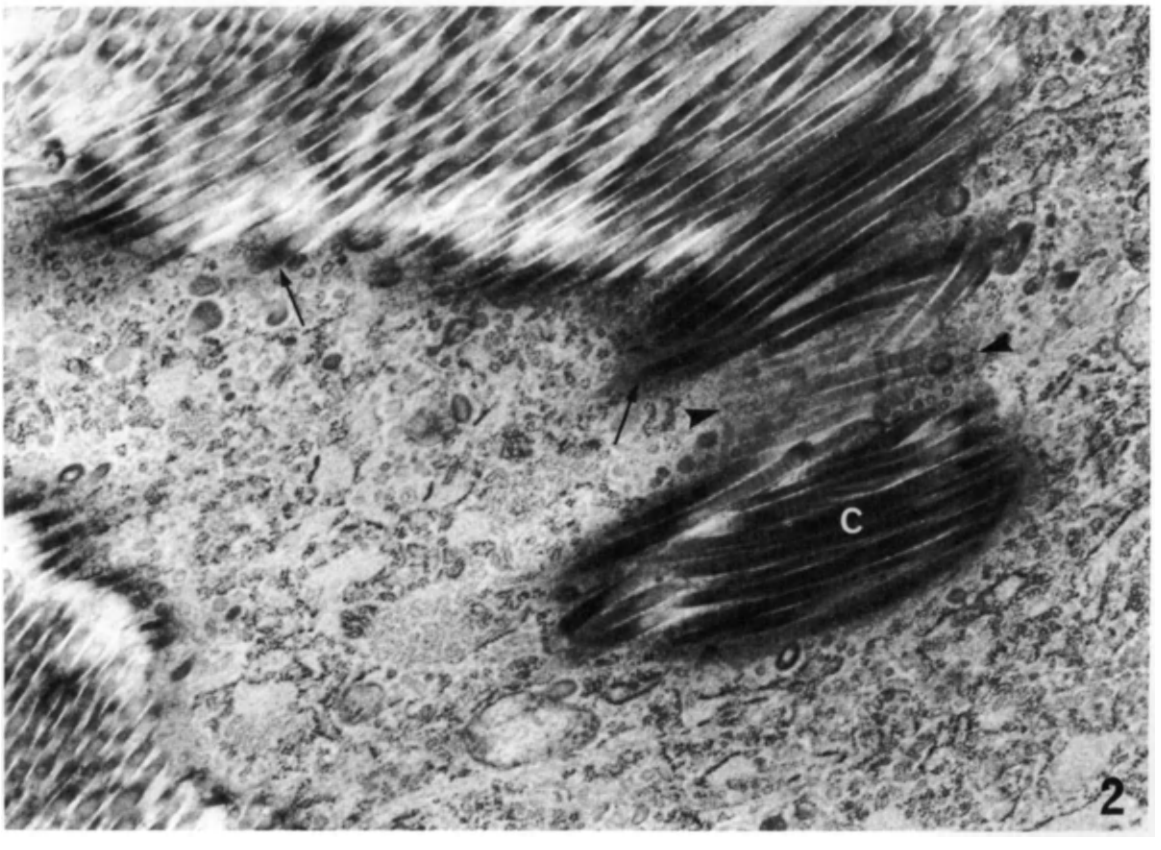
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vinblastine (Ehrlich *et al.*, 1974; Olsen and Prockop, 1974; Scherft and Heersche, 1975; Fernandez-Madrid *et al.*, 1980).

Examples of intracellular collagen which have been interpreted as phagocytosed collagen are dealt with on pages 716–719, but a few authors have interpreted intracellular collagen as evidence of both synthesis and/or phagocytosis; such examples include: (1) fibroblasts of cruciate and wing ligaments of rat and chick embryo (Oakes, 1974); (2) fibroblasts of peridontal ligament of mouse, guinea-pig and monkey (Ten Cate, 1972; Deporter and Ten Cate, 1973); (3) fibroblasts and myofibroblasts from torn human semilunar cartilages (Ghadially *et al.*, 1980) (*Plate 425*); and (4) smooth muscle of occluded rat artery and ureter and human varicose and atherosclerotic vessels (Staubesand, 1977). Intracytoplasmic collagen fibrils (i.e. lying free in the cytoplasm) assumed to have been synthesized in the cell have been seen in: (1) human hepatocytes from cases of chronic aggressive hepatitis, β-thalassemia and Wilson's disease (Gmelin *et al.*, 1973; Groniowski and Walski, 1975); (2) fibroblasts from a fibroma of human nasopharynx (Seifert, 1971); (3) fibroblasts from the radula of the common garden snail, *Helix aspera* (Meek, 1968). On the other hand, the idea that intracytoplasmic collagen resulted from penetration of collagen fibrils into the cell or secondary incorporation of matrical collagen fibrils into cells has been advocated for intracytoplasmic collagen fibrils seen in: (1) hepatocytes from active chronic hepatitis (Gerlach *et al.*, 1969); and (2) fibroblasts from normal human endometrium (Dubrauszky and Schmitt, 1960). An unusual manner in which collagen may become incorporated in the cell cytoplasm has been suggested by Azar and Lunardelli (1969) who found that the asteroid bodies in the giant cells of sarcoid granuloma are composed of collagen fibrils*. They suggest that here extracellular collagen fibrils are trapped between cells that undergo fusion to form giant cells and subsequent dissolution of the intervening cell membranes liberates the collagen into the cytoplasm of the giant cell.

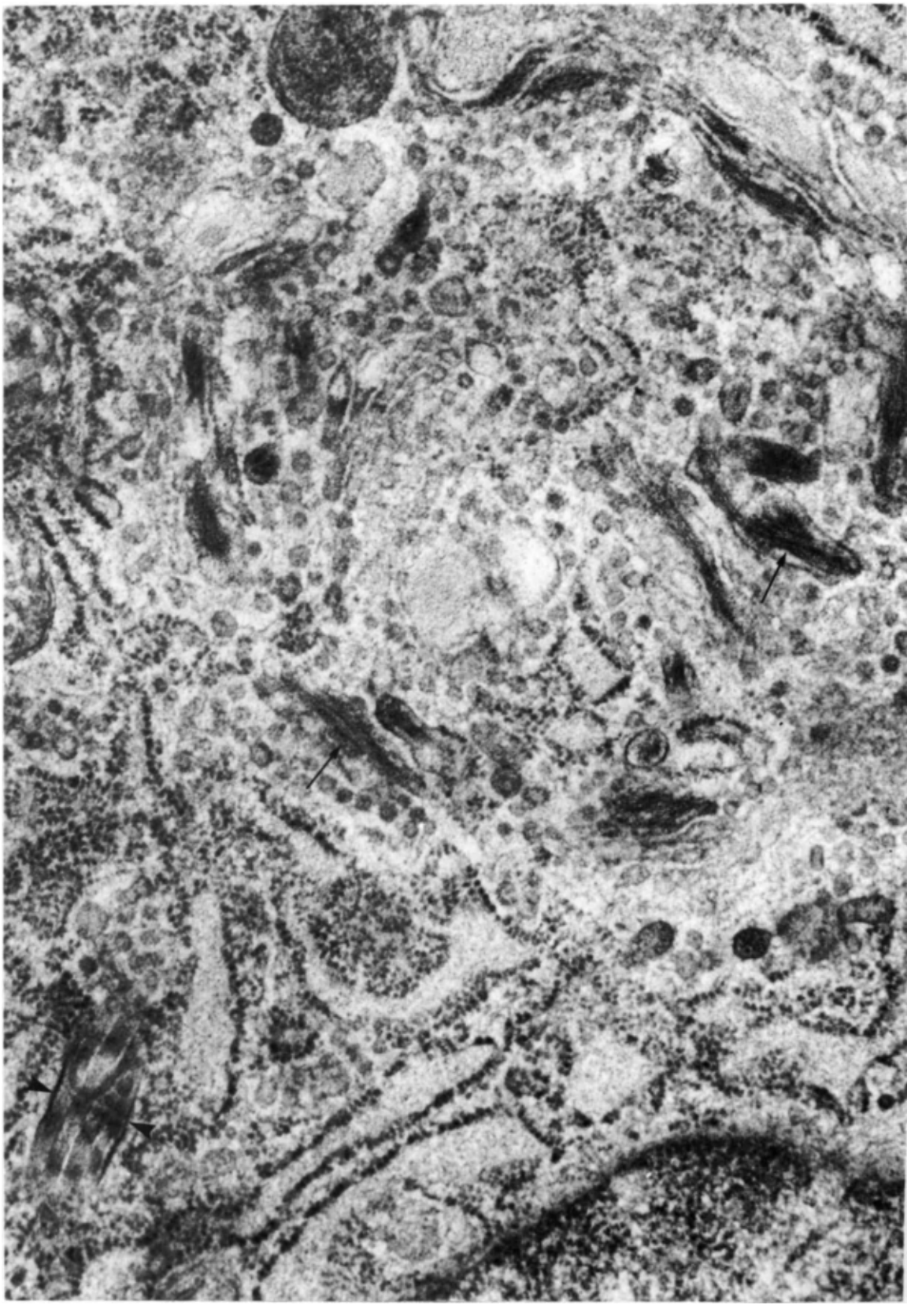
Let us now return to the various suggestions regarding the interpretation and significance of intracytoplasmic and intracellular collagen listed on page 996. The first problem that one faces is deciding whether the collagen fibrils are really within the cell or cell cytoplasm or whether these appearances are a product of sectioning geometry. There is little doubt that collagen fibrils can lie very close to the surface of fibroblasts (*Plate 423, Fig. 2*) and at times they are clearly seen to be in bays and invaginations of the cell membrane. Images suggesting that cell processes of fibroblasts have wrapped around collagen fibrils or that collagen fibrils pass right through the cell are also seen. However, it is difficult to believe that the long lengths of collagen lying in tubular structures such as that shown in *Fig. 10* in the paper by Allegra and Broderick (1973) or the collagen lying in meandering channels (*Fig. 13* in Tannenbaum, 1971) represent anything but collagen lying either in the endoplasmic reticulum or in some other membrane system within the cell. Such images and also others where the membrane-bound collagen is seen in the depths of the cell and not near the edge are difficult to explain away on the basis of collagen lying in bays and invaginations of the cell membrane.

The problem of overlying collagen fibrils is not relevant when membrane-bound collagen is seen, because it is difficult to see how an overlaying of collagen fibrils over a cell surface could produce a 'membrane-bound' appearance. This would be relevant in the case of intracytoplasmic collagen and such a phenomenon is not uncommon near the edge of a cell.

*However Cain and Kraus (1977, 1983) find that asteroid bodies are composed of vimentin filaments, microtubules and centrioles. This supports Altmann's (1960, 1964) opinion that asteroid bodies are malformed cytospheres. There are various other views regarding the nature of asteroid bodies (*see* page 920).

Plate 424

A chondrocyte from a rabbit semilunar cartilage. The Golgi complex contains electron-dense filamentous material (arrows). Also seen are some obliquely cut, presumably collagen fibrils flanked by membranous profiles (arrowheads). ×58 000 (*From Ghadially, Thomas, Yong and Lalonde, 1978*)



However, in the illustrations presented by Meek (1968) long lengths of collagen are seen well within the cytoplasm which would be difficult to explain away on the basis of an overlaying phenomenon.

If one concedes that in some instances at least collagen fibrils occur in an intracellular site and are not a mirage of sectioning geometry, then one of the possibilities one should consider is that collagen fibrils can form in the cell after removal from the body. This hypothesis stems from the well-known fact that collagen fibrils can be formed *in vitro* from collagen solutions but there is little else that supports this idea. One can argue against this hypothesis by pointing out that: (1) if tissues are fixed rapidly (as they usually are for electron microscopy) there would be little time for such polymerization to occur; (2) this is a hypothetical argument for which there is no real evidence; and (3) if such a phenomenon did occur one would see intracellular collagen quite frequently in many sites and situations, and this would have been reported in many studies by now; but this is not so.

Next one should consider the possibility that intracellular collagen is phagocytosed collagen. There is no doubt that this occurs (*see* pages 716–719). In such instances one expects to see also various stages of disintegration of collagen fibrils in bodies acceptable as lysosomes. As is to be expected in most instances such a phenomenon is seen in macrophages but resorption of collagen by fibroblasts also certainly occurs.

Finally, we come to the vexatious question: can fibril synthesis occur in an intracellular location? Despite support from various morphological observations (listed above), there is a great reluctance in accepting this idea, largely because it conflicts with the biochemical dogma which maintains that procollagen peptidase which cleaves the terminal extensions of the procollagen molecule resides outside the cell (Layman and Ross, 1973) although certain steps in the cleavage process and some modifications of procollagen molecules does occur in intracellular locations (Bornstein, 1974).

However, there are good morphological grounds for believing that intracellular synthesis of collagen fibrils does sometimes occur because filaments and unbanded and faintly banded fibrils acceptable as stages of collagen fibril assembly have been seen in Golgi vacuoles and endoplasmic reticulum (e.g. Sheldon and Kimball, 1962; Tannenbaum, 1971; Ghadially *et al.*, 1978, 1980). Further, it has been shown that drugs (antitubulin agents) such as colchicine and vinblastine, which interfere with the transport and secretion of newly synthesized procollagen (Dehm and Prockop, 1972; Diegelmann and Peterkofsky, 1972; Ehrlich and Bornstein, 1972) lead to the appearance of filaments and fibrils in Golgi-associated vacuoles within cultured osteoblasts (Ehrlich *et al.*, 1974; Olsen and Prockop, 1974; Scherft and Heersche, 1975). These have a morphology similar to that illustrated in *Plates 424* and *425* and give the staining reactions for collagen. When cultured fibroblasts from embryonic chick are treated with colchicine, intracytoplasmic vacuoles containing a variety of fibrous structures (filaments, banded and unbanded fibrils and segment long-spacing collagen) develop (Fernandez-Madrid *et al.*, 1980).

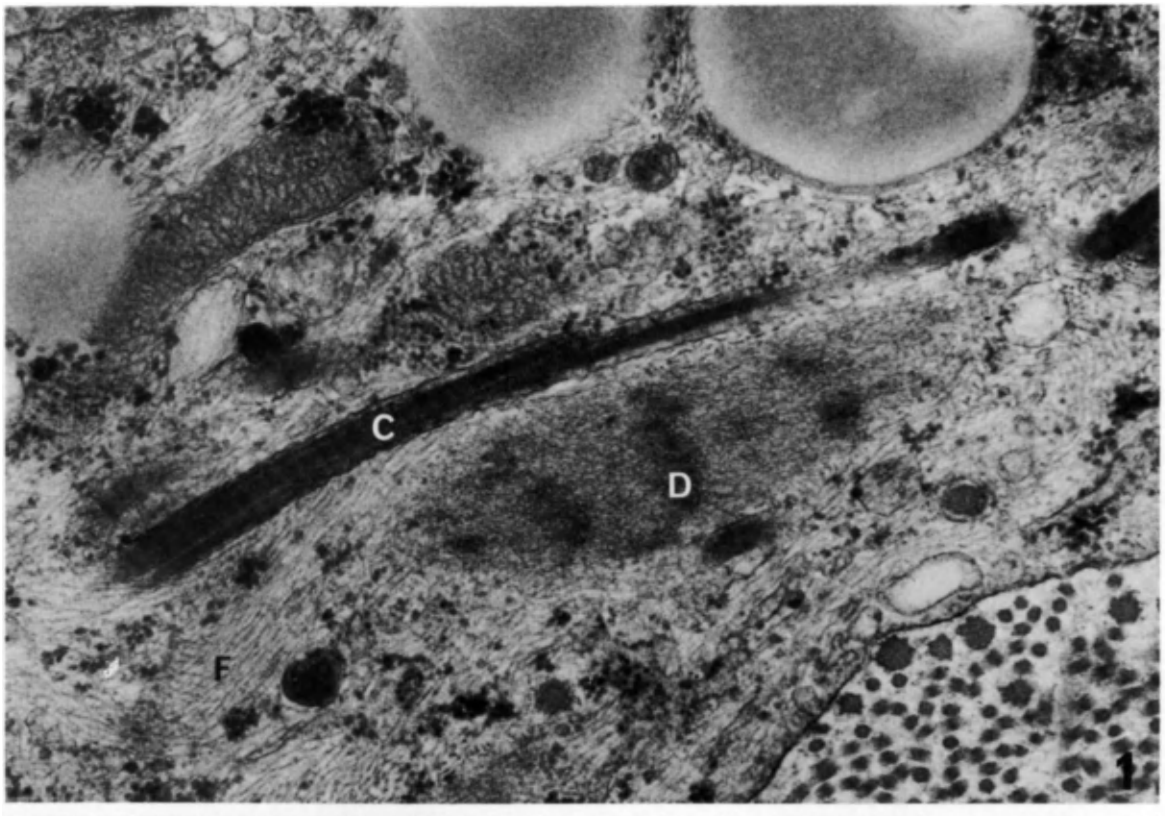
Hence one may speculate that in certain pathological states where the secretion of procollagen is hampered, or in normal states where procollagen synthesis is stimulated and occurs too rapidly for the transport mechanism to cope, intracellular collagen fibrils may develop.

Plate 425

Myofibroblast and fibroblast from torn human semilunar cartilage (*From Ghadially, Lalonde and Yong, 1980*)

Fig. 1. Myofibroblast showing intracytoplasmic collagen (C). Note also the thin filaments with focal densities (D) seen in such cells and the coarser intermediate filaments (F). ×41 000

Fig. 2. Fibroblast showing filamentous material (arrowhead) in an expanded Golgi sac and a fibril (arrow) in a Golgi-associated vacuole. ×91 000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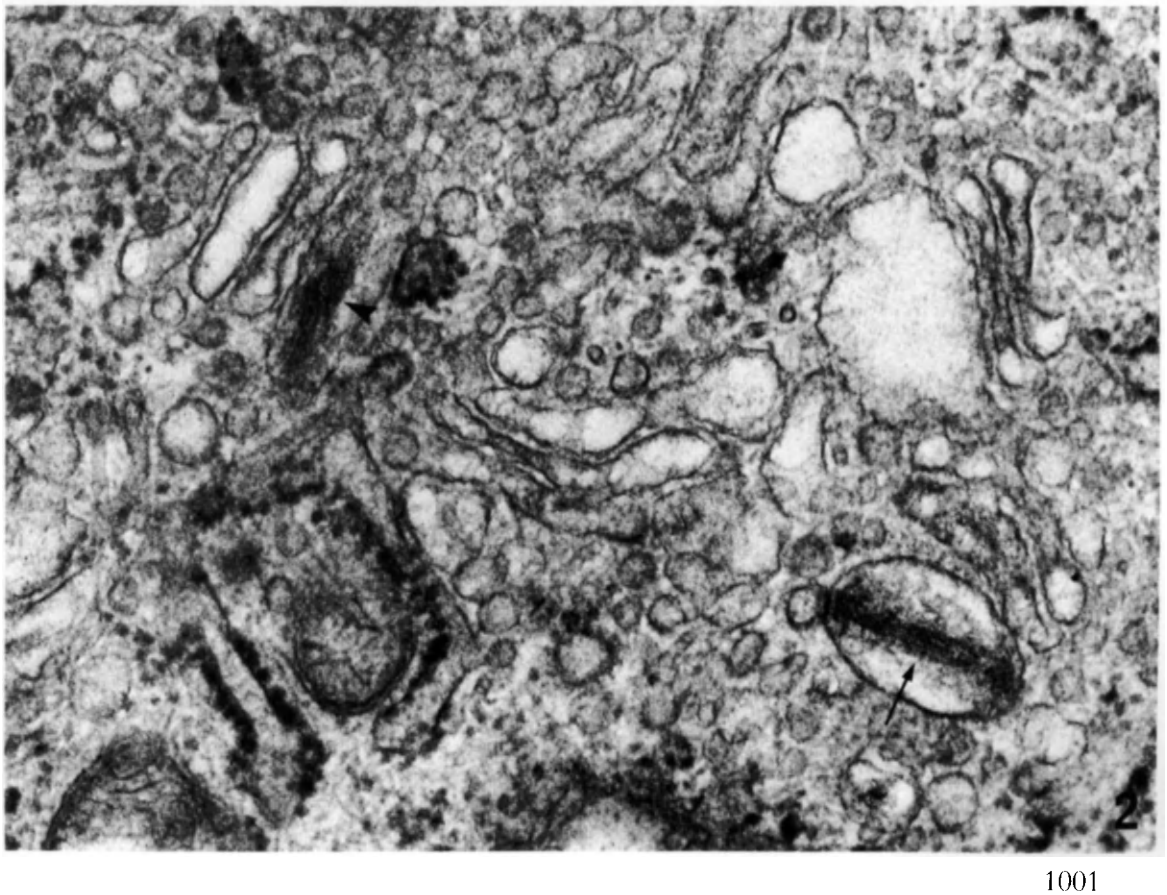
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Intracytoplasmic banded structures

In this section we deal with a heterogeneous collection of intracytoplasmic structures, which share a common morphological feature, namely that they present a banded, cross-striated or laminated appearance. The nature, significance and composition of most of these structures is not well understood.

One group of banded structures occurs in striated muscle cells (*Plate 426, Fig. 1*). Here they have been referred to by various terms such as 'zebra bodies', 'ladders', 'microladders', 'banded structures', 'leptomeric structures', 'leptomeric fibrils' and 'leptomeric complexes'. It is the latter three terms which are commonly used now. The term 'leptomeric structures' is a collective term which includes 'leptomeric fibrils' and 'leptomeric complexes'. A leptomeric fibril (can be several μm long and about 0.2–0.5 μm wide) is a cross-striated bundle of thin filaments. The periodicity of banding (i.e. distance between the dense lines or dense striae) is about 100–180 nm. The thickness of the filaments is about 4–6 nm. A collection of closely apposed, randomly orientated, leptomeric fibrils comprises a leptomeric complex.

Leptomeric structures have been seen in: (1) extra-fusal muscle fibres in the latissimus dorsi of the thrush (Ruska and Edwards, 1957); (2) extensor longus digitorum of the frog (Katz, 1961); (3) muscle spindle of man, rat and frog (Gruner, 1961; Karlsson and Andersson-Cedergren, 1968; Rumpelt and Schmalbruch, 1969; Ovalle, 1972; Foroglou and Winckler, 1973); (4) heart (Purkinje cells and/or ordinary myocardial cells) of pigeon, budgerigar, rabbit, hen, sheep, rat, mouse, dog and monkey (Caesar *et al.*, 1958; Theones and Ruska, 1960; Virágh, 1968; Virágh and Challice, 1969; Bogusch, 1975a; Ono *et al.*, 1978); (5) crop muscle of the cockroach (*Leucophaea maderae*) after severance of the recurrent nerve (Taylor, 1967); (6) ciliary muscle of the chick (Zenker and Krammer, 1967); (7) human extra-ocular muscle (Mukuno, 1966; Mair and Tomé, 1972); (8) myotendinous junction of the first dorsal interosseus muscle of human foot (Mair and Tomé, 1972); (9) diseased human skeletal muscle (Zebra body myopathy and Pompe's disease) (Lake and Wilson, 1975; DiMauro *et al.*, 1978); (10) cardiomyopathy (Fujita *et al.*, 1979); (11) human rhabdomyomas and rhabdomyosarcomas (Fenoglio *et al.*, 1976; Silverman *et al.*, 1978; Carstens and Martin, 1986; Duyvené de Wit, 1986).

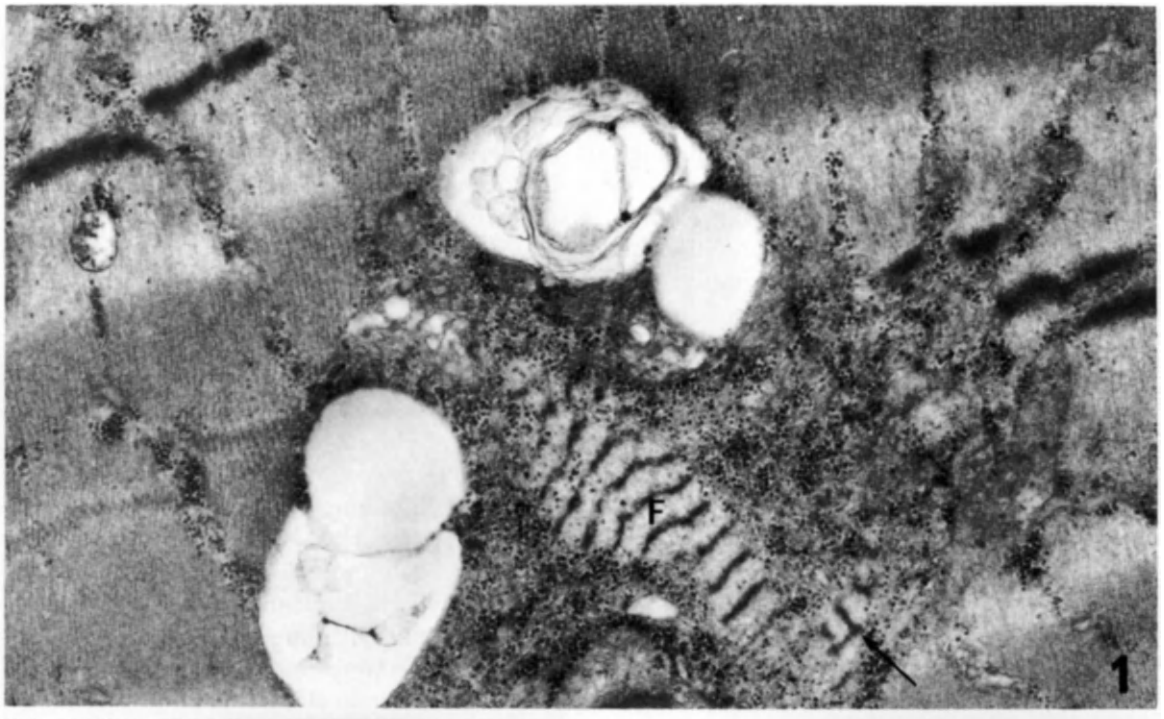
Leptomeric fibrils have been regarded as 'myoid type' structures (i.e. composed of myofilaments – presumably thin actin filaments) or as intracellular examples of fibrous long-spacing collagen (Taylor, 1967). Although the 'dense lines' or 'dense striae' of leptomeric fibrils morphologically resemble the Z-lines of striated muscle, they are chemically different (Bogusch, 1975b). There is no consensus about their function either. Katz (1961) thinks that they are 'microtendons', Karlsson and Andersson-Cedergren (1968) believe that they are 'shock absorbers', while Virágh and Challice (1969) suggest that they provide mechanical rigidity. In the mouse myocardium leptomeric fibrils are increased in number by anoxia (Ono *et al.*, 1978) and reduced in number by adriamycin (Payne, 1982).

Plate 426

Fig. 1. A leptomeric fibril (F) found in a dystrophic human skeletal muscle. A small portion of probably another leptomeric fibril (arrow) is also seen. ×25 000

Fig. 2. A banded structure is seen in a cell found in a pellet prepared from a pulmonary lavage obtained from a rat one day after intratracheal instillation of a solution of neutralized uranium acetate. The cell could not be confidently identified but it had a couple of structures resembling the secretory granules of type II pneumocytes. ×80 000

Fig. 3. A banded structure found in an unidentified necrotic cell. From same specimen as *Fig. 2*. The dark area at the top right of the figure is part of an erythrocyte. ×94 000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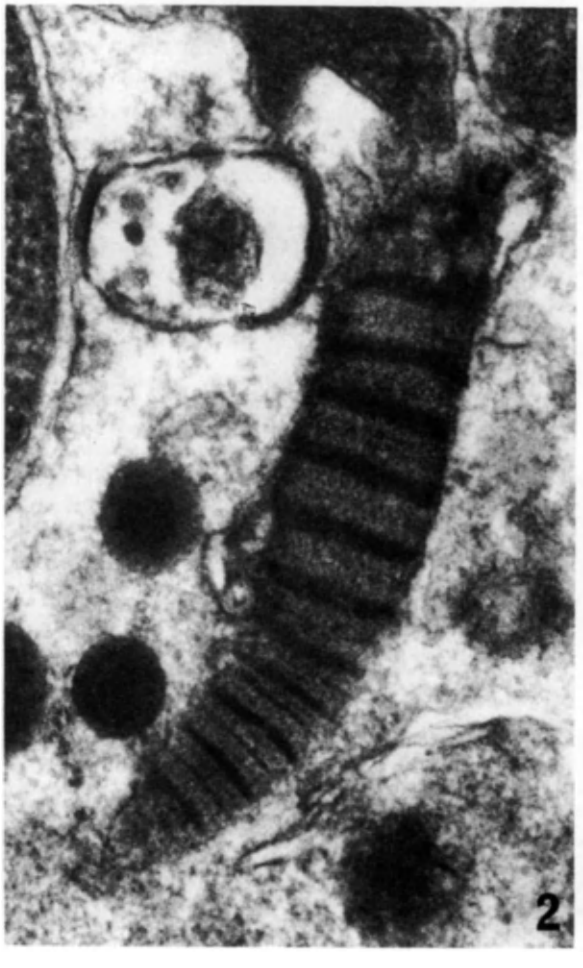
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Structures variously referred to as 'cross-striated fibrils', 'fibrous banded structures' and 'laminated cytoplasmic inclusions' have been found in: (1) lateral geniculate body neurons of healthy cats (Morales *et al.*, 1964); (2) sensory epithelium of macula of patients with Menière's disease (Hilding and House, 1964; Friedmann *et al.*, 1965); (3) human utricle in a patient with neuroma of the eighth nerve (Hilding and House, 1965); (4) ependymal cells of rat brain (Brightman and Palay, 1963); (5) hepatocytes from cases of chronic aggressive hepatitis (Groniowski and Walski, 1975); (6) human spermatocytes (Nagano, 1962); (7) podocytes of renal glomeruli of rats treated with daunomycin (Sternberg, 1970); (8) embryo chick ganglion cells incubated with colchicine or vinblastine (Thyberg and Hinek, 1977); (9) cells from pulmonary washings after administration of neutralized uranium acetate (Ghadially, Lalonde and Harley, unpublished observation) (*Plate 426*); and (10) several unicellular organisms, including various species of *Giardia (Plate 427)* (Cheissin, 1964; Friend, 1966; Kulda and Nohýnková, 1978; Barlough, 1979; Nemanic *et al.*, 1979; Sheffield, 1979).

The reported periodicity of the laminated structures in this group ranges from about 90–160 nm. The laminated bodies found in the neurons by Morales *et al.* (1964) can reach up to 5 μm in size. However, they can be seen with the light microscope only in osmicated specimens. The dense bands in these bodies contain microtubules about 25 nm in diameter which were probably continuous with the endoplasmic reticulum. This situation is difficult to evaluate. The reported diameter of these structures is that of microtubules, but their osmiophilia and probable continuity with the endoplasmic reticulum suggests that they are membrane-bound tubules (*see* page 938 for the differences between tubules and microtubules).

In the case of the striated bodies in the ependyma of rat brain Brightman and Palay (1963) have shown that they are the basal feet* (one foot per cilium) of cilia. Sternberg (1970) believes that the banded structures found by him in podocytes are contractile and contain myofibrils. The banded structures seen in ganglion cells after administration of antimicrotubular agents† (Thyberg and Hinek, 1977) and those found in the cirrhotic liver (Groniowski and Walski, 1975) are thought to be collagenous in nature, some of them bear a remarkable resemblance to fibrous long-spacing collagen. However, in most instances there is much doubt and speculation about the nature of these intracytoplasmic banded structures.

As stated at the beginning of this section, we are dealing here with a heterogeneous collection of 'inclusions', which have aroused much speculation but about whose nature and significance we have yet much to learn.

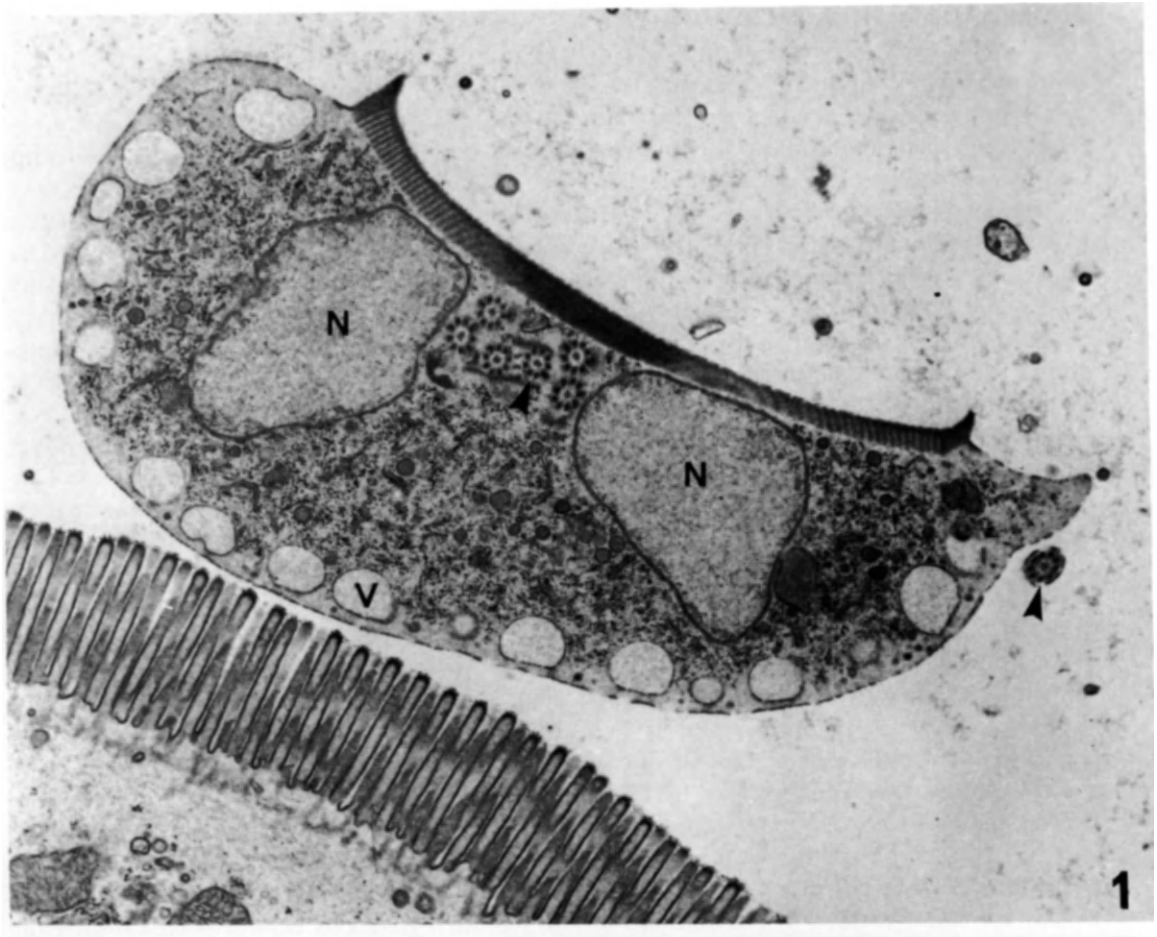
*A note on the nomenclature used here is essential. Filaments extend laterally and basally from the basal body of the cilium. In some sites and species an aggregate of lateral filaments forms a small structure referred to as a 'spur'; in others a more prominent striated structure called the 'basal foot'. Filaments extending downwards from the basal body of the cilium often aggregate to form a rootlet (*see Plate 497*). In some species and in some sites the rootlet has a cross-striated appearance. Some authors do not make such distinctions. They call both the lateral and basal filamentous appendages 'rootlets', while others refer to the filaments themselves as 'rootlets'.

†Secretion of procollagen is impeded by antitubulin drugs such as colchicine and vinblastine. In such a situation collagenous fibrils may form within the cell (*see* page 1000 for more details).

Plate 427

A detached *Giardia* found in the lumen of the gut of a rat.

Figs. 1 and 2. In the cytoplasm adjacent to the ventral surface of the parasite (but upper surface in the illustration) is seen a fibrous banded structure (periodicity about 90 nm) called the 'adhesive disc' or 'striated disc'. This is composed of a single layer of microtubules (not seen in these electron micrographs because of unfavourable plane of sectioning) from which arise regularly spaced electron-dense bands or ribbons (arrows). Note also the nuclei (N), transversely cut flagella (arrowheads), the vacuoles (V) in the dorsal cytoplasm (characteristic of the genus *Giardia*) and the absence of mitochondria. ×15 500; ×61 000



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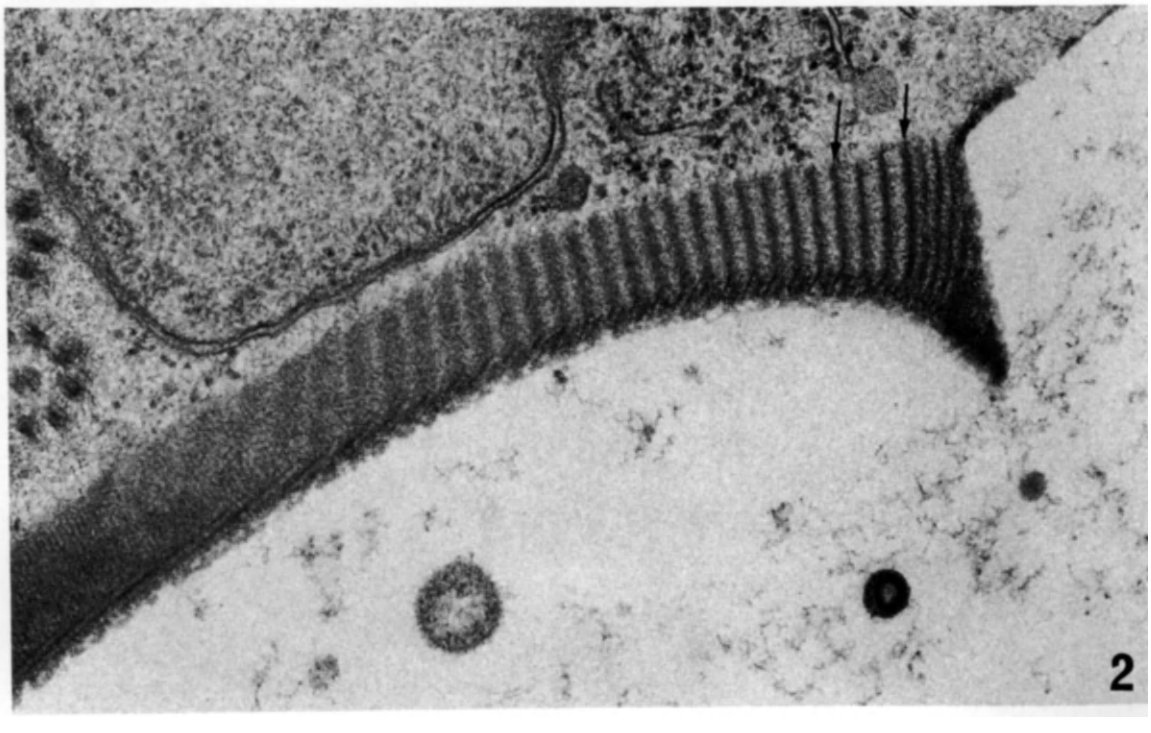


2

Intracytoplasmic desmosomes

One of the ways in which intracytoplasmic desmosomes may occur is when cells connected by desmosomes fuse to produce multinucleated giant cells or syncytial sheets. After fusion the intervening cell membranes suffer fragmentation and dissolution but the less vulnerable desmosomes tend to persist awhile and are hence seen lying free in the cytoplasm.*

Perhaps the best-known example of intracytoplasmic desmosomes is that known to occur in syncytiotrophoblasts which develop by fusion of cytotrophoblasts (Pierce *et al.*, 1964; Enders, 1965; Boyd and Hamilton, 1966; Okudaira and Strauss, 1967; Garancis *et al.*, 1970). After such fusion one may find: (1) fragments of cell membranes lying within the cytoplasm; (2) intrasyncytial clefts with desmosomes (i.e. paired membranes with accompanying desmosomes); (3) intracytoplasmic desmosomes; and (4) electron-dense rods (thought to be derived by degradation of desmosomes).

In primary cultures of human amnion cells infected with varicella zoster virus (DNA virus of herpes type), giant cells (polykaryocytes) containing intracytoplasmic desmosomes (*Plate 428*), were found by Cook and Stevens (1970). These authors postulate the same mechanism for the formation of intracytoplasmic desmosomes as mentioned earlier, but add the interesting observation that 'the formation of polykaryocytes and 'cytoplasmic desmosomes' may be mediated by the exportation of hydrolytic enzymes since we have seen acid phosphate positive deposits between the plasma membranes of adjacent cells in infected cultures'.

Multinucleate cells produced by repeated division of the nucleus without cell division can hardly be expected to contain intracytoplasmic desmosomes. Hence when intracytoplasmic desmosomes are found in a multinucleate cell this argues strongly in favour of the idea that such a giant cell has resulted from a fusion of cells. However, not all multinucleate cells produced by fusion can be expected to show intracytoplasmic desmosomes, for, clearly, desmosomes linking the cells have to be present in the first instance. An example of this is seen in developing muscle at the stage when myotubes are formed by fusion of myoblasts. Attachment plaques (described as close junctions) develop between the adjacent cell membranes of the myoblasts, which are about to fuse (Mendell *et al.*, 1972), but such junctions do not persist within the cytoplasm of the multinucleate myotube which develops. Cell junctions other than desmosomes have not been convincingly demonstrated in an intracytoplasmic position†. One would imagine that they are far more vulnerable than desmosomes.

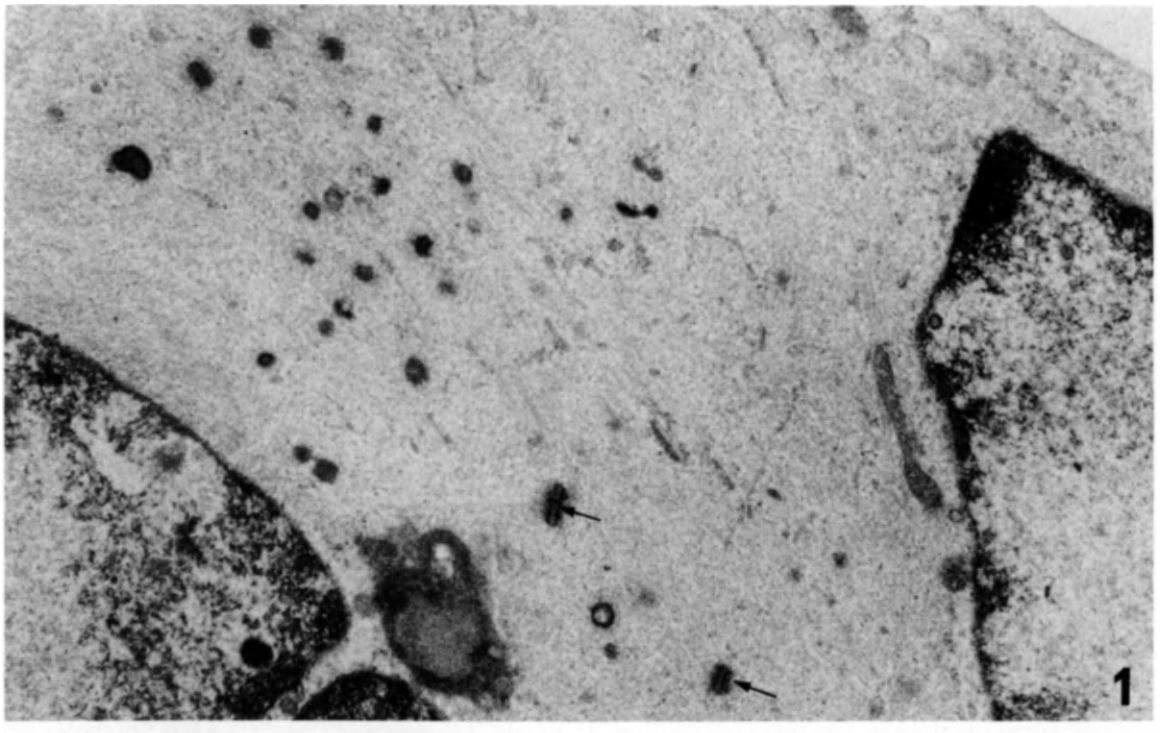
*Although the term 'intracytoplasmic desmosome' is convenient and describes the situation adequately, it should be remembered that a major part of even the normal desmosome (i.e. dense plaques and filaments) is intracellular.

†Leach and Oliphant (1984) describe gap junctions within the cytoplasm of horse hoof epidermal cells. The illustrations are not 100 per cent convincing, nor was it clearly shown that the annular gap junctions were not still connected to the cell membrane; however, the illustrations are certainly suggestive.

Plate 428

Fig. 1. Intracytoplasmic desmosomes (arrows) found in a varicella zoster virus-infected human amnion cell in culture. The cell appears to have at least two nuclei. Some of the intracytoplasmic desmosomes lie in the intervening cytoplasm. ×21 000 (*From Cook and Stevens, 1970*)

Figs. 2 and 3. High-power view of intracytoplasmic desmosomes. The dense plaques and radiating filaments characteristic of this type of junction are clearly visualized. ×98000; ×98000 (*From Cook and Stevens, 1970*)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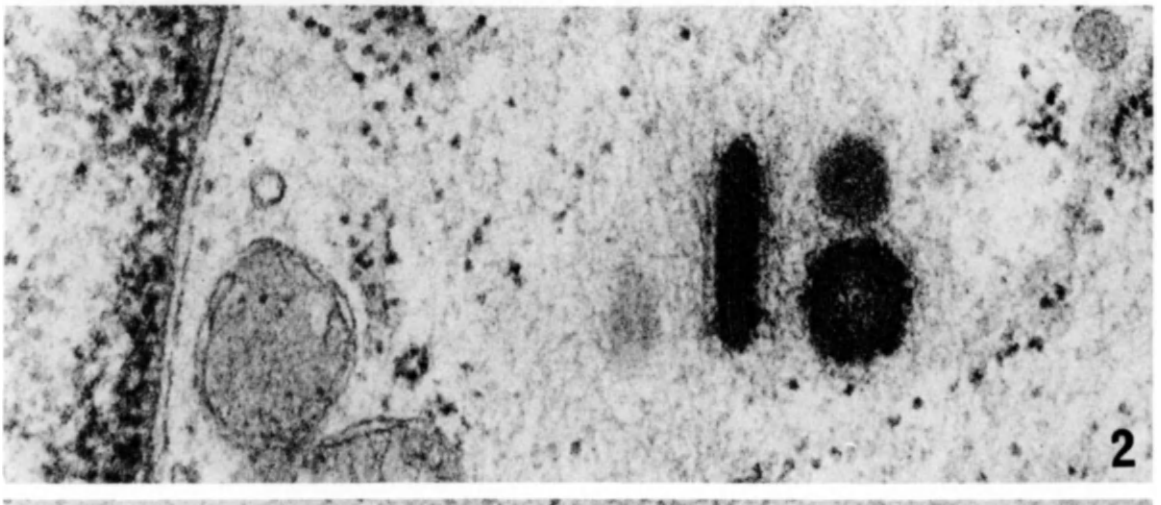
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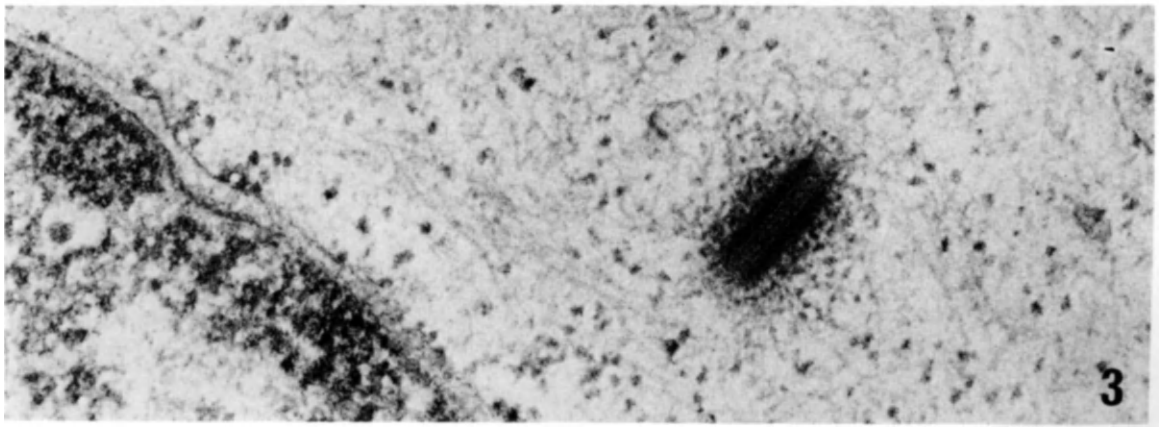
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The occurrence of intracytoplasmic desmosomes does not always indicate cell fusion, for this phenomenon is not restricted to multinucleate cells. For example, intracytoplasmic desmosomes have been seen in: (1) keratinocytes of stripped epidermis (Mishima and Pinkus, 1968); (2) dyskeratotic cells in contact dermatitis (Kobayasi and Asboe-Hansen, 1974); (3) dyskeratotic cells in graft versus host reaction (De Dobbeleer *et al.*, 1975); (4) dyskeratotic cells in erythematous fixed drug eruption (De Dobbeleer and Achten, 1977); (5) dyskeratotic cells in Bowen's disease (Seiji and Mizuno, 1969); (6) keratinocytes in Paget's disease (Ishibashi *et al.*, 1972); (7) cells of squamous cell carcinoma (Hirone and Eryu, 1970; Klingmüller *et al.*, 1970); (8) cells of keratoacanthoma (von Bülow and Klingmüller, 1971; Takaki *et al.*, 1971; Fisher *et al.*, 1972); (9) cells of Gottron's carcinoid papillomatosis (Caputo and Prandi, 1972); (10) cells of keratoma palmo-plantare (Klug and Haustein, 1974); (11) keratinocytes in malignant melanoma (Klug and Haustein, 1974); (12) cells of an anaplastic carcinoid of the oesophagus (Ghadially, 1980) (*Plate 429, Fig. 1*); (13) cells of a phaeochromocytoma (*Plate 429, Fig. 2*); (14) cells of nitrosamine-induced tumours in nasal olfactory region of the Syrian golden hamster (Reznik-Schüller, 1978).

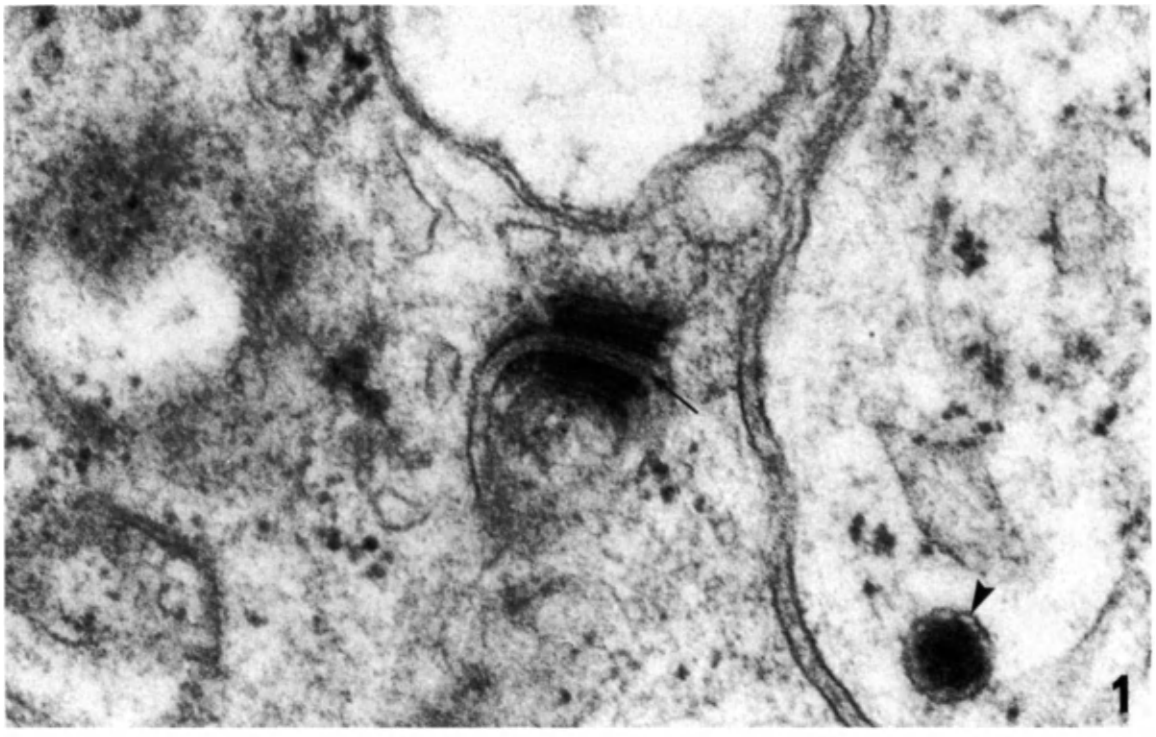
It would appear that intracytoplasmic desmosomes have been sighted more frequently in cutaneous keratinocytes (particularly dyskeratotic cells) than in any other cell type. This is not too surprising when one considers that desmosomes are best developed and most frequent in cutaneous squamous epithelium. Intracytoplasmic desmosomes have been seen more often in a variety of epithelial tumours than in any other pathological state.

The collective evidence seems to suggest that when cells connected by desmosomes break away from each other during mitosis (particularly neoplastic proliferation of cells) they are likely to incorporate desmosomes in their cytoplasm. However, the exact mechanism by which this occurs is not known.

Plate 429

Fig. 1. An intracytoplasmic desmosome found in a secondary deposit of an anaplastic carcinoid of the oesophagus in a lymph node. The intermediate line (arrow) is just discernible. Note also the neuroendocrine granule (arrowhead). ×89000 (*From Ghadially, 1980*)

Fig. 2. Intracytoplasmic desmosomes found in a phaeochromocytoma (arrowheads). ×35000 (*From Ghadially, 1980*)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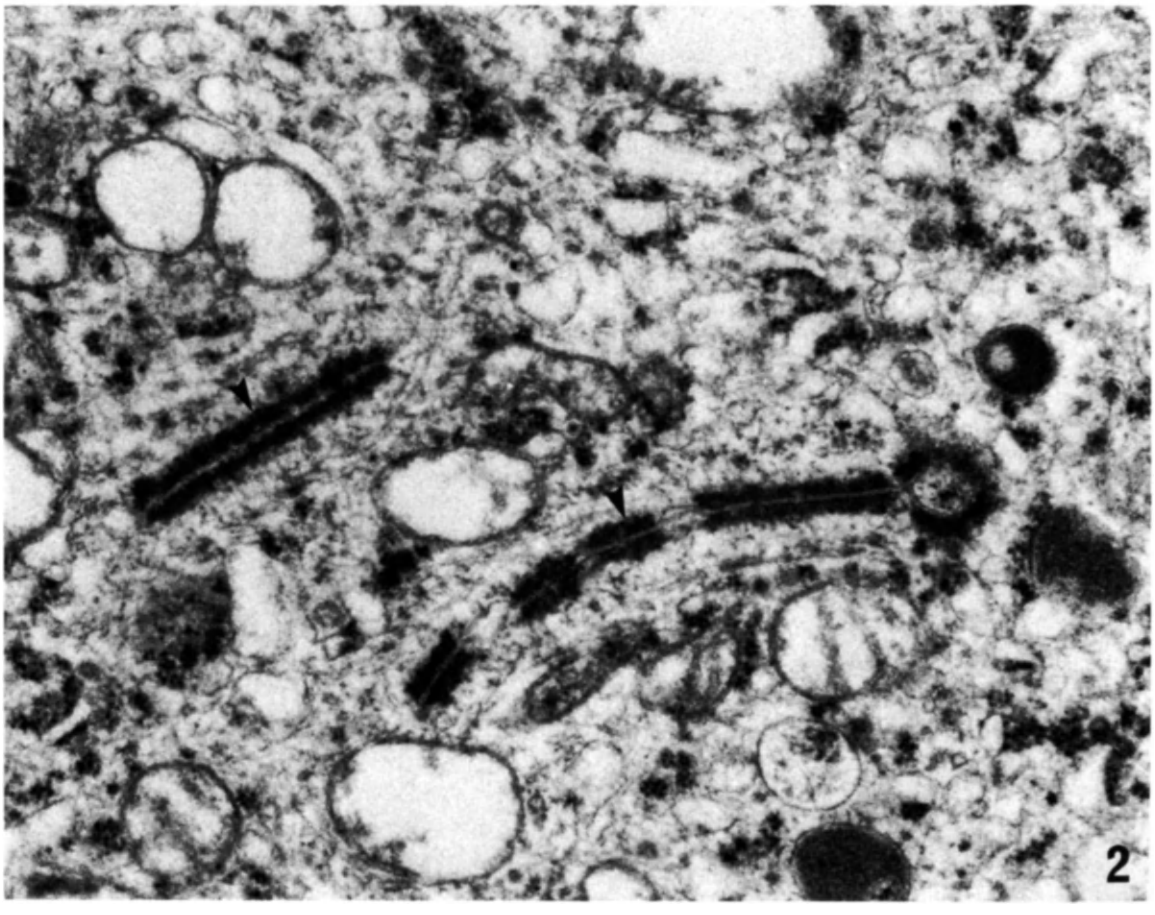
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Intracytoplasmic canaliculi and lumina

Canaliculi which transport secretory products (*Plate 430, Fig. 1*) lie as a rule between secretory cells and not within them. However at times such structures are found within the cell cytoplasm (*Plate 430, Fig 2*)*. In quite a few instances it is difficult to decide whether these intracellular structures are true canaliculi which conduct material to the exterior or isolated cystic spaces containing sequestrated secretory products. Hence various terms such as intracellular or intracytoplasmic 'canaliculi', 'lumina', 'microlumina', 'alveolus', 'spaces', 'cavities', 'cysts', 'microcysts', 'ductules' and 'microductules' have been used to describe them. In sectioned material these structures present as single membrane-bound cystic spaces bearing a few or many microvilli. At time such structures appear 'empty' and one is tempted to think that this is an invagination of the cell membranes bearing microvilli, while in other instances the structure is so distended with secretory material that one may be tempted to conclude that no communication with the exterior exists.

Perhaps the best known example of true intracellular canaliculi (i.e. communicating with the exterior) is found in gastric parietal cells. An extensive, elaborate intracellular canalicular system lined by numerous microvilli is a striking but normal feature of this cell type (*Plate 430, Fig. 3*). This is the only cell type in which the intracellular canaliculus is such a constant prominent feature, but an occasional intracytoplasmic canaliculus may be found in the cells of quite a few normal glands. Most reports, however, deal with such canaliculi or lumina in tumour cells.

Intracellular or intracytoplasmic caniculi or lumina (*Plates 430–434*) have been seen in: (1) hepatocytes in, (a) liver of goldfish, (b) fetal liver of rats and humans, and (c) pathological livers (for references *see* Koga, 1971); (2) pancreatic acinar cells (*Plate 430, Fig. 2*); (3) gastric parietal cells (*Plate 430, Fig. 3*); (4) gastric parietal cell carcinoma (Capella *et al.*, 1984); (5) adenocarcinoma of stomach (Kondo *et al.*, 1970; Nevalainen and Järvi, 1976); (6) adenocarcinoma of palatal salivary glands (Mohamed and Cherrick, 1975); (7) various types of carcinomas of the breast (Wellings and Roberts, 1963; Sykes *et al.*, 1968; Carter *et al.*, 1969; Goldenberg *et al.*, 1969; Csuka and Sugár, 1971; Ozzello, 1971; Erlandson and Carstens, 1972; Tobon and Price, 1972; Ahmed, 1974; Gould *et al.*, 1975; Spriggs and Jerrome, 1975; Harris and Ahmed, 1977; Harris *et al.*, 1978; Sobrinho-Simões *et al.*, 1981) (*Plate 431*); (8) adenocarcinoma and adenosquamous carcinoma of lung (Spriggs and Jerrome, 1975; Akagi and Kimoto, 1976; McDowell *et al.*, 1978; Dingemans, 1983b) (*Plate 432, Figs. 1 and 2*); (9) adenocarcinoma of

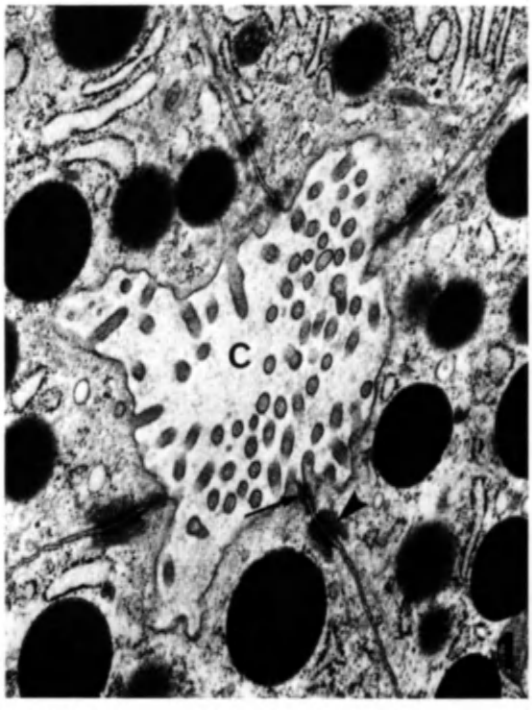
*Intracellular canaliculi are distinguishable from intercellular canaliculi by the absence of junctions around the former and the presence of junctions around the latter (*Plate 430* and *Plate 432, Fig. 2*).

Plate 430

Fig. 1. An intercellular canaliculus (C) bound by pancreatic acinar cells, the borders of which are connected by terminal bars: each comprising a tight junction (arrow) and a desmosome (arrowhead). From a case of insulinoma. There was no detectable abnormality of the exocrine pancreas. ×21 000

Fig. 2. Intracytoplasmic canaliculus (between arrowheads) in a pancreatic acinar cell. Note the microvilli protruding into the lumen. Same case as *Fig. 1* ×28 000

Fig. 3. Normal human gastric parietal cell showing intracytoplasmic canaliculi (C). ×13 500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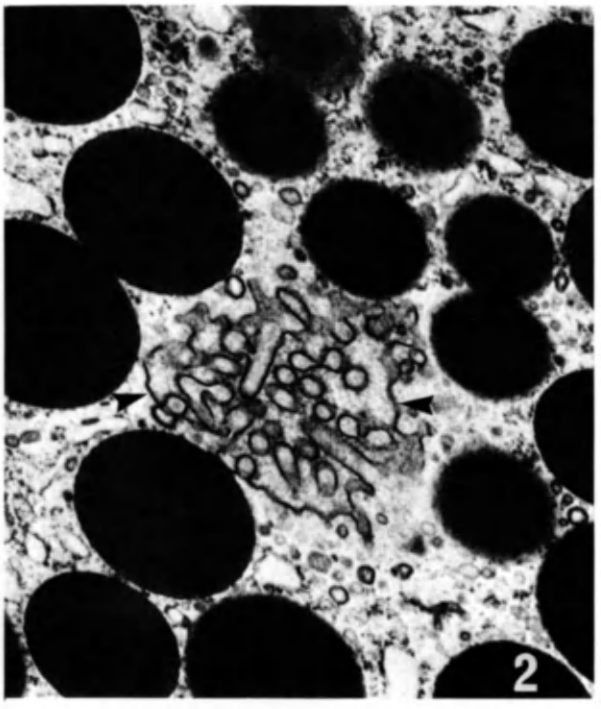
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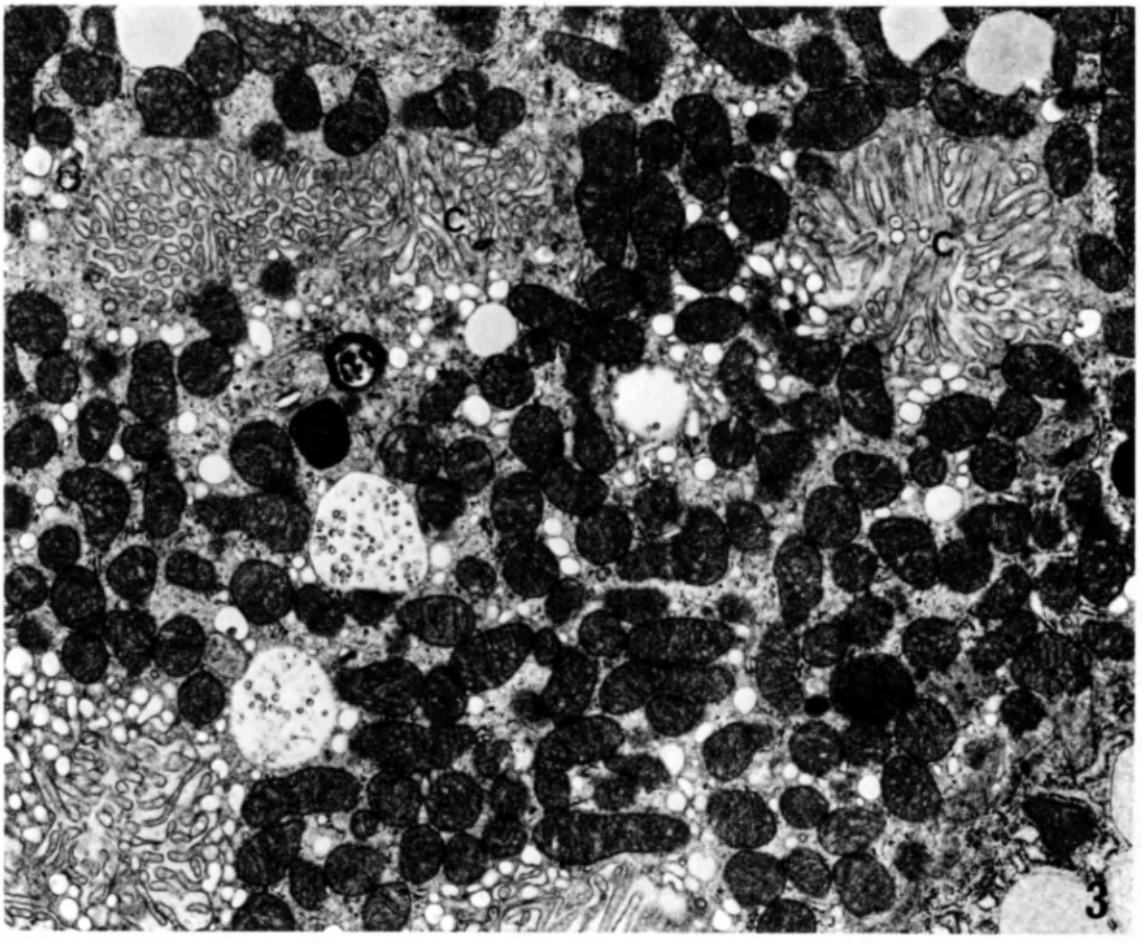
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prostate (Ghadially, unpublished observation); (10) mucus secreting elements in ameloblastoma (mucoepidermoid ameloblastoma) (Mincer and McGinnis, 1972); (11) functioning beta cell tumours (insulinomas) of the pancreatic islets (Suzuki and Matsuyama, 1971); (12) cells of the corpus luteum of term pregnancy (Crisp *et al.*, 1973); (13) clear cell adenocarcinoma of the female genital tract (Roth, 1974); (14) fallopian tube carcinoma (Johnson *et al.*, 1978); (15) ovarian adenocarcinomas (Spriggs and Jerrome, 1975; Johannessen, 1978) (*Plate 432, Fig. 3*); (16) normal and hyperplastic breast epithelium (Carter *et al.*, 1969; Kern and Dermer, 1972); (17) epithelial cells of fibroadenoma (Archer and Omar, 1969); (18) *in vitro* cultures of breast carcinoma cells (Ozello, 1972); (19) cells of the thyroid follicle (*in vivo* and *in vitro*, normal and pathological, embryonic and adult, of various species; for references *see* Remy *et al.*, 1977a, b; Ericson, 1979); (20) thyroid carcinomas (Valenta *et al.*, 1974; Johannessen *et al.*, 1978; Gould *et al.*, 1981); (21) oncocytic thyroid carcinomas (Valenta *et al.*, 1974; Johannessen *et al.*, 1978) (*Plate 433, Fig. 1*); (22) a visceral epithelial cell of human glomerulus in a case of AIDS (Stanley, personal communication) (*Plate 433, Fig. 2*); (23) renal adenoma and carcinoma (human and experimentally produced tumours in rats) (Cooper and Waisman, 1973; Tannenbaum, 1971; Pratt-Thomas *et al.*, 1973; Johannessen *et al.*, 1980; (24) urinary bladder epithelium (Alroy *et al.*, 1979); (25) urinary bladder carcinoma (Alroy *et al.*, 1979); (26) cells of intra-epidermal eccrine duct in the embryo and in eccrine poroma (Hashimoto *et al.*, 1965; Hashimoto and Lever, 1969; Turner *et al.*, 1982); (27) spiradenoma (Castro and Winkelmann, 1974); (28) syringocystadenoma papilliferum (apocrine duct tumour) (Niizuma, 1976); (29) sweat gland carcinoma (Yeung and Stinson, 1977; Sobrinho-Simões *et al.*, 1981); (30) meibomian gland carcinoma (Sobrinho-Simões *et al.*, 1981); (31) experimentally produced adenocarcinoma of the glandular stomach of rats (Hananouchi *et al.*, 1974); (32) diarrhoeogenic tumours of the pancreas (Verner-Morrison syndrome) (Greider *et al.*, 1974); (33) primary ovarian carcinoid tumours (Sforza *et al.*, 1984); (34) phaeochromocytoma (Oliva and Usera, 1974); (35) canine aortic body tumour (Cheville, 1972); (36) mesotheliomas (Wang, 1973; Legrand, 1974; Legrand and Pariente, 1974; Suzuki *et al.*, 1976) (*Plate 433, Fig. 3*); (37) adenomatoid tumours of the genital tract (ultrastructural studies support the concept of a mesothelial origin) (Taxy *et al.*, 1974; Nistal *et al.*, 1978); (38) meningioma (Kepes, 1975); (39) metastatic melanoma (Yum *et al.*, 1984;

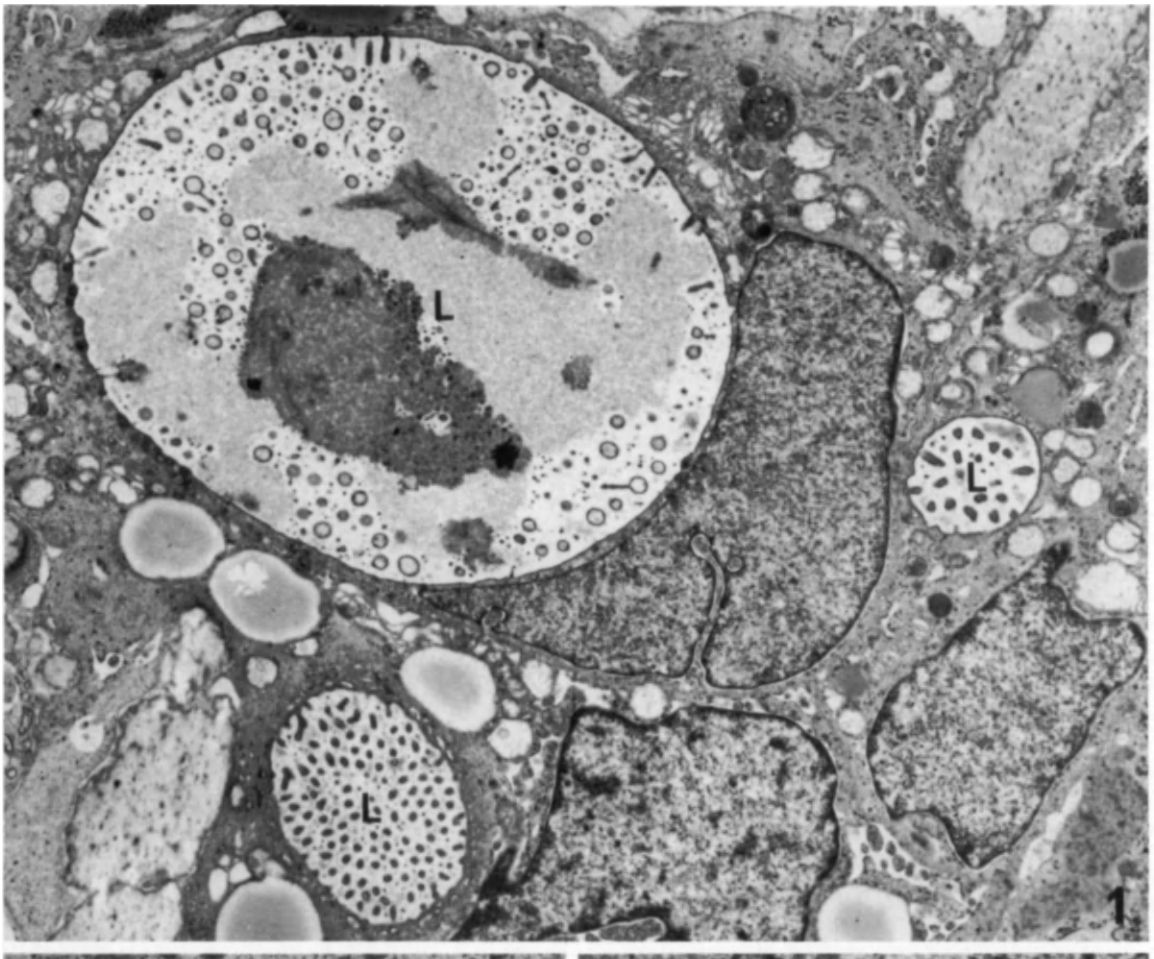
Plate 431

Intracytoplasmic lumina in breast carcinoma (*From Ghadially, 1985*)

Fig. 1. Infiltrating duct carcinoma of female breast. Three intracytoplasmic lumina (L) with microvilli cut in various planes. The largest contains material acceptable as secretory product and debris derived from swelling and breakdown of microvilli. ×7700

Fig. 2. Intraduct papilloma of female breast. This illustration shows continuity between an intercellular lumen (A) and an intracellular lumen (B). The microvilli in the intracellular lumen are more prominent than those in the intercellular lumen. ×18000

Fig. 3. Carcinoma of male breast. A cell containing an intracytoplasmic lumen. ×10500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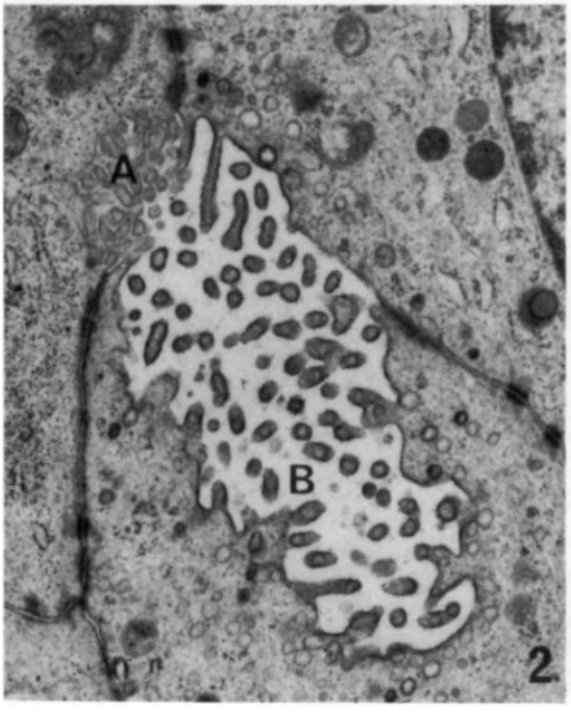
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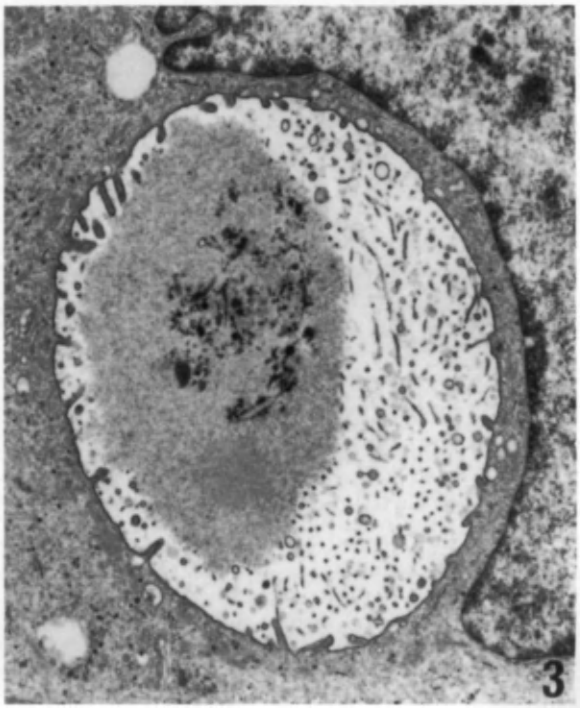
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Stanley, personal communication, including electron micrographs); (40) thymic corpuscles of the guinea pig and mouse (Kohnen and Weiss, 1964); (41) nasal mucosa of rat (Stockinger, 1963); (42) morphologically normal, and metaplastic and dysplastic nasal mucosa of nickel workers (Boysen and Reith, 1980)*; and (43) malignant fibrous histiocytoma (*Plate 433, Fig. 4*).

It will be noted from the list presented above that intracytoplasmic lumina have been seen quite frequently in various types of breast carcinoma. Hence it was thought that intracellular lumina were pathognomonic of breast carcinoma or diagnostic of breast carcinoma. The demonstration of such lumina in normal and hyperplastic breast epithelium shows that this is not strictly speaking correct, however, the presence of large numbers of such lumina would in the case of a breast tumour support the diagnosis of carcinoma for such structures are rare in normal or hyperplastic breast tissue and adenoma. In this connection it is also worth noting that Erlandson and Carstens (1972) found that such lumina were more abundant in an anaplastic than in a well-differentiated carcinoma.

The idea that intracellular lumina, if found in a tumour biopsy, would be diagnostic of adenocarcinoma is also of course not strictly speaking correct for such lumina have on rare occasions been seen in several varieties of tumours. However, the probability of finding intracytoplasmic lumina is infinitely greater in adenocarcinoma than other tumours such as melanoma or malignant fibrous histiocytoma. Further, the lumina in adenocarcinoma often contain quite characteristic secretory material. Thus an intelligent analysis of lumina combined with other ultrastructural features (and histological analysis to narrow down the diagnosis) often permits one to arrive at a diagnosis of adenocarcinoma.

The diagnostic electron microscopist should be cognizant of another situation which can lead to an erroneous diagnosis, namely the trapping or accidental inclusion of non-neoplastic adenomatous cells bearing lumina in a tumour. This is especially true of pulmonary neoplasms, where type II alveolar cells trapped in a squamous cell carcinoma can lead to an erroneous diagnosis of adenocarcinoma (Dingemans, 1983). McDowell *et al.* (1978) have shown that cells

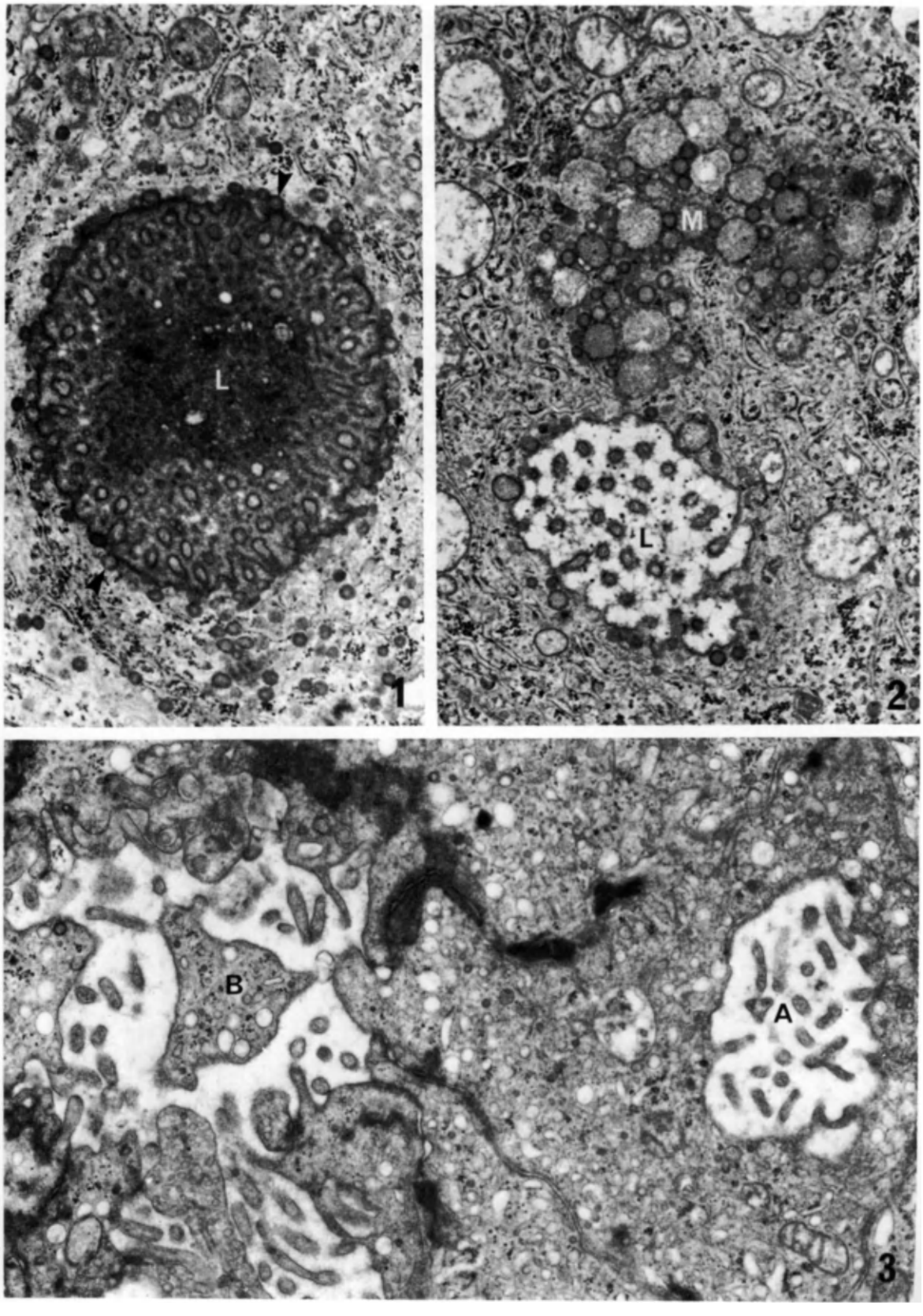
*Two types of intracytoplasmic lumina were present. One type contained microvilli, and the other contained microvilli and cilia. Intracytoplasmic lumina containing cilia were also seen in rat nasal mucosa (item 41).

Plate 432

Fig. 1. Poorly differentiated adenosquamous carcinoma of the lung that was diagnosed as a squamous cell carcinoma by light microscopy. Numerous quite electron-dense mucous granules (arrowheads) appear to be discharging into an intracytoplasmic lumen (L) with dense contents. Note also the numerous microvilli cut in various planes. ×18 500 (*From Ghadially, 1980*)

Fig. 2. Same tissue as *Fig. 1* showing an intracytoplasmic lumen (L) which contains little besides transversely sectioned microvilli. A collection of pleomorphic mucous droplets (M) is seen in the cell cytoplasm. ×19 000 (*From Ghadially, 1980*)

Fig. 3. Poorly differentiated mucinous cystadenocarcinoma of the ovary showing an intracytoplasmic lumen (A) with associated microvilli and an intercellular lumen containing a cell projection or fragment (B) and microvilli. The main feature which distinguishes this intercellular lumen from the intracellular lumen is the presence of modified junctions seen in association with the former. ×21 000 (*From Ghadially, 1980*)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containing lumina can be found in some lung tumours with areas of squamous differentiation. As these authors point out, these are adenosquamous carcinomas which show both epidermoid and adenomatous differentiation and not just squamous cell carcinomas.

The diagnostic significance of intracytoplasmic lumina has been assessed by Sobrinho-Simões *et al.* (1981). These authors studied 61 consecutive unselected metastatic neoplasms in the lymph nodes and in other sites where the light microscopic diagnosis was 'metastatic tumour of unknown primary site'. They also 'studied 52 cases of primary or metastatic melanoma, 27 cases of primary or metastatic squamous cell carcinoma and 71 cases of lymphoma to see if intracytoplasmic lumina were present in these neoplasms'. These authors conclude that if intracytoplasmic lumina are present in a tumour biopsy, one may rule out squamous cell carcinoma, melanoma* and lymphoma because not a single intracytoplasmic lumen was found in these tumours. They state that if intracytoplasmic lumina are present in a metastatic carcinoma, then 'the breast is the most likely site of the primary tumour but that the clinical context may, and often does, modify the validity of this criterion'. They found intracytoplasmic lumina in adenocarcinomas of sweat and meibomian glands and hence warn pathologists not to jump to the conclusion that a secondary carcinoma of the breast is present when intracytoplasmic lumina are found in a tumour of the skin.

Thus one may generalize by saying that there are two principal diagnostic implications of intracytoplasmic lumina. If several intracytoplasmic lumina are found: (1) in a biopsy of a breast lesion, then the lesion is most likely malignant; and (2) in a metastatic tumour of unknown origin, the most likely site of the primary would be breast.

*There is no doubt that melanomas as a rule do not contain intracytoplasmic lumina. I have not seen one, nor did Mazur and Katzenstein (1980) find any in the 26 cases of metastatic melanoma they studied. In fact I am aware of only two cases (item 39) where intracytoplasmic lumina have been seen in melanoma. Therefore while one cannot completely exclude melanoma if intracytoplasmic lumina are sighted, it does make this an unlikely diagnosis.

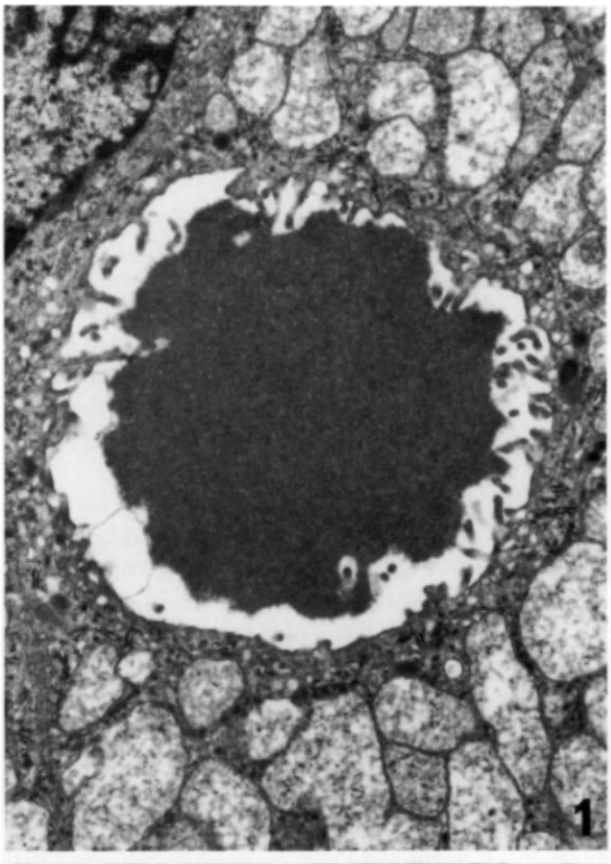
Plate 433

Fig. 1. Malignant oncocytoma of thyroid gland with secondary deposits in the liver. This section from a secondary deposit shows an intracytoplasmic lumen containing a homogeneous electron-dense secretory product resembling the colloid seen in thyroid follicles. ×10 500

Fig. 2. Renal biopsy from a case of AIDS. Glomerular visceral epithelial cell containing an intracytoplasmic lumen. Note the microvilli and absence of secretory product. Appearances seen here (arrow) make one suspect that in another plane of sectioning continuity between lumen and the cell surface might be demonstrable. ×19 000 (*Dr T. Stanley, unpublished electron micrograph*)

Fig. 3. Mesothelioma. A cell containing an intracytoplasmic lumen. Note the rather long microvilli (arrows) and the prominent proteoglycan particles (arrowheads). These features are rather characteristic of mesothelioma. ×14 500

Fig. 4. Malignant fibrous histiocytoma. This tumour cell contains a fair amount of rough endoplasmic reticulum. Therefore, it is acceptable as a fibroblast-like cell. Three profiles of intracytoplasmic lumina are seen in this cell. They contain several microvilli but no secretory product. ×13 000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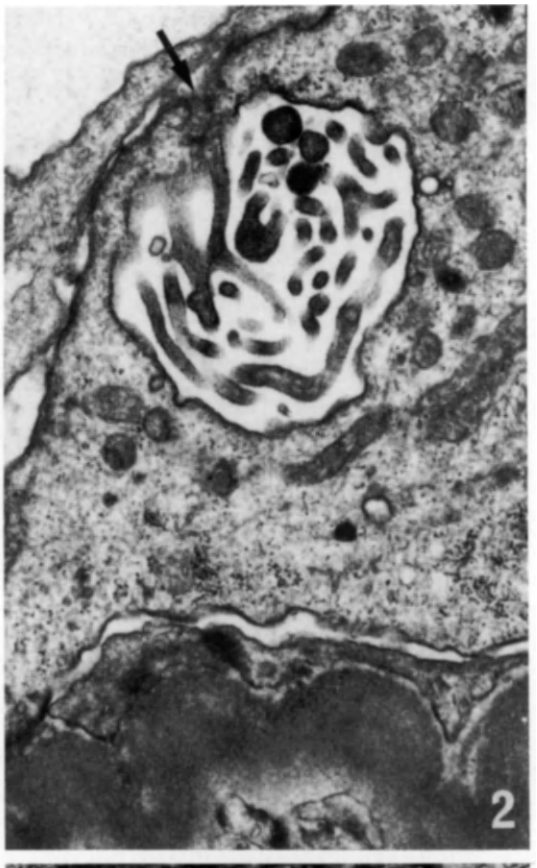
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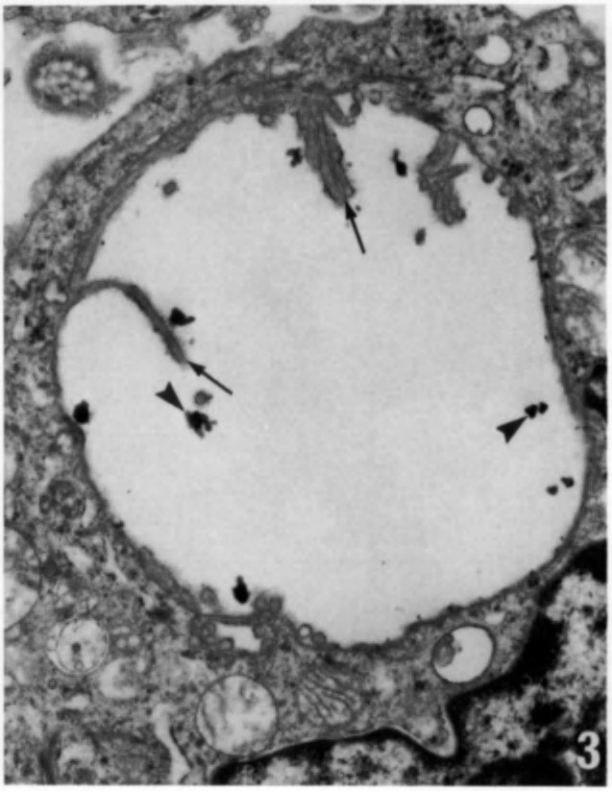
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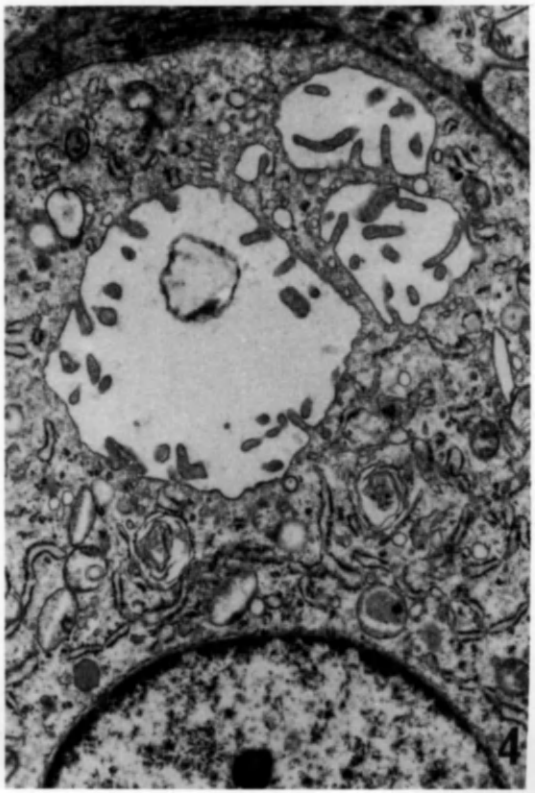

4

Various possibilities have to be considered regarding the nature and origin of intracellular canaliculi and lumina. In some situations these structures are clearly little more than simple or elaborate invaginations of microvilli-bearing cell surfaces; and the lumina of such canaliculi are on serial section found to be continuous with an intercellular canaliculus. It is possible that just as in the case of a pseudoinclusion in the nucleus (page 74), the invaginating membranes at the neck of the intracellular canaliculus may fuse, so that an intracellular cystic space or lumen lined by microvilli is produced.

It is also conceivable that an intracellular lumen loaded with secretory material may arise by conglomeration of mucous granules within the cell. In this connection it is worth noting that the signet-ring cell found in some adenocarcinomas comprises either a large collection of mucous granules displacing the nucleus to the periphery, or a large intracytoplasmic lumen distended by mucus. In thyroxin-treated rats, intracellular lumina develop in thyroid follicle cells and Ericson (1979) states 'Intracellular fusion of exocytotic vesicles appears to be the most likely mechanism of formation but the possibility of formation by invagination of the apical cell surface cannot be excluded'.

Yet another attractive possibility has been noted by Harris *et al.* (1978) who present a series of electron micrographs which suggest that at least some of these intracytoplasmic lumina derive from distended Golgi cisternae and/or vacuoles (*Plate 434*). Support for such a concept also comes from studies on porcine thyroid follicle cells in culture (Remy *et al.*, 1977a, b) where intracellular lumina were found to derive by modification of the Golgi zone. Later, two cells with such lumina were found to establish tight junction-like contacts between them and the migration and opening of the intracellular lumina into the closed intercellular space between the cell membranes led to the formation of an intercellular lumen.

Some of the intracytoplasmic lumina illustrated in the literature are seen to be surrounded by intracytoplasmic filaments. Their concentric arrangement suggests that as these lumina expand in size a rearrangement of the intracytoplasmic filaments in the cell cytoplasm occurs. However, such filaments are not seen around all intracellular canaliculi and lumina. The final impression that one gets is that we are dealing with at least two distinct entities, an intracellular canaliculus or lumen which communicates with the exterior and another which does not. Since in routine sections it is often impossible to distinguish between these two alternatives, it seems advisable, at least in most instances, to use the non-committal term 'intracytoplasmic lumina', rather than 'intracytoplasmic canaliculi' which suggests intracellular canals conducting secretory products to the exterior.

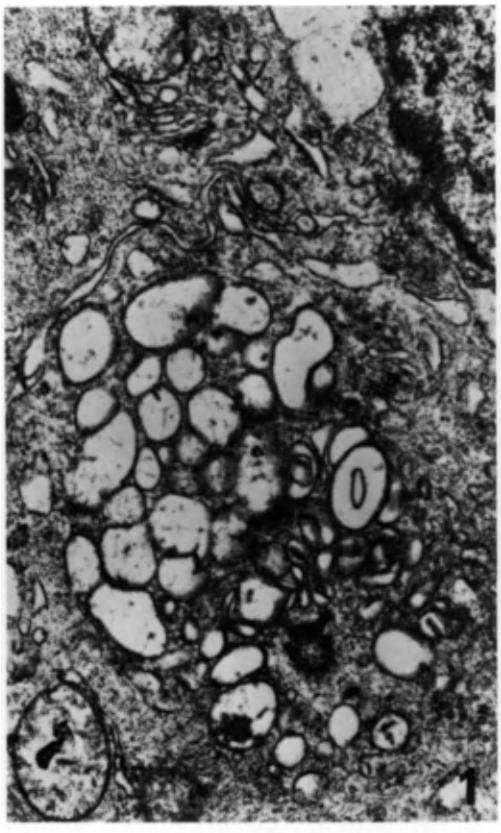
Plate 434

Infiltrating lobular carcinoma of the breast. This set of electron micrographs depicts the probable sequence of events leading to the formation of an intracytoplasmic lumen from dilated Golgi elements. Centrioles are frequently seen in close association with the Golgi complex in many cell types. It is therefore interesting to note the presence of centrioles (arrows) in *Figs. 1,2 and 4.* (*From Harris, Vasudev, Anfield and Wells, 1978*)

Fig. 1. Seen here are vacuoles probably derived by dilatation of elements of the Golgi complex. ×28 000

Figs. 2 and 3. Further stages of dilatation and coalescence of elements of the Golgi complex could produce the appearances seen here. ×15 000; ×31 000

Fig. 4. An intracytoplasmic lumen containing microvilli and mucin. ×16 000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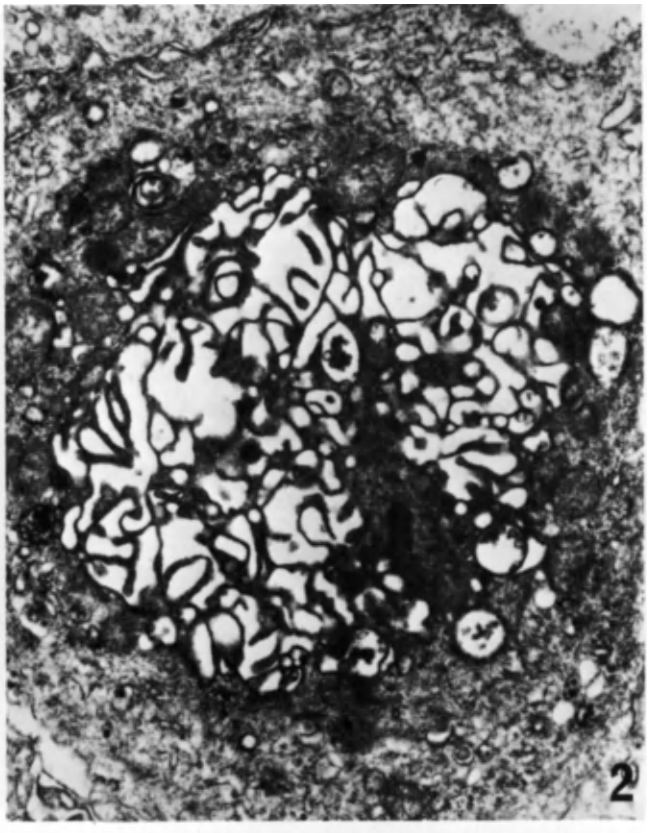
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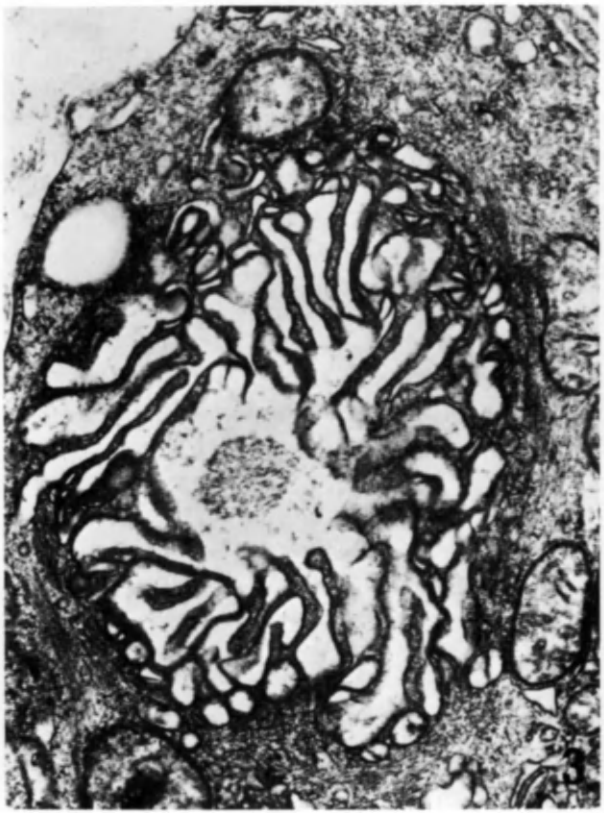
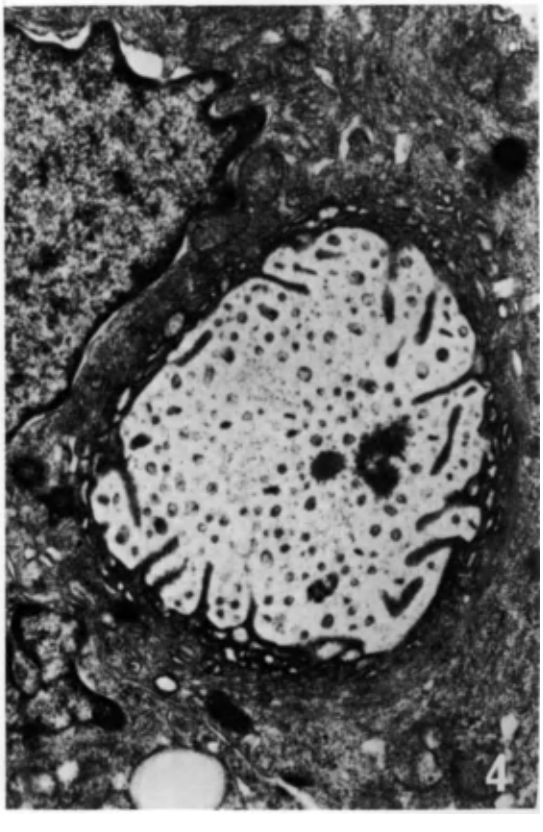



4

Intracytoplasmic nucleolus-like bodies (nematosome, nuage, dense body, honeycomb body, ribosomal body)

As their name implies, nucleolus-like bodies morphologically resemble nucleoli, the main difference being that while the nucleolus resides in the nucleus, nucleolus-like bodies occur as non-membrane-bound structures* in the cytoplasmic matrix. It will be recalled (page 56) that the nucleolus occurs in two principal forms: (1) the open nucleolus where the thread-like nucleolonema is spread out or opened out, and hence is easily visualized; and (2) the compact nucleolus where the nucleolonema is not visualized (presumably because it is compacted) or perhaps truly absent. However, in the compact nucleolus one does at times see a 'hole' or 'holes' (i.e. non-membrane-bound clear spaces containing nuclear matrix) presumably reflecting less than total compacting of the nucleolonema.

As one would expect, nucleolus-like bodies also come in two principal forms, and in analogy with the nucleolus I will refer to them as: (1) 'open nucleolus-like bodies'; and (2) 'compact nucleolus-like bodies'.† This simplifies the task of understanding and describing these bodies which have been called by at least a dozen different names (some good, most inept) in the literature. One such quite good term is 'nematosome' which has quite rightly been applied to bodies which resemble the open nucleolus, but unfortunately it has been applied often enough to bodies where no 'nema' (thread) is visible and which more resemble the compact nucleolus. Thus the value of this term is now debased. Other more or less commonly used terms include 'nuage' (Fr: cloud), 'glomerular body', 'honeycomb body', 'dense body', 'nuclear emission', 'nucleolar emission', fibrillo-granular body', and 'granulo-fibrillar body'. Most of these terms (e.g. 'dense body', 'glomerular body', 'honeycomb body') are non-specific and confusing because they have been used to describe several unrelated structures. The remainder have been used sometimes but not always to describe rounded or irregular shaped structures resembling the compact nucleolus.

The term 'ribosomal body' has been used (only on very few occasions) by those who believe that some of these bodies are composed of aggregates of cytoplasmic ribosomes. This, of course, is a possibility, but there is little to support such a concept. There is, however, much evidence supporting the idea that these bodies derive from the nucleolus (see below).

Open nucleolus-like bodies (usually about 0.3–1.5 μm in diameter but can be much larger) have been seen in: (1) oocytes of dragonfly (Halkka and Halkka, 1975, 1977); (2) ectodermal and mesodermal cells of rat embryo (Takeuchi, 1980); (3) pollen of *Campanulae* (Dunbar, 1973); (4) syncytial trophoblasts in placenta of mouse (Enders, 1965; Toro and Rohlich, 1966; Björkman, 1970; Hernandez-Verdun and Legrand, 1971; Hernandez-Verdun, 1972; Hernandez-Verdun and Bouteille, 1976; Ockleford *et al.*, 1987); (5) cells of middle trophoblastic layer of several species of myomorph rodents (King and Hastings, 1977); (6) syncytial trophoblasts in placenta of rat (Toro and Rohlich, 1966); (7) cytotrophoblasts in placenta of baboon (Wynn *et al.*, 1971); (8) cytotrophoblasts in placenta of humans (Martin and Spicer, 1973; Jones and Ockleford, 1985); (9) neurons in various ganglia, cerebral cortex, cerebellum and medial accessory olive of

*One hesitates to call these structures 'inclusions' because they may be organelles of as yet unknown function.

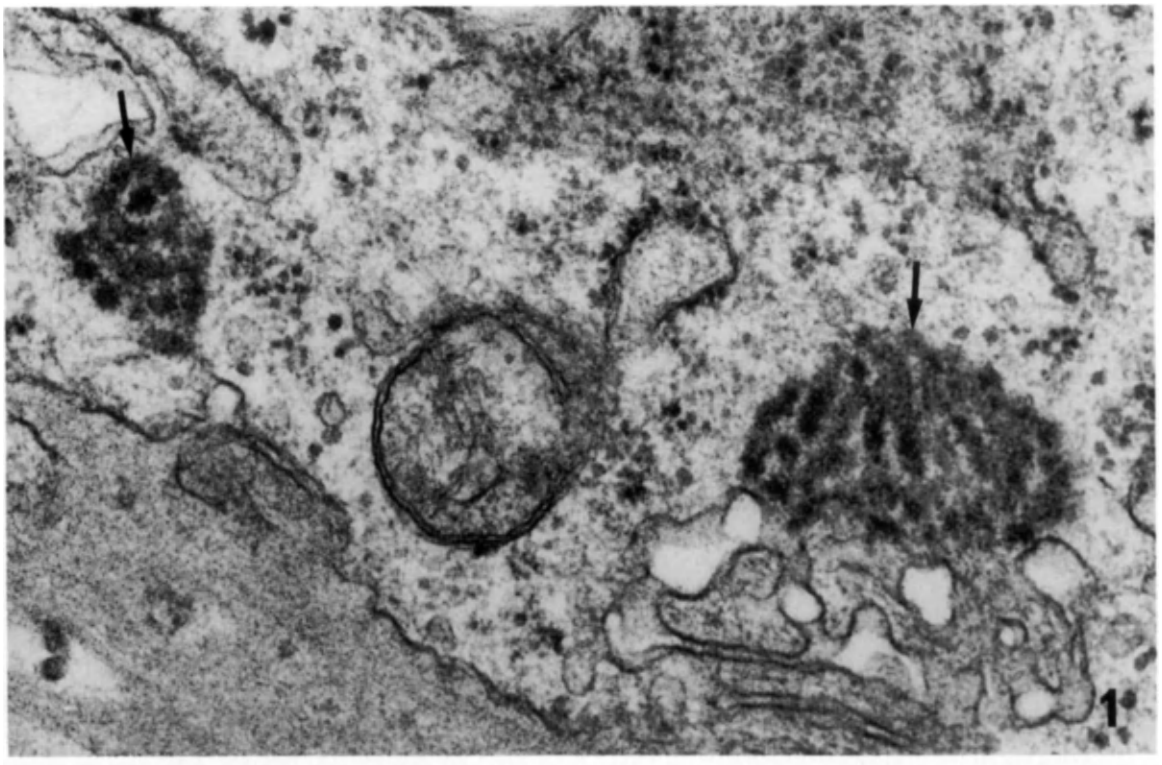
†In the lists which follow, I have reclassified these structures (on the basis of published illustrations) into two groups namely open nucleolus-like bodies and compact nucleolus-like bodies. Like the nucleolus, both types of bodies are composed of varying proportions of fine electron-dense filaments and electron-dense granules.

Plate 435

From full term human placenta obtained at caesarean section.

Fig. 1. Two open nucleolus-like bodies (arrows) in a cytotrophoblast. ×70 000

Figs. 2 and 3. Profiles of open nucleolus-like bodies showing the nema cut in various planes. ×77 000, ×81 000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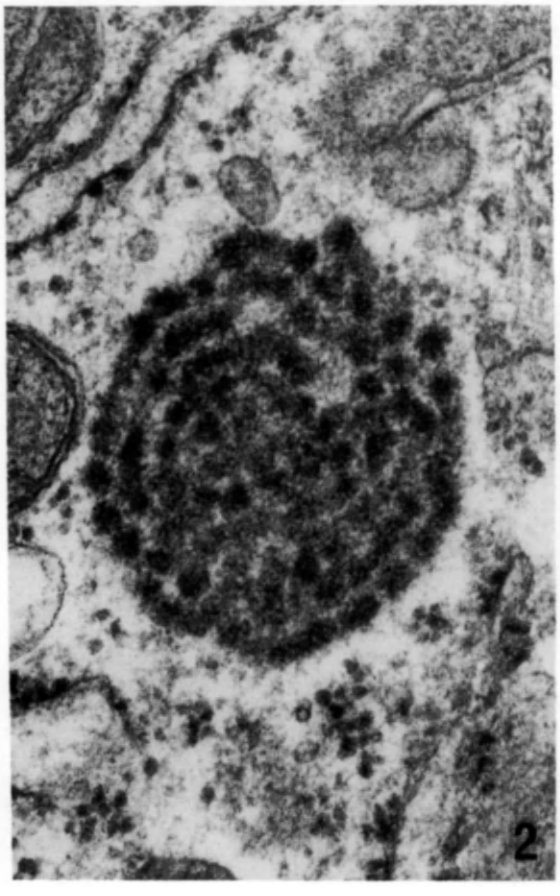
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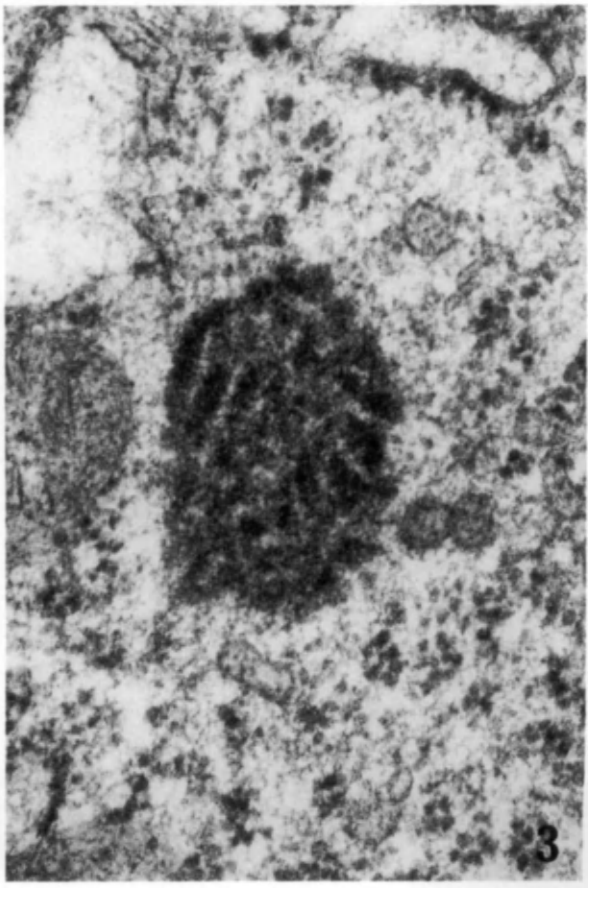
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rat (Grillo, 1970; Kanerva and Teräväinen, 1972; Peach, 1972; Chan-Palay, 1973: Routtenberg and Tarrant, 1974; Jacobs *et al.*, 1975; Knox *et al.*, 1980; Volk, 1980; van den Bosch de Aguilar and Vanneste, 1981; Heym and Addicks, 1982; Bourrat and Sotela, 1983); (10) neurons in cerebellum of reeler mutant mouse and *pcd* mutant mouse (Mariani *et al.*, 1977; Landis and Mullen, 1978); and (11) neurons in spinal ganglia of dog (Ferčáková and Marsala, 1983).

Compact nucleolus-like bodies* (usually about 0.3–1.5 μm in diameter but can be much larger) with or without 'holes' have been seen in: (1) oocytes of dragonfly (Halkka and Halkka, 1977); (2) oocytes of *Thyone briareus* (Kessel and Beams, 1963); (3) spermatocytes of *Drosophila melanogaster* (Kessel, 1981, 1985a); (4) primordial germ cells of *Tetrodontophora bielanensis* (Szklarzewicz and Klag, 1986); (5) oocytes of rat (Takeuchi, 1982); (6) ectodermal and mesodermal cells of rat embryo (Takeuchi, 1980); (7) neurons in hypothalamus of mouse (Anzil *et al.*, 1973); (8) neurons in mice after intracerberal injection of puromycin (Gambetti *et al.*, 1968); (9) neurons in medulla oblongata of rat (Kishi, 1972); (10) neurons in the hypothalamo-posthypophyseal complex of the Brattleboro rat (Tasso and Rua, 1978); (11) neoplastic cells of human hepatocellular carcinoma (Smetana *et al.*, 1972); (12) neoplastic cells of human neuroblastoma (Conde *et al.*, 1982); (13) neoplastic cells of metastasizing carcinoid of colon (Dingemans, 1983); and (14) striated muscle cells in reducing body myopathy† (Brooke and Neville, 1972; Hübner and Pongratz, 1981).

There is now ample evidence that both the compact and open nucleolus-like bodies contain RNA (Szollosi, 1965; Bernhard, 1969; Grillo, 1970; Takeuchi, 1982; Ockleford *et al.*, 1987). There is also now ample proof that nucleolar material is at times extruded from the nucleus into the cytoplasm, and indeed this is quite a common and constant phenomenon in oocytes. This phenomenon has been witnessed in living cultured cells and fixed sectioned and stained cells with the light and electron microscope (for references *see* Kessel and Beams, 1963 and Szollosi, 1965). Kessel and Beams (1969) found that small amounts of such material may pass through the nuclear pores, while quite large masses escape by evagination and rupture of the nuclear envelope followed by quick repair and reconstitution of the envelope. On the other hand, Szollosi (1965) has demonstrated that nucleoli can escape from the nucleus by budding. I have seen appearances suggesting nucleolar material escaping from the nucleolus (*Plate 436, Fig. 3*) in a seminoma, but no nucleolus-like bodies were found in the cell cytoplasm. The impression gained from a study of several images was that the escaped nucleolar material dispersed rapidly after leaving the nucleus.

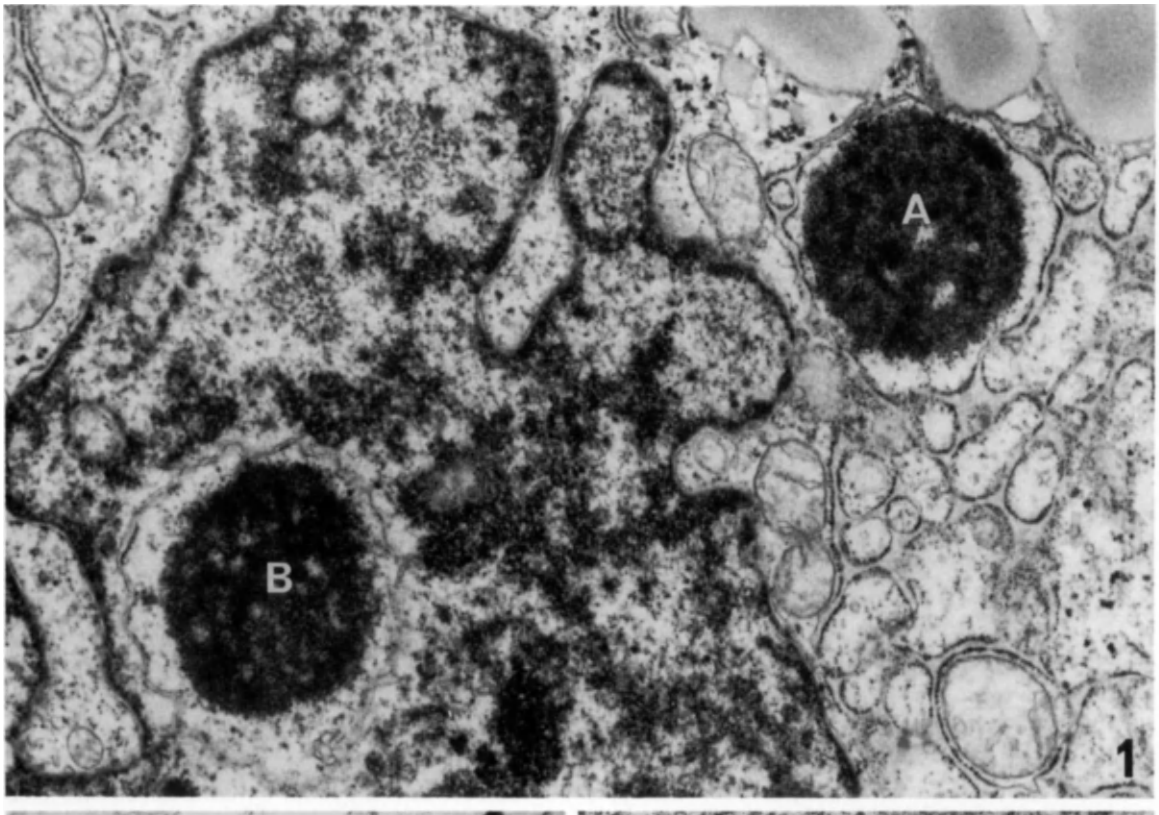
*Annulate lamellae at times develop in the compact nucleolus-like bodies in oocytes and spermatocytes (*see* page 582).
†The nature and significance of reducing bodies seen in these myopathies is not at all clear. These structures do look like nucleoli with 'holes' and the 'holes' contain glycogen, mitochondria and triads. Among other things these bodies have been shown to contain RNA. Therefore, they are probably acceptable as examples of compact nucleolus-like bodies.

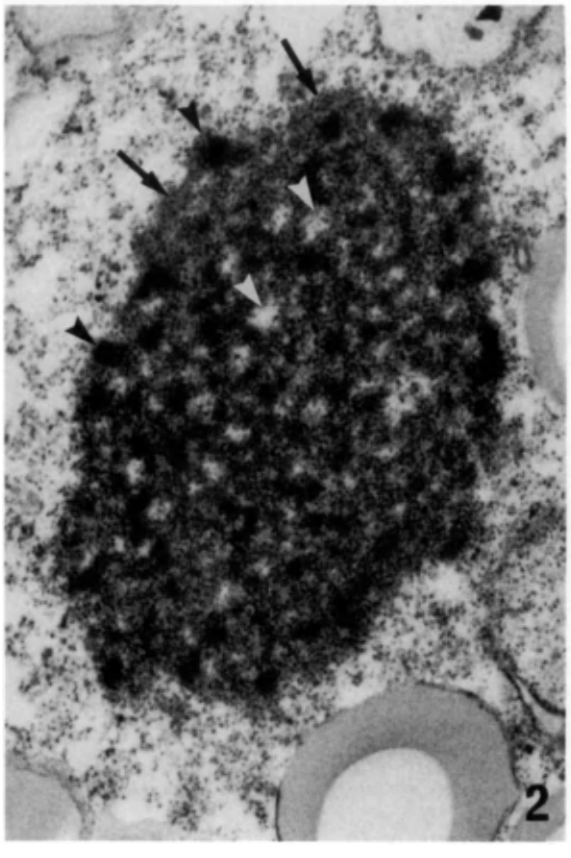
Plate 436

Fig. 1. Rhabdomyosarcoma. Two compact nucleolus-like bodies (about 1.6 μm in diameter) are seen here. One of them (A) is surrounded by rough endoplasmic reticulum, but from the position of the ribosomes on the membrane one can see that it lies in the cytoplasmic compartment and not in the intracisternal compartment. The other body (B) lies in a portion of cytoplasm lying within a double-membrane-bound intranuclear pseudoinclusion. ×21 000

Fig. 2. Same specimen as *Fig. 1.* A compact nucleolus-like body (about 3.5 μm in diameter) with several clear spaces (white arrowheads) in its substance. As in the nucleolus, one can discern here the dense filamentous component (black arrowheads) and the light granular component (arrows). ×24 000

Fig. 3. Seminoma. Seen here is a very open or meandering nucleolus (N), and what can be interpreted as nucleolar material (arrow) about to leave the nucleus. ×22 000


A
B
1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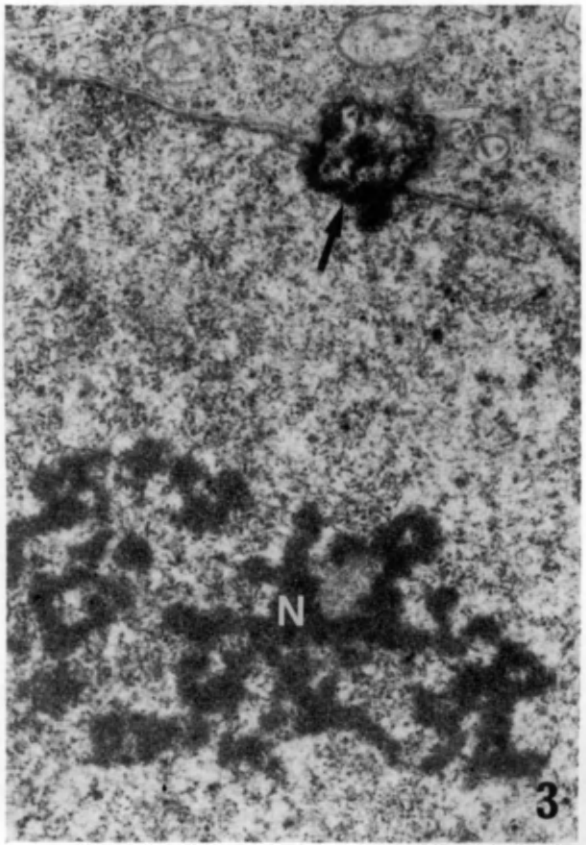

N
3

An examination of the lists presented above shows that open and compact nucleolus-like bodies have been found in essentially the same kinds of cells (namely embryonic cells, neurons and tumour cells). This, combined with the fact that open and compact nucleolus-like bodies have at times been seen in the same specimen or the same cell shows that all these structures are similar and collectively deserve to be looked upon as nucleolus-like bodies. Particularly interesting is the paper by Halkka and Halkka (1977) who show that from nucleolar material extruded from the nucleus both compact nucleolus-like bodies and open nucleolus-like bodies develop in the cytoplasmic matrix.

The significance of nucleolar extrusion and formation of nucleolus-like bodies is not known. So variable are the reports of close association of nucleolus-like bodies with various structures (e.g. mitochondria, endoplasmic reticulum, smooth surfaced and coated vesicles) that they provide no clue about their function. However, it has been suggested that they are: (1) a storage form of RNA (Dunbar, 1973; Halkka and Halkka, 1975); (2) an inhibitor of mitosis* (Hernandez-Verdun and Legrand, 1971; Hernandez-Verdun, 1972): and (3) a microtubule-organizing centre (Jacobs *et al.*, 1975; Jones and Ockleford, 1985).

Finally a discourse on structures called 'ribosome bodies' or 'ribonuclear bodies' is essential. The term 'ribosome body' was used by Jones (1962) to describe RNA positive structures found in the cytoplasm of mitotic erythroblasts. He notes that 'these cytoplasmic bodies looked almost exactly like nucleolar material'. With this one would concur and say that these structures are acceptable as compact nucleolus-like bodies. The term ribonuclear body was used by Flaks *et al.* (1970) to describe spider-like bodies† which they found in human ureteric tumours and which they consider to be an 'aggregate of ribosomes'. The structures found by Willett and Clayton (1985) in a papillary serous carcinoma are illustrated in *Plate 437*. They state that the 'inclusions consisted of branching and anastomosing cords'. These structures were up to 3 μm in diameter, strongly pyroninophilic, and ribonuclease digestion rendered them non-pyroninophilic. These authors suggest that they may be very large or giant polyribosomes.

However, the structures shown in *Plate 437, Fig. 1* are acceptable as open nucleolus-like bodies. It seems to me that the spider-like bodies described by Willett and Clayton (1985) and Flaks *et al.* (1970) could be a variation of the very open or meandering nucleoli seen in seminoma which at times show an arborescent or staghorn pattern (*Plate 32*). Nevertheless, one cannot totally rule out the possibility that some of the structures described in this section of the text derive by aggregation of cytoplasmic ribosomes or polyribosomes. The difference between this hypothesis and 'origin from extruded nucleolar material' hypothesis is not fundamental because after all ribosomes are derived from the nucleolus.

*Hernandez-Verdun and Legrand (1971) and Hernandez-Verdun (1972) base this on the observation that neither neurons nor syncytial cells normally undergo mitosis, and that centrioles and nucleolus-like bodies were not observed by them in the same cell. However, Jones and Ockleford (1985) have found centrioles and open nucleolus-like bodies in the same cell. This, plus the fact that these structures have been seen in tumours, does not help the idea that nucleolus-like bodies are inhibitors of mitosis.

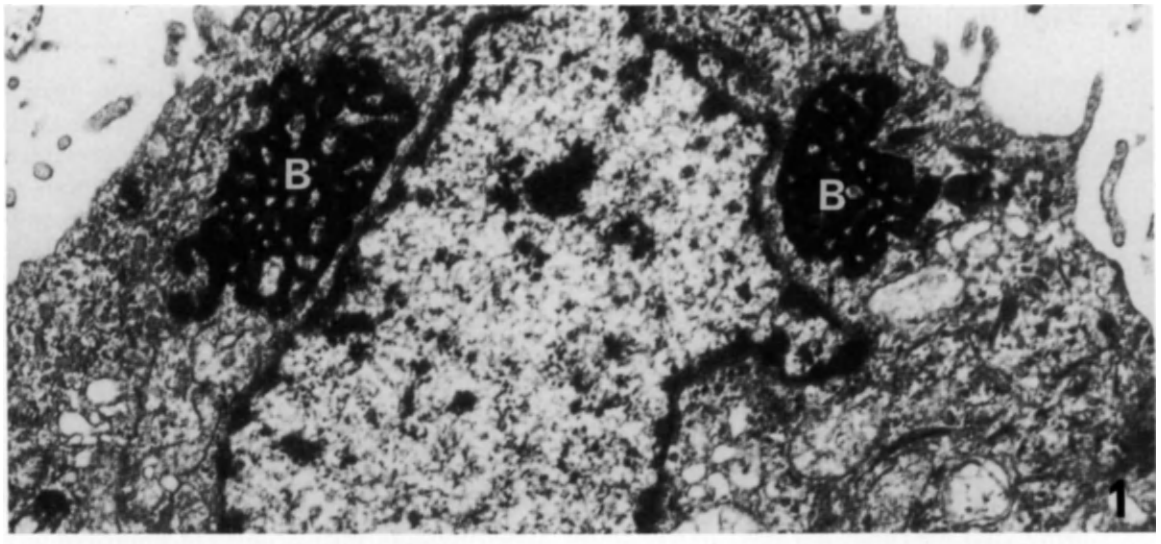
†The bodies illustrated by Falks *et al.* (1970) resemble the spider-like bodies found by Willett and Clayton (1985), shown in *Plate 437, Fig. 2.*

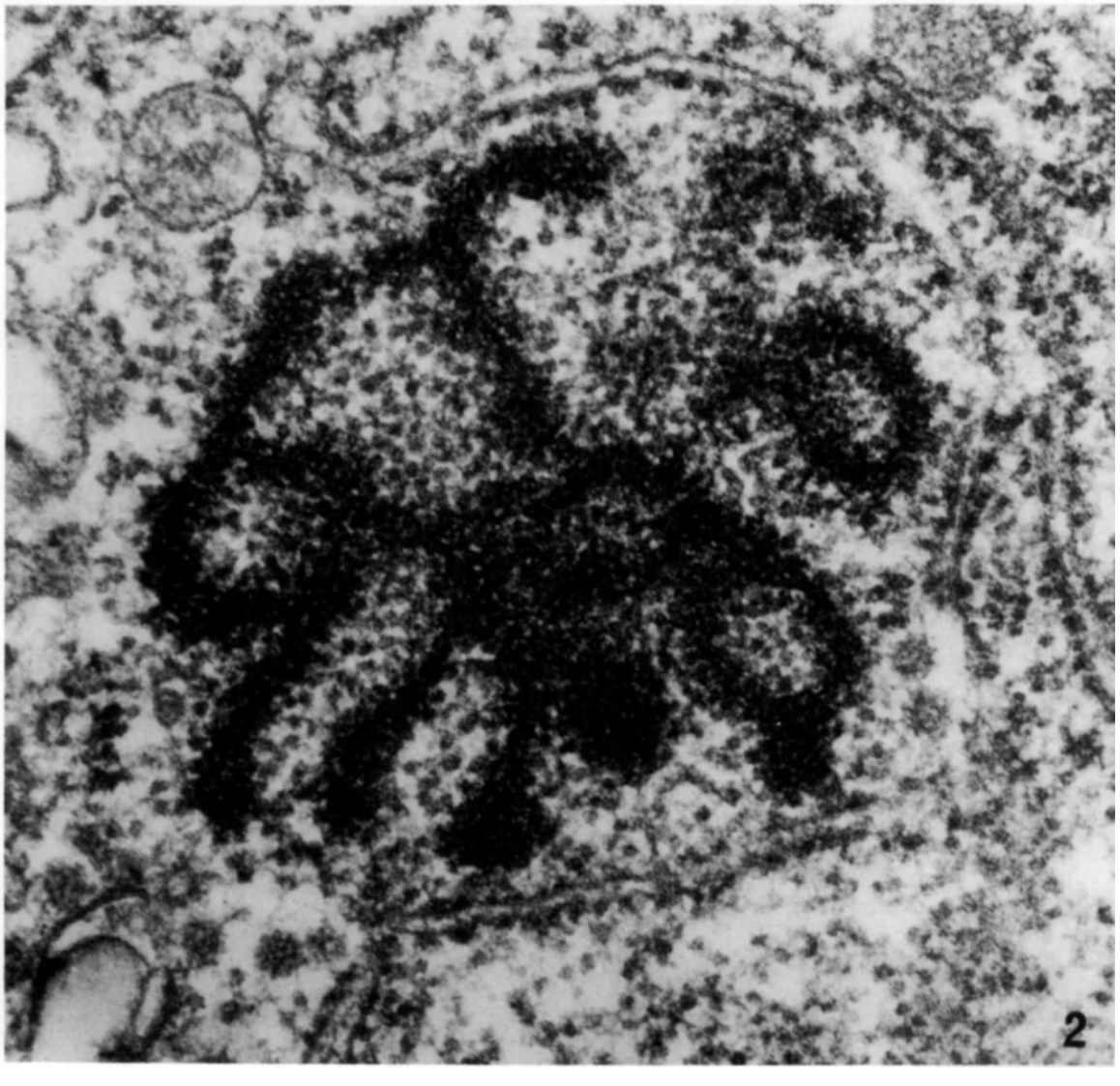
Plate 437

From a papillary serous carcinoma involving the surface of both ovaries, endocervix, endometrium, peritoneum and lymph nodes. (*From Willett and Clayton, 1985*)

Fig. 1. Two open nucleolus-like bodies (B) are seen in the cytoplasm of a tumour cell. ×21 000

Fig. 2. Seen here is a spider-like body which one may look upon as: (1) a very open nucleolus-like body; (2) a body produced by aggregation of cytoplasmic ribosomes. (Note similarity of size and morphology of the granules in the body and ribosomes on the rough endoplasmic reticulum); or (3) giant polyribosomes composed of messenger RNA and ribosomes. ×115 000


B
B
1


2

Viral inclusions

In previous chapters of this book we have dealt with (1) intranuclear viral inclusions (pages 130–139) and other nuclear changes (pages 42–45) produced by viruses; (2) viral inclusions in the endoplasmic reticulum and Golgi complex formed by budding of virus particles assembled on these membranes or in the adjacent cytoplasm (page 548); and (3) cytoplasmic inclusions of reovirus forming in association with microtubules (page 948). In order to avoid repetition only some remaining points of general interest will be mentioned here. Detailed references will not be quoted, since support for these statements may be found in many standard virology texts.

The term 'inclusion bodies' has long been employed by light microscopists to describe certain round, oval or irregular-shaped bodies found in the cytoplasm and/or nucleus of virus-infected cells. This morphological evidence of cell-virus interaction was first described by Findlay in 1938. Since then inclusion bodies have been examined extensively by light and electron microscopy as well as by fluorescent antibody staining methods. Although not all viral infections produce inclusion bodies and while structures resembling viral inclusions can at times be produced by other noxious agents, the value of such inclusion bodies in the diagnosis of many viral infections has been clearly demonstrated by light microscopy and improved and extended by electron microscopy.

Light microscopic studies have shown that inclusion bodies may be either basophilic or eosinophilic. Some show a clear halo around them but this is almost certainly a shrinkage artefact. Electron microscopy has clearly demonstrated that most but not all* inclusion bodies are sites of viral synthesis and/or maturation (referred to at times as 'factory areas' or 'viral factories'), as had indeed been suspected and in some instances fairly convincingly demonstrated long before the advent of electron microscopy.

Virtually all RNA viruses (except myxoviruses) are assembled in the cytoplasm and as such tend to produce inclusions in this region (*Plate 438*). However, inclusion bodies large enough to be seen with the light microscope are not formed in every case. Many of the small non-enveloped RNA viruses (e.g. poliovirus, Coxsackie virus and echovirus) and reovirus can, however, produce quite large inclusions which may progress to occupy most of the cytoplasm. Measles virus (an RNA virus) produces inclusions both in the nucleus and in the cytoplasm. At electron microscopy both the nuclear and cytoplasmic inclusions present as aggregates of microtubules composed of viral nucleoprotein (*Plate 68*).

*For example, some of the prominent cytoplasmic eosinophilic inclusions of pox-virus infected cells (called 'A' bodies) are thought to reflect a late cellular response rather than a site of viral replication. (For details and references, *see* Fenner, 1968.)

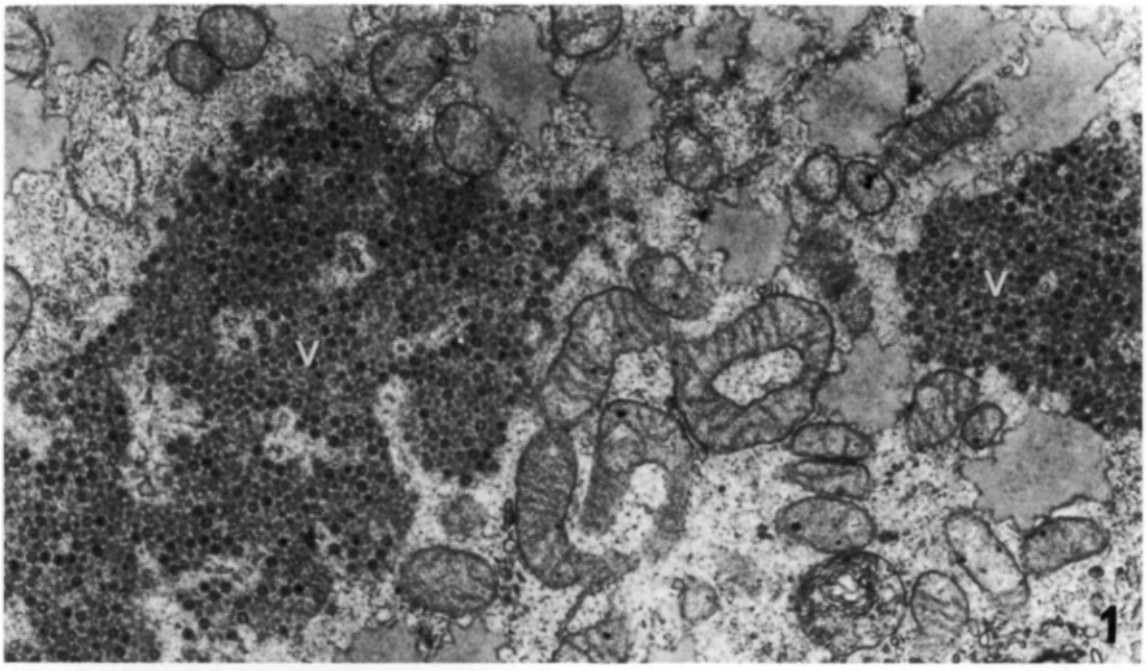
Plate 438

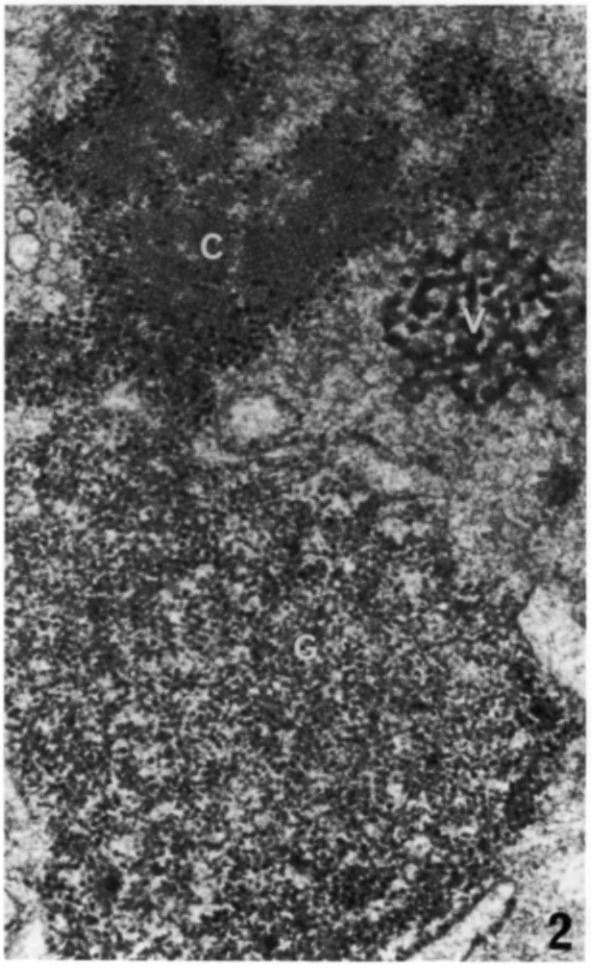
Intracytoplasmic viral inclusions (*Grodums, unpublished electron micrographs*)

Fig. 1. Reovirus inclusions (V) in LLCMK cell (a continuous cell line derived from monkey kidney). ×22000

Fig. 2. Picornavirus (echo II) replicating in the cytoplasm of LLCMK cell. Granular (G), crystalline (C) and vermicellar (V) inclusions are seen in the cytoplasm of the infected cell. ×50000

Fig. 3. Rhabdovirus inclusions (V) in an ependymal cell from the brain of a three-week-old mouse. ×50000


V
V
1


C
V
G
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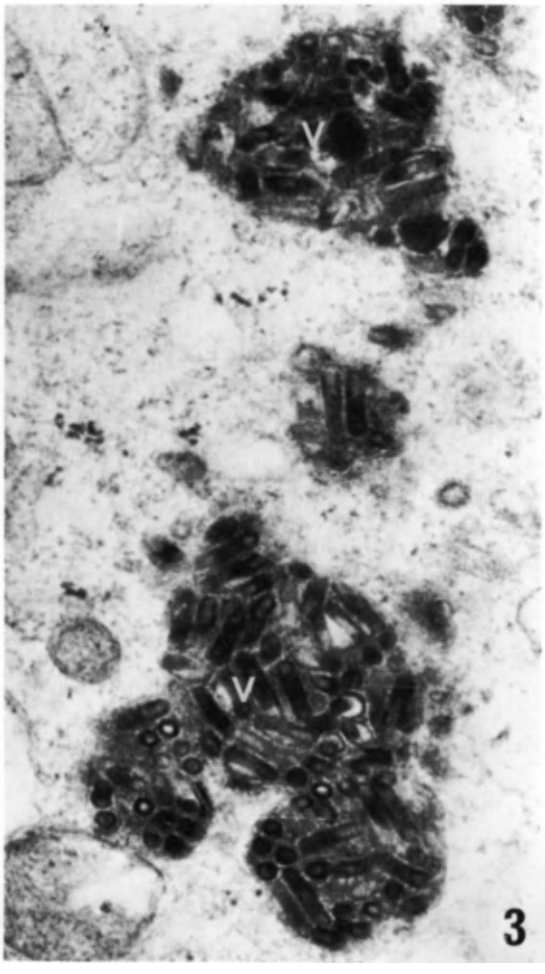
V
V
3

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14

Cell membrane and coat

Introduction

At the cell surface or the limiting boundary which demarcates the cell from its surroundings, two structures are usually seen: a membrane composed of lipids and proteins called the 'cell membrane', 'plasma membrane', 'plasmalemma' or 'plasmalemmal membrane' (which shows a characteristic trilaminar structure, *Plate 439*) and frequently but not invariably* also a polysaccharide-rich layer adjacent to or attached to the external surface of the cell membrane called the 'cell coat' or 'glycocalyx'.

In the pre-electron-microscope era, Danielli and Davson (1935) proposed a theory of membrane structure, based on the then available evidence about the chemical composition, surface tension, permeability and electrical properties of membranes. According to this theory the membrane was thought to consist of a central lipid layer covered by monolayers of protein. With the advent of electron microscopy the proposed trilaminar structure was visualized in sections through the cell membrane as two dense laminae separated by a lucent lamina. The overall thickness of the cell membrane was found to be about 8–10 nm, each layer being about 3 nm in thickness. This characteristic trilaminar structure is sometimes referred to as the 'unit membrane' or 'Robertson's unit membrane'.

Based on extensive studies on the structure of cell membranes and other cytomembranes, Robertson (*see* his 1969 review for history and details) proposed that the central lipid layer is a bimolecular lipid leaflet (with the polar groups pointing outwards) and that the boundary protein layers are chemically asymmetrical (i.e. they have a different chemical composition). In keeping with this is the fact that in quite a few membranes a morphological asymmetry is also demonstrable, the inner lamina (adjacent to cytoplasm) being thicker than the external lamina (Koss, 1969).

*It would be more accurate to say that a cell coat is always present but often it is so insignificant that it is not visualized in routinely processed tissues.

Various other concepts and models of membrane structure have been proposed (*see* the review by Stoeckenius and Engelman, 1969) but space permits the mention of only one other, called the 'fluid mosaic model of membrane structure' (Singer and Nicolson, 1972). This theory views various cytomembranes (i.e. cell membrane and various intracellular membranes) as a mosaic composed of globular molecules of proteins embedded in a phospholipid matrix, with the polar groups (with attached carbohydrates) protruding from the membrane into the aqueous phase. The bulk of the phospholipid is thought to be organized as a discontinuous fluid bilayer. Thus the matrix of the mosaic is thought to be lipid in which float the proteins which constitute the pieces of the mosaic. A somewhat similar concept of membrane structure is also proposed for mitochondrial membranes by Packer (1972).

As mentioned earlier a carbohydrate-rich layer is demonstrable on the outer surface of the cell membrane of many cells with the electron microscope. Because of its extracellular position, Brandt (1962) called it the 'extraneous coat'; while Rambourg and Leblond (1967) suggested that it be called the 'cell coat'. Its carbohydrate content led Bennett (1963) to call it the 'glycocalyx' (meaning 'sweet husk').

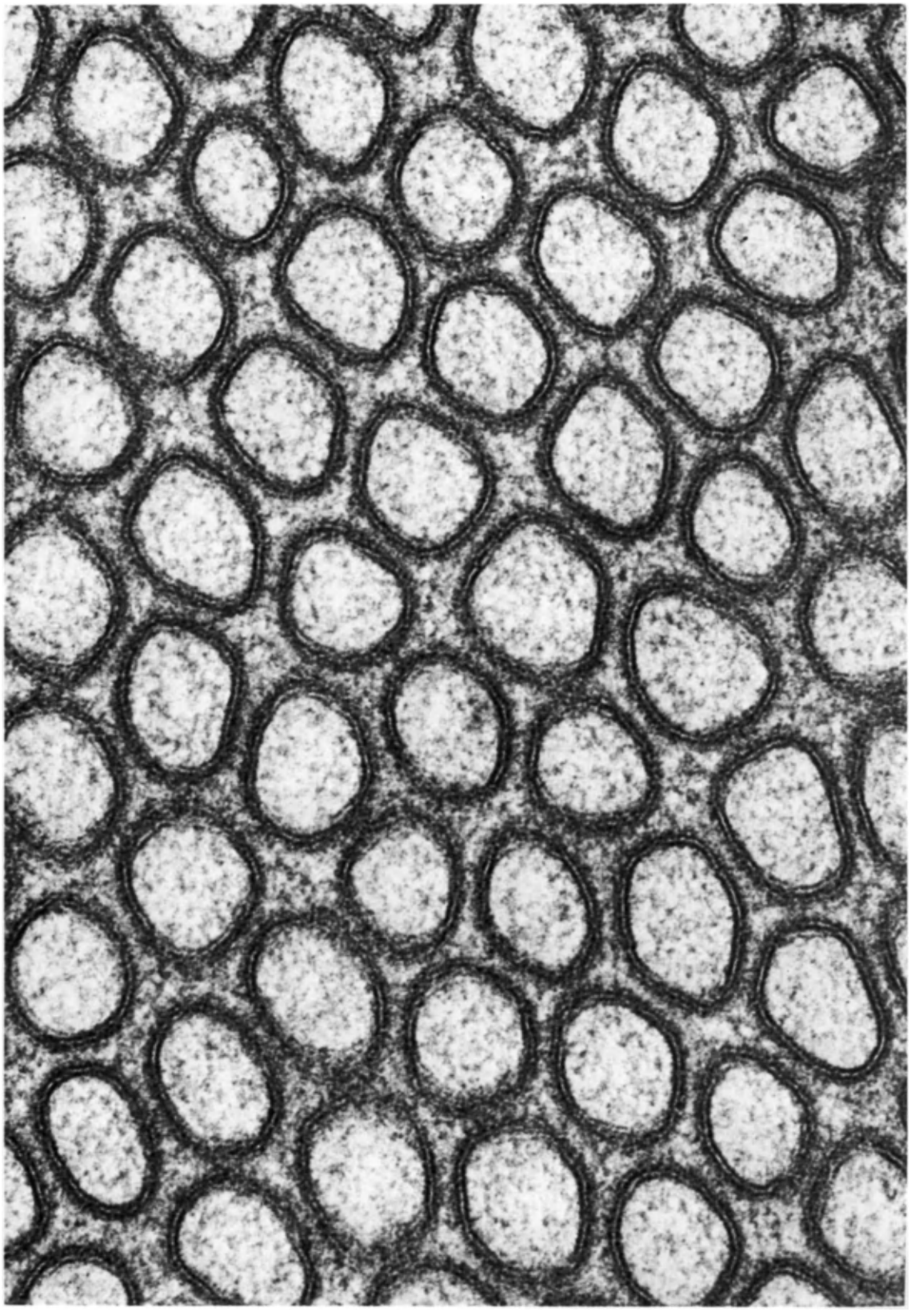
Sugars of some sort or other are a constant component of the cell coat but it also contains various other substances such as amino acids, proteins and lipids. The cells of plants are characteristically surrounded by a rigid envelope; the cell wall. The cell wall is formed outside the cell membrane and is hence a manifestation of the glycocalyx. A common polysaccharide usually found here in woody plants is cellulose (Kreger, 1969). The glycocalyx of animal cells is, of course, never so robust but it does show many site-related variations in structure and thickness.

At the surface of some epithelia (e.g. intestinal epithelium) the glycocalyx (called also the 'free surface coat') presents as a filamentous coat intimately attached to the cell surface. At the base of epithelia and also in some other sites the glycocalyx forms a lamina (called the 'basal lamina') which is usually only about 30–100 nm thick but it may attain a thickness of up to 300 nm or more in certain sites even in the normal state (e.g. glomerular basal lamina). A somewhat thinner but still easily discernible glycocalyx (called the 'external lamina') invests some cells such as muscle cells and Schwann cells, but on erythrocytes the glycocalyx is so tenuous that it is not seen in ordinary electron micrographs but it can be demonstrated by cytochemical and immunological techniques (Kabat, 1956; Lee and Feldman, 1964).

Such matters and the alterations in the cell coat or glycocalyx engendered by age and pathological states form the major topic of this chapter.

Plate 439

Cross-section of human intestinal microvilli showing the characteristic trilaminar structure (i.e. two dense 'lines' separated by a light intermediate zone). ×180 000



Cell membrane

It has already been noted that the cell is demarcated from its environment by a trilaminar membrane and also by a cell coat (*Plates 439* and *440*). However, this trilaminar structure of the cell membrane is not easily demonstrated in many routinely prepared tissues used for electron microscopy. Excellent visualization is obtained when fixed blocks of tissue are stained with uranium (*en bloc* staining) prior to dehydration.

Besides the method of tissue preparation, one must also consider the problems of sectioning geometry. It takes little imagination to see that unless the cell membrane is cut at right angles (or close to a right angle)* to its surface and the segment of membrane lying in the thickness of the section is straight and does not bend or fold, there will be little chance of demonstrating the characteristic trilaminar structure. Yet another factor is the intensity of staining. The trilaminar structure is difficult to demonstrate in heavily stained sections. The particles of stain deposit can reach a diameter of 2–3 nm which would easily 'obliterate' the lucent lamina in the cell membrane. It is therefore common experience that in many routine preparations, the trilaminar structure is difficult to demonstrate, and is at best visualized only in small segments of the cell membrane. More frequently, the cell membrane presents as a single dense line or as a blurred line or band, depending upon the plane of sectioning.

The chances of demonstrating the trilaminar structure of the membrane† are enhanced when a relatively flat expanse of membrane is encountered, as when a cell is apposed to another cell or to some firm structure (*Plate 440, Fig. 2*). Favourable also is the situation in the case of microvilli covering the surface of intestinal mucosa. Here an accurately placed transverse section gives a beautiful demonstration of the trilaminar structure, even in routinely fixed and stained material (*Plate 439*), for a straight tubular segment of the microvillus lies vertically within the section thickness.

Although the morphological features of cell membranes from various cells show a remarkable similarity, detectable differences in overall thickness and in the symmetry, thickness and spacing of the laminae have been noted in different parts of the same cell and in cells from various tissues and organs (Yamamoto, 1963). For example, the membrane covering the free surface of a cell is usually slightly thicker than that covering its lateral surface. In keratinizing epithelia the more mature keratinizing cells have a thicker cell membrane, with a wider lucent lamina than the cell membrane of the basal cells from which they stem.

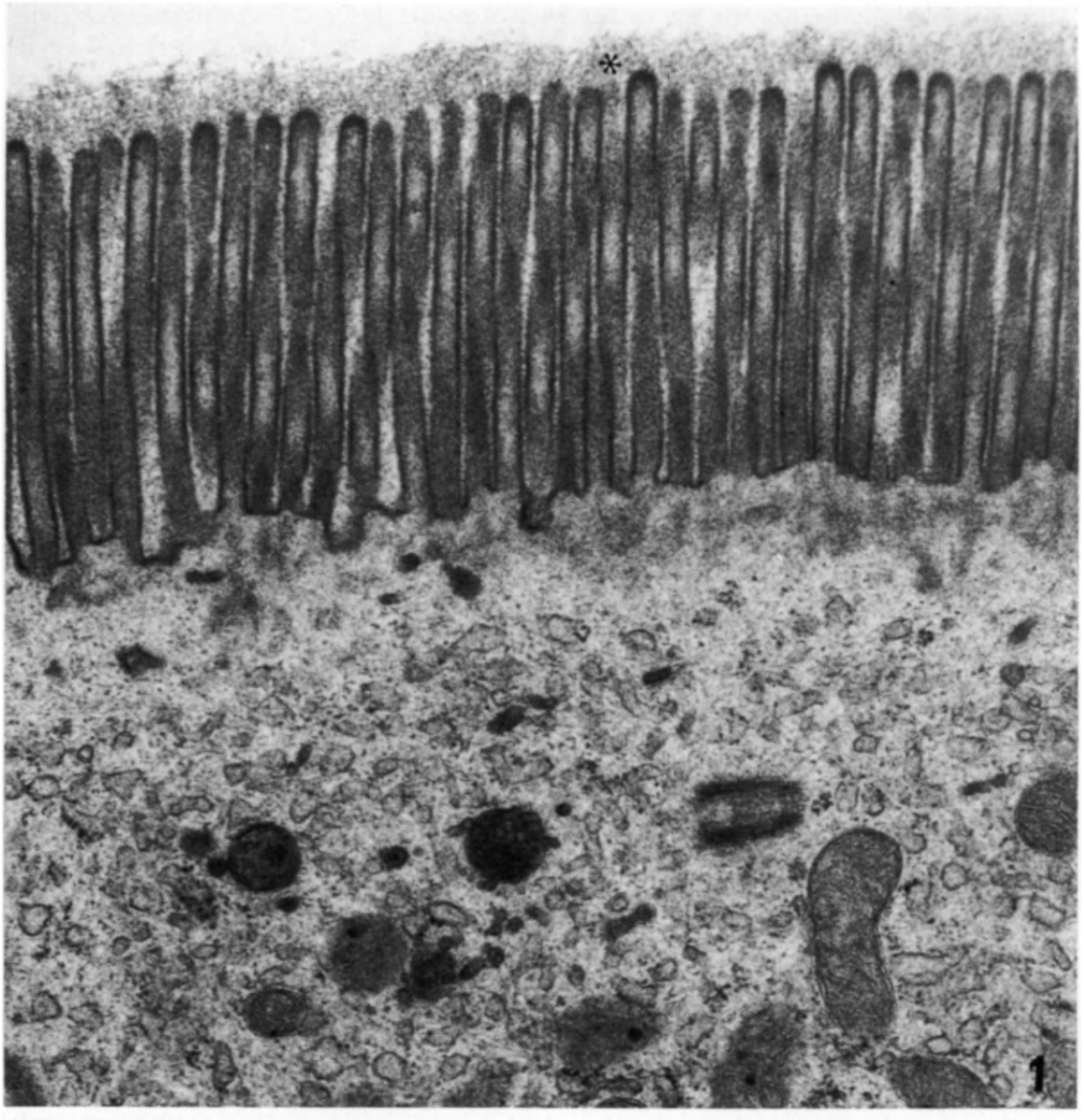
*However, sectioning geometry has less effect than one would imagine, because stains do not penetrate clear through the section thickness; in fact only the very superficial part of a section is normally stained. If this were not so the chances of seeing the trilaminar structure would be very small indeed. Simple calculations show that if a 60 nm thick section were to be stained throughout, it would be impossible to demonstrate the trilaminar structure if the membrane was off vertical by as little as 3°.

†In passing one may note that the ability to resolve the trilaminar membrane structure is a good rough and ready test of microscope performance (e.g. absence of significant stage or specimen drift) and resolution. In ultrathin sections the resolution attainable even with the best electron microscope is not much better than 2–3 nm. (For more details *see* Ghadially, 1985.)

Plate 440

Fig. 1. Longitudinal section of microvilli of intestinal brush border, showing the surface coat (★). ×37 000 (*Ghadially and Ailsby, unpublished electron micrograph*)

Fig. 2. Endothelial cell (E) from a fenestrated capillary, showing the characteristic trilaminar structure of the cell membrane and some 'fuzzy' material which represents the cell coat. The diaphragm (arrow) stretching across the pore does not have a trilaminar structure. Vascular lumen (L). From the kidney of a desert rat (*Perognathus baylei*). ×235 000 (*Newstead, unpublished electron micrograph*)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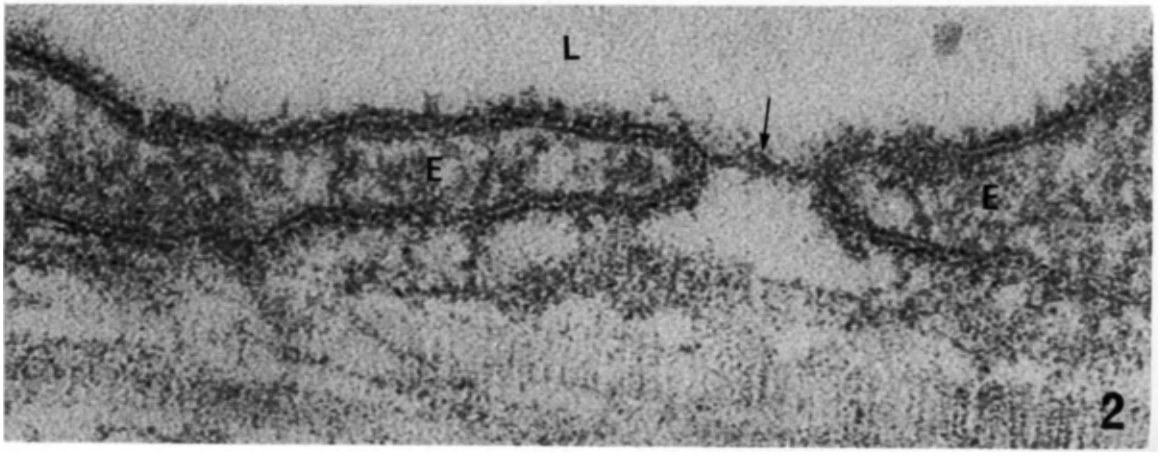
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Singularly interesting are the studies with freeze-cleaving and freeze-etching techniques. With this method (freeze-cleaving) the specimen, frozen in liquid nitrogen, is cleaved and the fractured surface replicated by platinum-carbon shadowing. Such preparations show surfaces (fracture-faces) covered by 8–10 nm diameter particles.

Although at first it was not at all clear exactly where these particles lay (a fundamental difference of opinion had developed as to the plane along which cleavage occurs) (Friederici, 1969), it is now thought that the fracture splits the membrane down its hydrophobic centre (Branton, 1966; Muhlethaler, 1971) and that the particles seen are protein components protruding into the central regions of the membrane. Freeze-etching, a process whereby ice is allowed to sublime from the fractured specimen after cleaving, exposes the membrane surfaces (Tillack and Marchesi, 1970). Such surfaces are, relatively speaking, quite smooth (*Plate 441*).

The varying physiological properties and functions of cell membranes from different sites clearly indicate that within the basic unit membrane lie different systems of lipids and enzymes, although these are not demonstrable in routine electron micrographs. That specialized areas exist in or on cell membranes is, however, shown when ultrastructural studies are combined with ferritin labelling (Lee and Feldman, 1964; Nicolson *et al.*, 1971) and cytochemical techniques. Such studies show, for example, that blood group antigens and some enzymes have a characteristic pattern of distribution on or in the cell membrane.

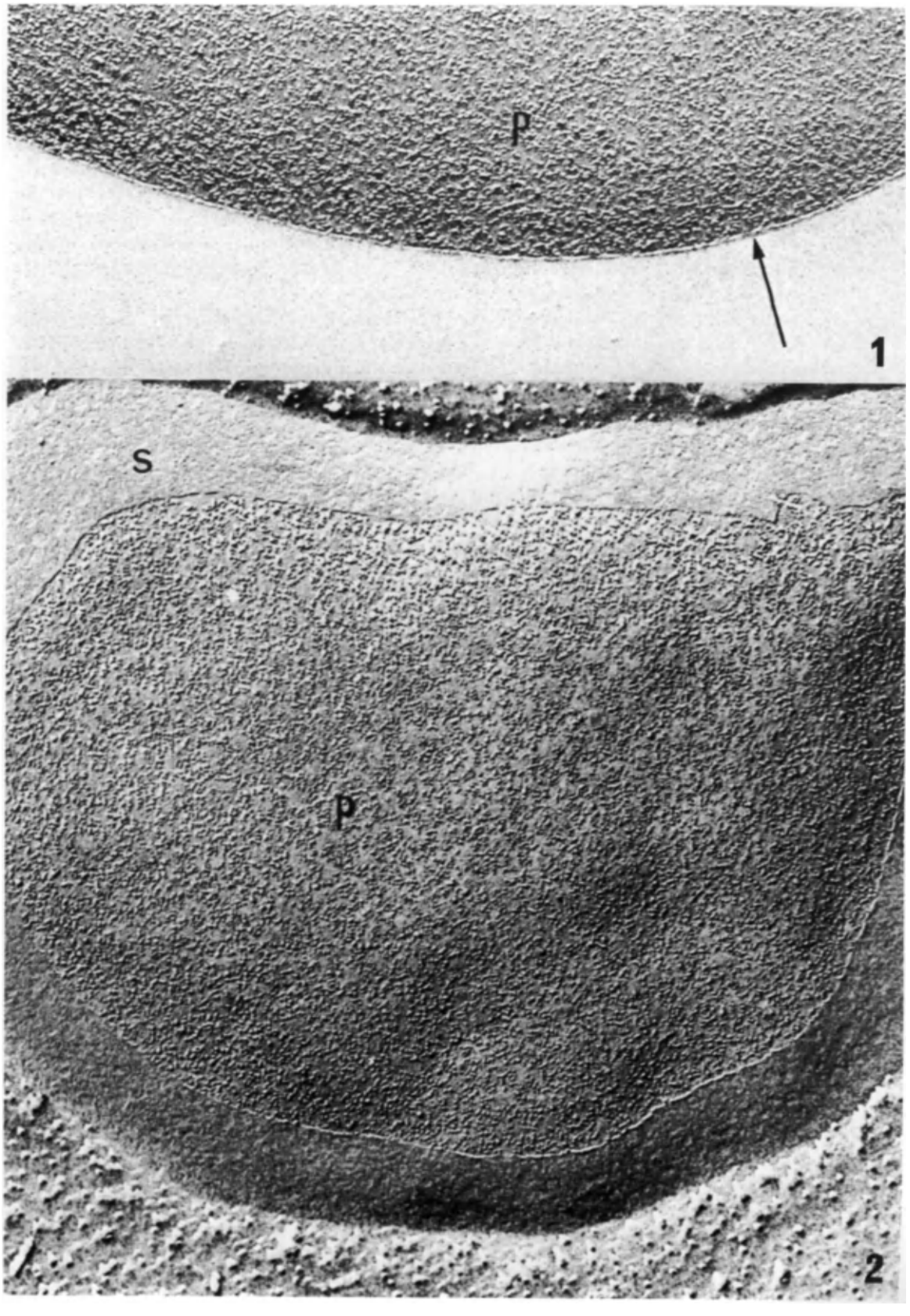
Active transport (i.e. against a concentration gradient) of ions across cell membranes requires expenditure of energy, so also does the transport of particulate material and fluid by mechanisms such as phagocytosis and micropinocytosis. In keeping with this is the demonstration of ATPase activity on the inner or outer surfaces of the cell membrane of various cell types by cytochemical methods (Ashworth *et al.*, 1963; Marchesi and Barrnett, 1963; Bartoszewicz and Barrnett, 1964; Marchesi *et al.*, 1964; Otero-Vilardebo *et al.*, 1964; Torack and Barrnett, 1964; Wachstein and Besen, 1964; Kaye and Pappas, 1965; Farquhar and Palade, 1966; Kaye and Tice, 1966; Kaye *et al.*, 1966; Santos-Buch, 1966; Campbell, 1968). The distribution of the reaction product is variously described by different authors but, generally, micropinocytotic vesicles show much activity, as also do membranes of cells known to be engaged in active transport. Furthermore, it would appear that in the case of some epithelia, only certain segments of the cell membrane exhibit overt ATPase activity and that this can be correlated with the known functional activity of the organ.

Thus in the normal liver (Wills and Epstein, 1966) ATPase activity is mainly localized at the luminal aspect of the bile canalicular membrane and microvilli (i.e. the part of the cell membrane actively involved in the transport of bile). In obstructive jaundice, both microvilli and enzyme activity tend to disappear from the distended bile canaliculi.

Plate 441

Fig. 1. Platinum-carbon replica of a freeze-cleaved human red blood cell ghost suspended in distilled water. The internal layer of the cell membrane bearing 8.5 nm particles (P) is revealed by this procedure. The junction of the particulate face and the surrounding ice is indicated by the arrow. ×60 000 (*From Tillack and Marchesi, 1970*)

Fig. 2. Platinum-carbon replica of a freeze-cleaved and deep-etched red blood cell ghost. The cloven convex face of the ghost is again seen to be covered with 8.5 nm particles (P). However, sublimation of the ice during the etching process has revealed a smooth surface (S) which is the actual outer surface of the red blood cell membrane. ×60 000 (*From Tillack and Marchesi, 1970*)


P
1
S
P
2

Several past light microscopic studies (Bourne, 1943; Deane and Dempsey, 1945) have demonstrated the presence of alkaline phosphatase in the brush border of the intestine and Moog and Grey (1967) have shown the presence of different isoenzymes of alkaline phosphatase in the cells lining the crypt and the villus. Ultrastructural studies show that alkaline phosphatase is a common component of mammalian cell membranes and not just that of the intestinal brush border (Clark, 1961). However, the tremendous mass of membrane at the brush border makes this the predominant location (Crane, 1968). According to Ito (1956) 'alkaline phosphatase is localized in or on the trilaminar plasma membrane and in the immediately adjacent region of the surface coat'. However, Mukherjee and Swift (unpublished observations) have found that most of the enzyme activity is located on the inner surface of the membrane lining the microvillus. Furthermore, they have found that the enzymatic activity varies greatly among intestinal cells lining even the same region of the villus (*Plate 442*).

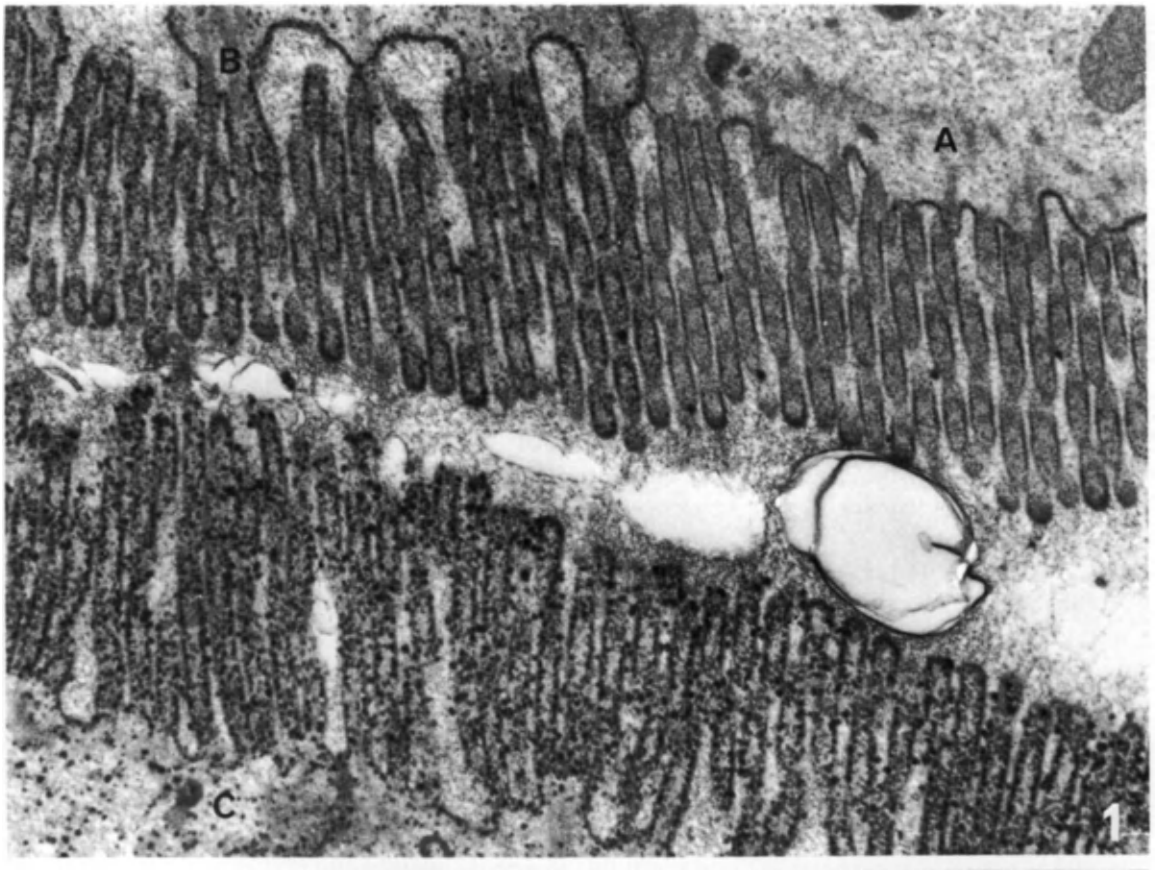
Few authors comment about the structure of the cell membrane (or alterations that might have occurred) when describing tissues of experimental animals or pathologically altered human tissues. To some extent this is due to the difficulty of demonstrating the trilaminar structure in routine preparations, and also because most biological work is done at quite low magnifications. Little information therefore is available on this point. The diminished power of adhesion of cancer cells, and the loss of contact inhibition shown by certain strains of cancer cells in tissue culture do not appear to be correlated with a detectable morphological alteration in the cell membrane itself. Such phenomena are probably explicable on the basis of alterations in the cell coat and/or cell junctions (*see* page 1110).

Plate 442

Brush border of mouse small intestine. The electron-dense lead precipitate (reaction product) indicates the site of alkaline phosphatase activity.

Fig. 1. This electron micrograph shows the marked differences in alkaline phosphatase activity between epithelial cells lining intestinal villi. On the basis of size and frequency of occurrence of electron-dense particles (i.e. reaction product) on the microvilli one may surmise that one of the cells (A) shows very little enzyme activity, an adjacent cell (B) shows a modest degree of enzyme activity, while the cells (C) on the adjacent villus show well marked activity. ×34 000 (*From a block of tissue supplied by Drs Mukherjee and Swift*)

Fig. 2. Higher-power view of microvilli. Much of the electron-dense reaction product (arrows) (and hence presumably the enzyme activity) appears to be located on the inner surface of the plasma membrane. ×93 000 (*Mukherjee and Swift, unpublished electron micrograph*)


B
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C
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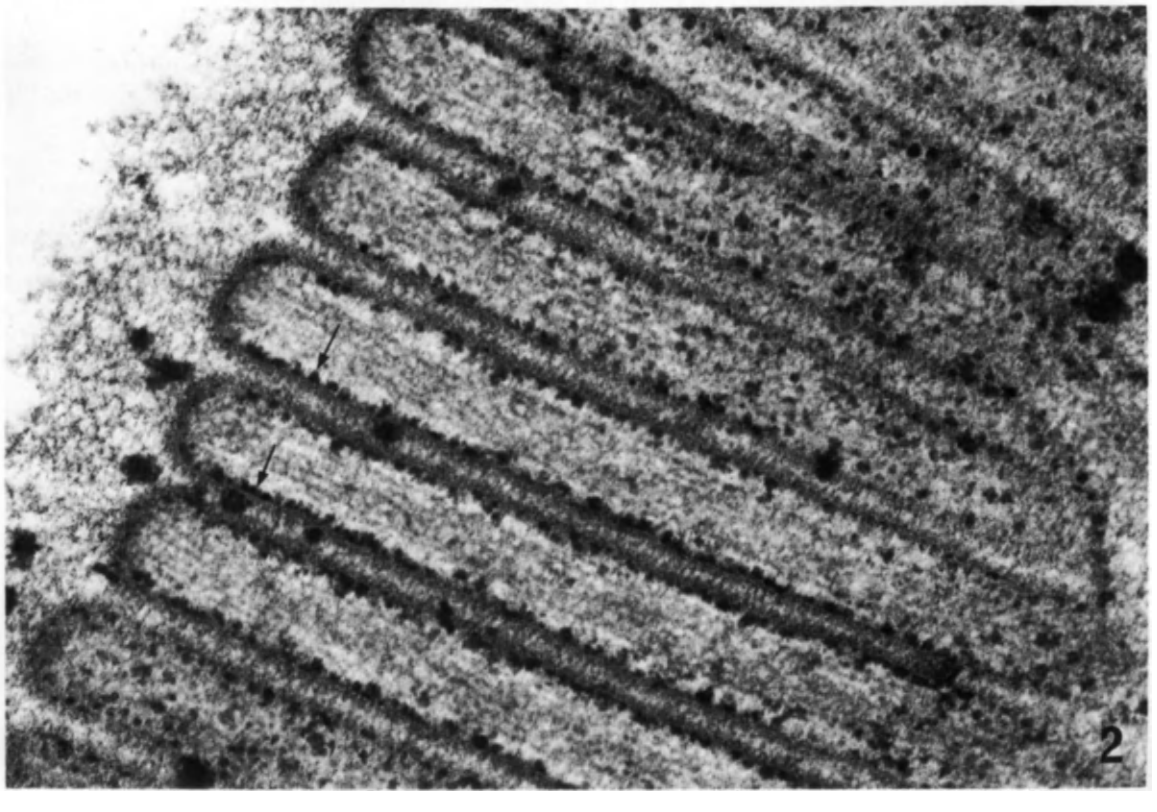
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T-tubule networks

Proliferation of T-tubules of striated muscle leads to the formation of a honeycomb-like or hexagonal network (reticulum) of tubules (about 30–40 nm in diameter). The overall size of the T-tubule network* varies from about 0.2 × 0.2 μm–1.3 × 4.9 μm. This reticulum of tubules usually lies in a single membrane-bound space. Sometimes, however, no limiting membrane is detectable, and on rare occasions two or more limiting membranes are present (*Plate 443*). Continuities between the walls of the reticulated tubules and the wall of the dilated T-tubule in which they lie are at times seen. Further, studies with electron-dense tracers have shown that, like the lumina of normal T-tubules, the lumina of the tubules of the network are continuous with the extracellular compartment (Huxley, 1964; Ishikawa, 1968; Schotland, 1970). Here then we have an example of a tubuloreticular structure which derives from the cell membrane, for the T-tubule is little more than a tubular extension of the cell membrane. Since tubular and/or tubuloreticular structures arising from the inner membrane of the nuclear envelope (*Plates 46–48*), mitochondrion (*Plate 93*) and endoplasmic reticulum (*Plates 211–214*) are known to occur, it would appear that proliferation of virtually any cytomembrane is capable of producing such structures.

T-tubule networks have been seen in skeletal muscle from cases of: (1) neurogenic atrophy (Shafiq *et al.*, 1967; Tomé and Mair, 1970); (2) hyperkalemic and hypokalemic periodic paralysis (Macdonald *et al.*, 1968; Schutta and Armitage, 1969; Engel, 1970); (3) myotonic dystrophy (Schröder and Adams, 1968; Schotland, 1970); (4) periodic paralysis (Macdonald *et al.*, 1969; Schutta and Armitage, 1969; Engel, 1970); (5) polymyositis (Chou, 1969; Mastaglia and Walton, 1971); (6) idiopathic myoglobinuria (Schutta *et al.*, 1969); (7) lupus erythematosus (Dr Y.S. Lee, unpublished observation, *Plate 443*); (8) late onset acid maltase deficiency (Engel and Dale, 1968); (9) muscular dystrophy (Fardeau, 1970); (10) reducing body myopathy (Neville, 1973); (11) hypothyroid myopathy (Afifi *et al.*, 1974); (12) rhabdomyoma (Cornog and Gonatas, 1967); (13) denervation atrophy of rats (Pellegrino and Franzini, 1963); (14) tissue cultures of developing chick skeletal muscle (Ishikawa, 1968).

The significance of T-tubule networks is obscure. However, the various speculations on this point are that their presence reflects: (1) a degenerative change; (2) unbalanced growth; (3) regeneration; or (4) hyperexcitability of muscular tissue.

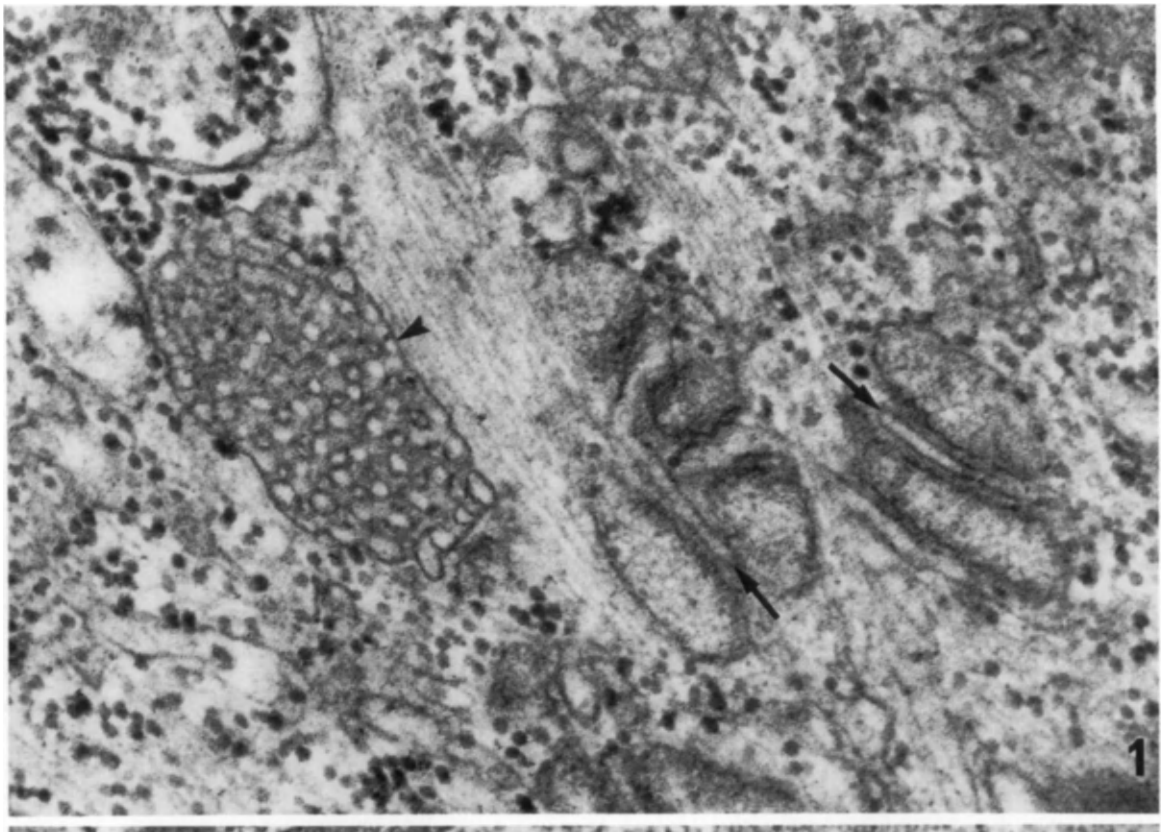
*These T-tubule networks are clearly different from the aggregates of parallel smooth membrane-bound single-walled or double-walled tubules (45–200 nm in diameter) which have been seen in patients with: (1) periodic paralysis (Bradley, 1969; Bergman *et al.*, 1970; Engel *et al.*, 1970; Schröder and Becker, 1972; Meyers *et al.*, 1972; Papadimitriou and Mastaglia, 1982); (2) hereditary myotonia (Schröder and Becker, 1972); and (3) the exertional muscle cramp syndrome (Brumback *et al.*, 1981). Most authors believe that these tubular aggregates are derived from the smooth endoplasmic reticulum but on the basis of enzyme histochemical studies Pearse and Johnson (1969) claim that 'the tubules are of mitochondrial origin and that they are not proliferated components of the SR- or T-systems'. Tubular aggregates, originating from the endoplasmic reticulum, are found in various other cell types besides muscle. This is dealt with on pages 478–485.

Plate 443

Muscle biopsy from a case of lupus erythematosus. T-tubule networks were found principally in atrophic muscle fibres containing few myofilaments and much glycogen. The quite distinct and different lupus-type inclusions (microtubuloreticular structures, (*see* pages 496–504) were present in vascular endothelial cells. (*From a block of tissue supplied by Dr Y.S. Lee*)

Fig. 1. A typical single-membrane-bound (arrowhead) T-tubule network is seen lying adjacent to triads. The T-tubules in the triads are indicated by arrows. ×104 000

Fig. 2. Two somewhat atypical double-membrane-bound (arrowheads) T-tubule networks are seen here. ×72 000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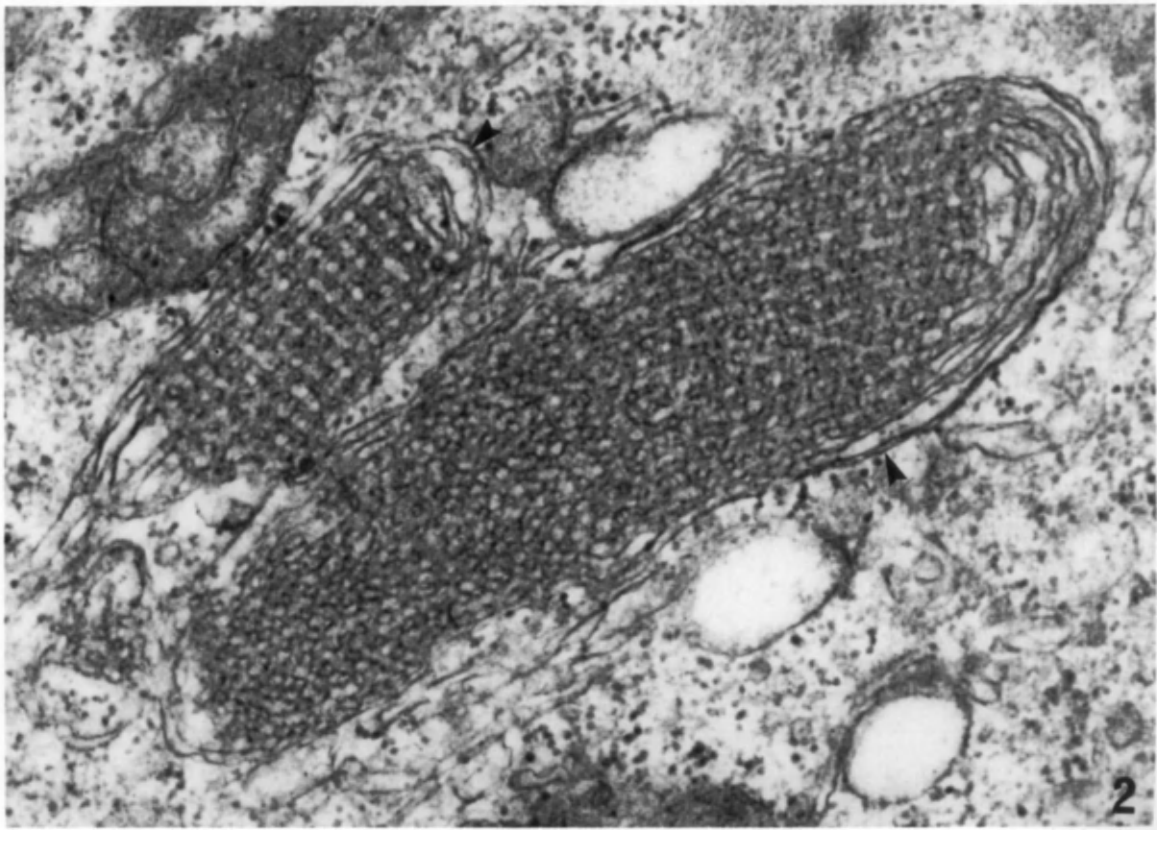

2

Basement membrane and basal lamina

At the junction of epithelia and underlying connective tissue lies an extracellular supporting layer that has long been called the 'basement membrane' by light microscopists. The history of its discovery is not too clear but about this matter Ham (1969) states 'classical histologists noted that a thick "structureless" membrane could be seen in ordinary sections just below the tracheal epithelium – a location where it may be seen as an unstained band. It was later believed that all epithelia rested on such a membrane although generally less thick than the trachea. The name basement membrane was adopted'.

The basement membrane is often difficult to see in routine H and E preparations but it can be demonstrated by silver impregnation methods and the periodic acid-Schiff reaction. According to Bloom and Fawcett (1975) the former (silver impregnation) probably reflects mainly the presence of reticular fibres (collections of very fine collagen fibrils) while the latter (PAS reaction) reflects mainly the presence of a polysaccharide-rich ground substance.

With the electron microscope one finds that the basement membrane is not a single structural entity but has two or more components. They are: (1) the basal lamina; and (2) the reticular lamina. At low magnifications the basal lamina presents as a 50–100 nm thick band adjacent to the basal portion of the cell membrane of epithelial cells. At higher magnifications it is seen to consist of 3–4 nm thick filaments set in an amorphous matrix. In some sites (e.g. human glomerulus) the basal lamina can be quite thick and attain widths of up to about 300 nm.

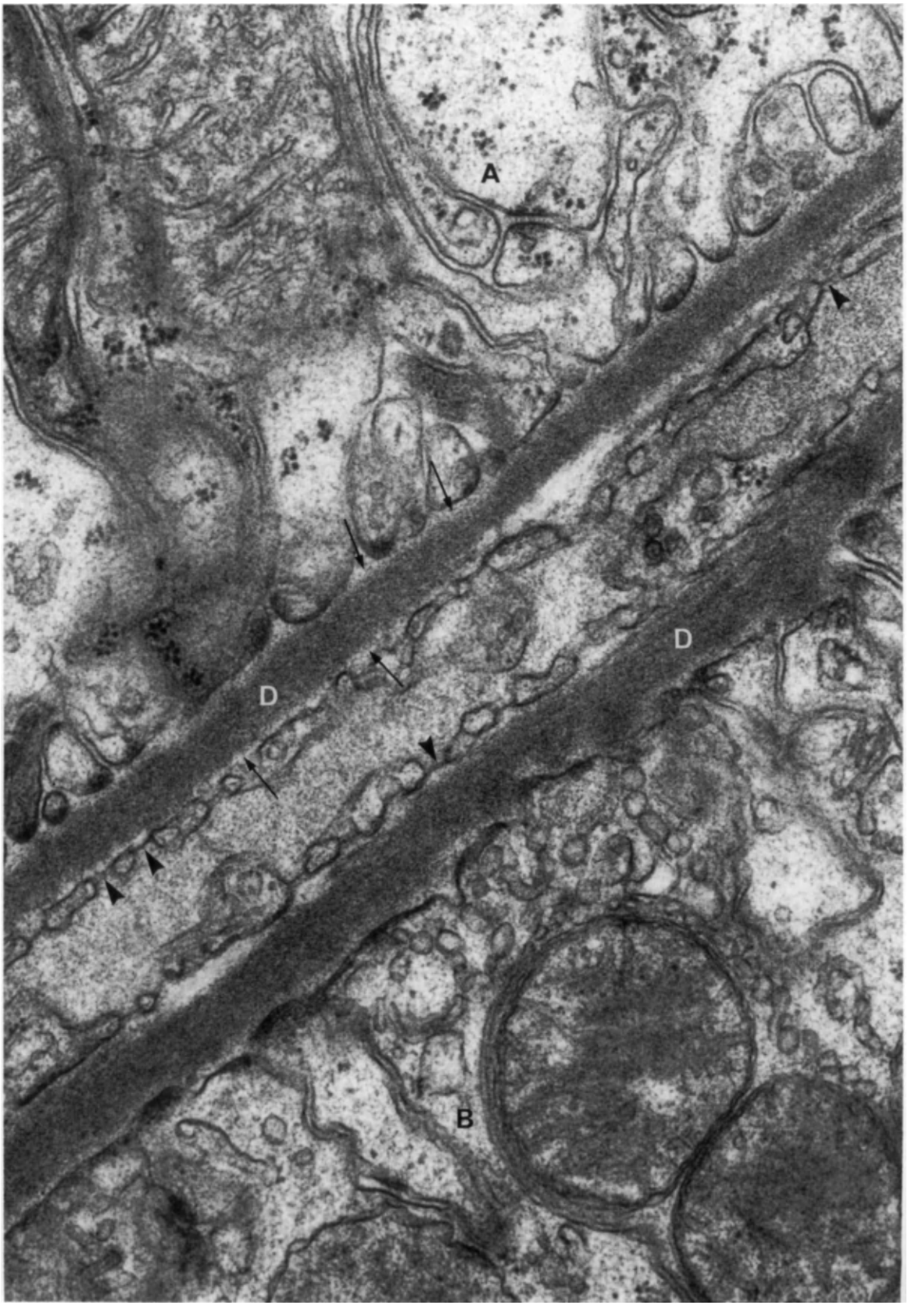
The density of the basal lamina is low immediately adjacent to the epithelial cells but increases a short distance away from the cell membrane. Hence in electron micrographs one often sees a medium density band running parallel to the cell surface some distance away (30–40 nm) from the cell membrane of the overlying epithelial cells. This relatively clear zone immediately adjacent to the cell membranes is referred to as the 'lamina lucida' or 'lamina rara' while the denser band is referred to as the 'lamina densa'.

Underneath the basal lamina lies the reticular lamina whose thickness and composition varies from site to site, but usually it contains fine collagen fibrils. In the trachea, the reticular lamina is hundreds of times thicker than the basal lamina, and it contains innumerable very fine collagen fibrils and cell processes of fibroblasts; while in the skin, the prominent reticular lamina contains some elastic fibres as well.

In situations where a capillary lies close to epithelial cells, the basal lamina of the capillary may fuse with the basal lamina under the epithelium (*Plate 444*). In such instances a single rather thick lamina densa flanked on either side by a lamina rara (one 'belonging' to the capillary, the other to the epithelium) may usually be discerned. This sort of situation is seen in the renal glomerulus where much of the glomerular basal lamina is in fact a lamina produced by the fusion of the basal lamina of the glomerular capillary and the basal lamina under the epithelial cells in this region (i.e. foot processes of podocytes). Much rarer is the situation where a capillary lies close to the renal tubules, but in this instance also a fusion of dense laminae occurs (*Plate 444*).

Plate 444

A fenestrated capillary (fenestrae indicated by arrowheads) is seen lying between cells (A and B) belonging to two renal tubules. Note the prominent lamina densa (D). A lamina lucida (arrows) may just be discerned under the epithelium and another lamina lucida (arrows) is seen adjacent to the capillary endothelium. From a normal rat kidney. ×56 000


A
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D
B

Descemet's membrane is essentially a very thick (5–10 μm) basal lamina (*Plate 445*). With the light microscope it presents as a homogeneous band separating the posterior surface of the corneal stroma (*substantia propria*) from the corneal endothelium. However, the electron microscope shows quite a complex substructure in the Descemet's membrane of older individuals. In cross-sections through Descemet's membrane a striated pattern of bands (about 10.7 nm apart) connected by 10 nm thick filaments is seen. In tangential cuts a pattern of nodes connected by 10 nm filaments is revealed. Histochemical, biochemical and x-ray diffraction studies have led to the conclusion that these filaments are an atypical form of collagen (Bloom and Fawcett, 1975).

Some electron microscopists refer to the basal lamina as the basement membrane. This not too uncommon practice is confusing and deplorable for it is obvious that the basal lamina is usually too thin to be resolved by the light microscope. What one sees as the basement membrane with the light microscope is a combination of the basal lamina and reticular lamina. Further, one must object also to calling a 'lamina' a 'membrane'. In electron microscopy, the term 'membrane' is best reserved for structures that show the characteristic trilaminar appearance such as that shown by the plasma membrane and various other cytomembranes.

Chemical, histochemical and immunological studies have been carried out on the basal lamina in the glomerulus, the very thick basal lamina (lens capsule) of the vertebrate lens and the basal lamina of the cornea (Dische, 1970; Kefalides, 1973; Trelstad *et al.*, 1974). Such studies show the presence of glycoproteins and type IV collagen in the basal lamina. Also present are acid mucopolysaccharides (glycosaminoglycans) in the lens basal lamina (Dische, 1970) and chondroitin sulphate in the embryonic corneal basal lamina (Trelstad *et al.*, 1974).

The type IV collagen in the basal lamina is richer in hydroxylysine and sugar (glycosylgalactose) than most other collagens. It also seems to retain a telopeptide (extension peptide) which probably prevents it from polymerizing into banded fibrils. The filaments seen in the basal lamina probably represent linked collagenous and non-collagenous proteins (glycoproteins).

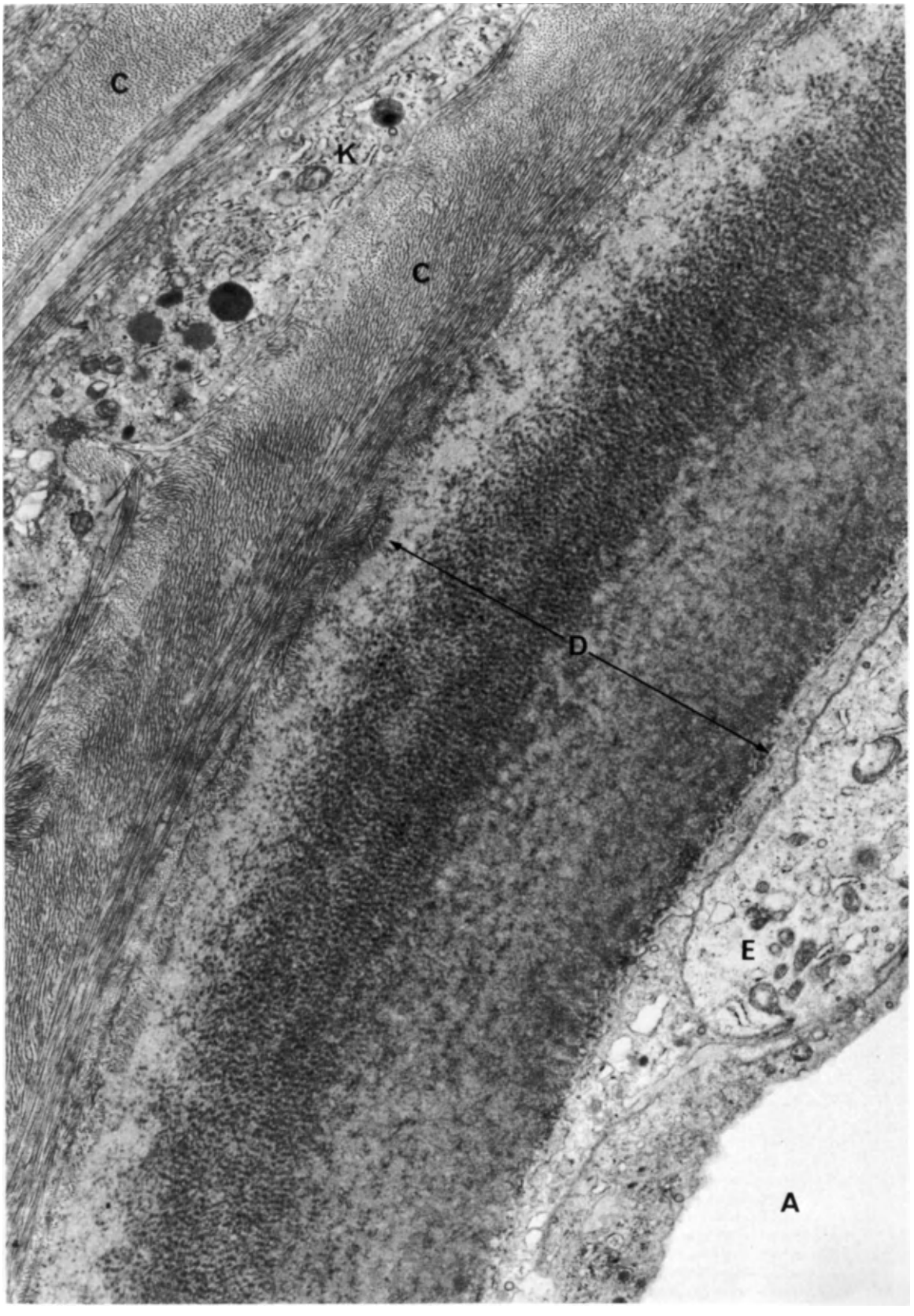
At one time it was thought that the basal lamina resulted from a focal condensation of matrix material and that the materials comprising the basal lamina were synthesized by fibroblasts in the underlying connective tissues. Such a view is no longer tenable for there is now considerable evidence supporting the idea that epithelia can make their own basal lamina (*see* review by Hay and Revel, 1969).

The basal lamina is found in sites other than the base of epithelia. A knowledge of the presence or absence of the basal lamina around blood capillaries, vascular sinusoids, and lymphatic capillaries is helpful in recognizing these structures and understanding their functions. According to electron microscopic classification three kinds of vascular capillaries, defined primarily by the structure of the endothelium, are recognized: (a) continuous; (b) fenestrated; and (c) discontinuous (i.e. sinusoids).

Examples of continuous capillaries are seen in various sites such as skin, connective tissue, lung and smooth, skeletal and cardiac muscle. Such capillaries have an uninterrupted layer of endothelium. A continuous easily recognizable basal lamina is seen at the base (external surface) of the endothelial cells. Although the basal lamina closely follows the contours of the capillary it does not dip into the numerous micropinocytotic vesicles or the junctions between the endothelial cells, it just bridges across such surface irregularities.

Plate 445

Section through human cornea from a case of post-traumatic hyphaema. Adjacent to the anterior chamber (A) of the eye are seen the corneal endothelial cells (E) resting on Descemet's membrane (D. Limits indicated by arrows) which in this electron micrograph is about 6.5 μm thick. Anterior to this lie the collagen lamellae (C) of the corneal stroma and a fibroblast which in this region is often referred to as a 'keratocyte' (K). ×11 000


C
K
C
D
E
A

Fenestrated capillaries are seen in tissues where rapid exchanges of water and solutes occur (e.g. renal glomerulus; ciliary body; choroid plexus; synovial membrane). This type of capillary is characterized by the presence of pores or fenestrations in the endothelial lining. The pores are bridged by a diaphragm which is composed of a single lamina (not a trilaminar membrane). The basal lamina of a fenestrated capillary is continuous across the fenestrations. The situation in renal glomerular capillaries is more complex in that it shows species-related variations. Diaphragms seem to be the rule in the mouse but in human diaphragms are absent across some fenestrae and present across others (Ericsson, 1968; Rhodin, 1974). It is thought that the basal lamina of fenestrated capillaries (including glomerular capillaries) acts as a selective filtration 'membrane'.

Around the lymphatic capillary the basal lamina is absent or patchy and discontinuous, being interrupted by fine reticular (i.e. collagenous) fibrils (Rhodin, 1974). These features are of some importance because they assist in distinguishing blood capillaries from lymphatic capillaries. The vascular sinusoids of the spleen, bone marrow and lymph nodes are lined by littoral cells but quite large (relatively speaking) 'holes' exist in the wall of the sinusoid. A basal lamina is not seen around such sinusoids.

In mesothelia lining the peritoneal and pleural cavity, the mesothelial cells are separated from the underlying connective tissue by a thin but continuous basal lamina (Rhodin, 1974). Conflicting opinions have been expressed regarding the presence or absence of a basal lamina between the synovial intimal cells and subsynovial tissues. Most authors (Barland *et al.*, 1962; Coulter, 1962; Wyllie *et al.*, 1964; Ghadially and Roy, 1966, 1969; Roy *et al.*, 1966; Roy and Ghadially, 1967) have failed to find a basal lamina in human, rabbit, guinea-pig and rat synovial membrane but in calf, dog and guinea-pig, Langer and Huth (1960) claim that such a lamina exists.★

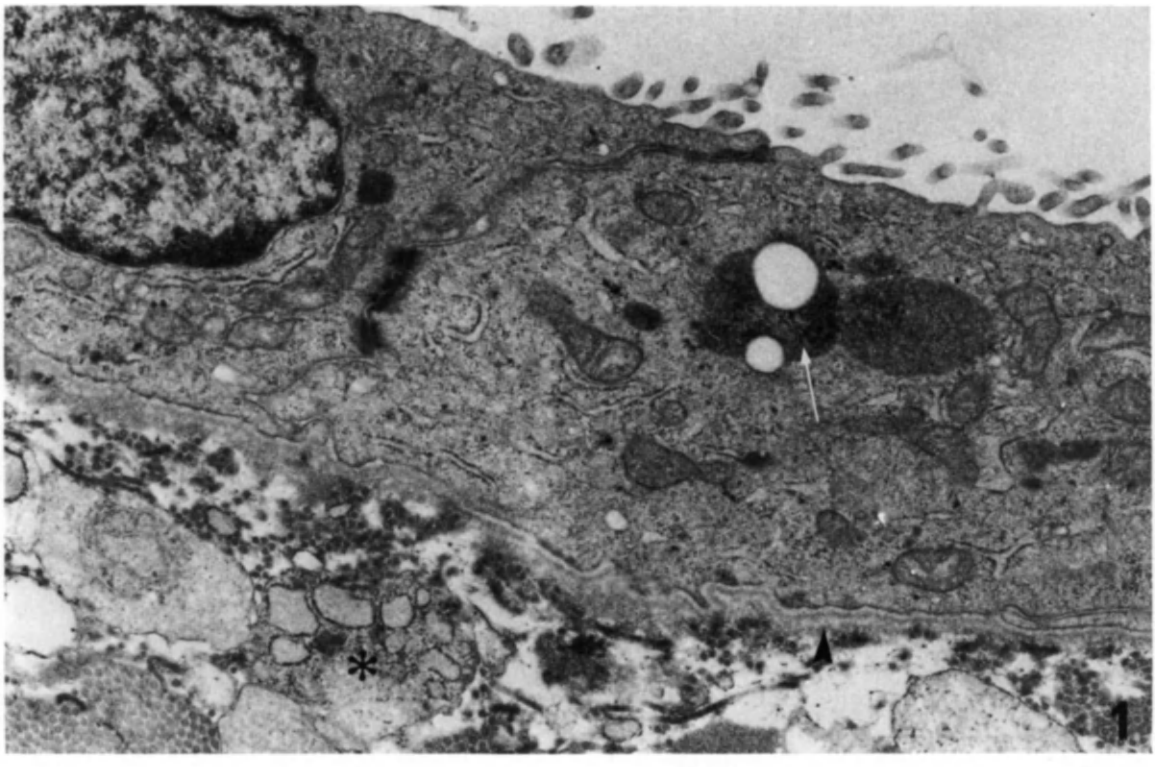
Neither my studies (Ghadially, 1983) nor published electron micrographs have persuaded me to accept that a true basal lamina exists in the synovial membrane between the synovial intima and subintimal tissues. However, at times patches of filamentous matrix material or a collagen fibril lying adjacent to a synovial intimal cell may create the illusion that an interrupted basal lamina or external lamina is present. Wyllie *et al.* (1966) also noted such a phenomenon in the rheumatoid synovial membrane. They state: 'These filaments may be arranged in short basement membrane-like formations when adjacent to the cell membrane'. However, it must be stressed that in fact neither a true external lamina nor a basal lamina is seen in normal or pathological human synovial membrane.

★However in a later study (Huth and Langer, 1965) on rabbit synovial membrane they state: 'As opposed to our previous investigation (Langer and Huth, 1960) we found no indication of basal membrane between these cells'.

Plate 446

Fig. 1. From a lung biopsy of a case of rheumatoid arthritis treated with gold. Pleural mesothelial cells are seen resting on a basal lamina composed of a lamina rara and a lamina densa. In one small area the basal lamina is reduplicated (arrowhead). The reticular lamina is disrupted by fragments (★) of a cellular exudate which comprised mainly of macrophages and plasma cells. The lysosome (arrow) in the mesothelial cell contains filamentous electron-dense profiles which represent gold deposits similar to those shown in *Plate 323, Fig 3.* ×17000

Fig. 2. Synovial membrane from the knee joint of a normal rabbit. Adjacent to the joint space (J) lie synovial intimal cells (S) set loosely in an abundant matrix (M). A cell process of a fibroblast (F) and a small portion of a lipocyte containing a large lipid droplet (L) are seen in the subsynovial region. No basal lamina demarcates the synovial intima from the subsynovial tissue. ×10000 (*From Ghadially and Roy, 1969*)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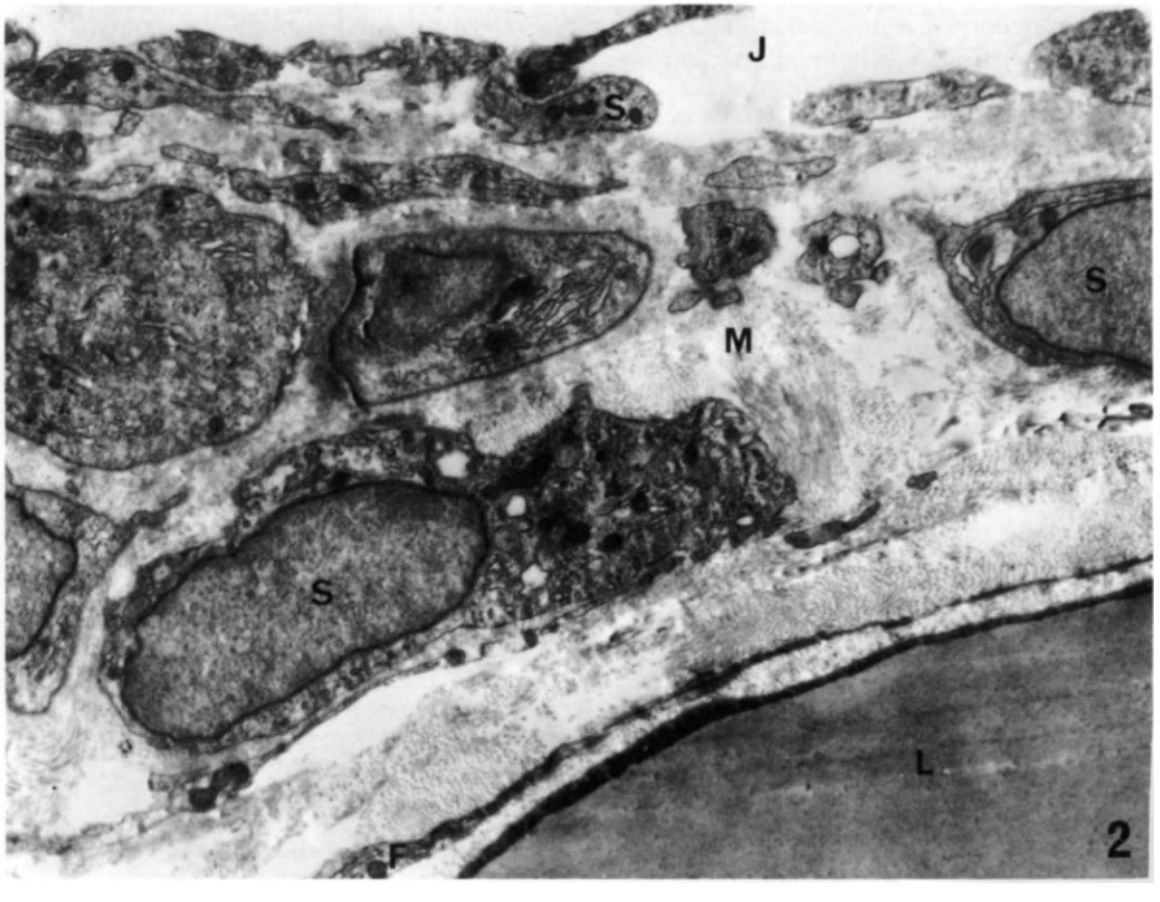
J
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2

Alterations in the basal lamina

A variety of morphological changes are known to occur in the basal lamina in several disease states. Most studies deal with changes in the basal lamina in: (1) tumours (*Plate 447*); (2) renal glomerulus (*Plate 448*); and (3) systemic vessels (found in biopsies of skin and muscle).

The morphological alterations may be classified as follows: (1) thinning or attenuation of the lamina with or without breaks in its continuity; (2) thickening of the lamina; (3) reduplication (also called 'duplication', 'splitting', 'lamination' and 'reticulation') of the lamina; (4) dense deposits in the lamina*; and (5) formed elements in the lamina such as spherical microparticles (pages 1080–1085) and crystals (pages 1086–1089).

These alterations are not specific for any disease state† although when taken in conjunction with other clinical and pathological findings some of them are of diagnostic import. It would be hopelessly repetitious to list the situations and diseases in which each of the above mentioned alterations has been seen because several of the above mentioned changes may be found in a single given disease process or even in a single specimen. Hence it would be better to look at some of the changes seen in certain disease states and note other instances where similar changes have been seen.

The chief interest in thinning and breaks in the lamina has been shown by students of neoplasia (Frithiof, 1969; Gould and Battifora, 1976) for one would imagine that the earliest attempts at invasion by a carcinoma would be heralded by an extension of tumour cell processes through breaks in the basal lamina. While broadly speaking it is true to say that the basal lamina is often thin and broken in many carcinomas, this is by no means invariably so; nor are such breaks diagnostic of malignancy or even of the neoplastic state.

It is now clear that small breaks in the lamina can in rare instances be found in the basal lamina under normal epithelia and one may also find a cell process traversing a gap in the basal lamina.‡ Indeed in both inflammatory and neoplastic states these features are more marked and

*Dense deposits in the renal glomerulus corresponding to the immunofluorescent deposits of immunoglobulin and complement components are seen in immune complex glomerulopathies. They are classified by their location as: (1) subepithelial; (2) subendothelial; (3) intralaminar (i.e. within the lamina densa); and (4) mesangial. It is beyond the scope of this book to deal with these dense deposits, for this the reader should consult standard texts (e.g. Jenis and Lowenthal, 1977). However, the somewhat different (in that they do not contain immunoglobulins or complement components) dense deposits in dense deposit disease are dealt with on page 1066.

†This statement is essentially true, but in Alport's syndrome and in dense deposit disease the ultrastructural changes in the glomerular basal lamina are so characteristic that they are almost diagnostic of these conditions. Hence, separate sections in this chapter are devoted to Alport's syndrome (pages 1064 and 1065) and dense deposit disease (pages 1066 and 1067).

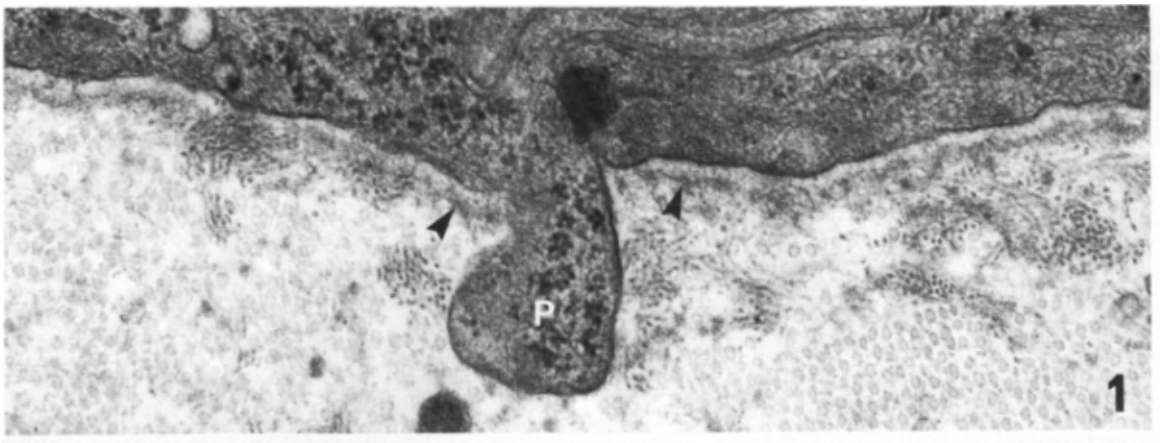
‡According to Rhodin (1967), in small arterioles one rather routinely finds cell processes of endothelial cells penetrating the basal lamina and establishing junctional contacts (called 'myoendothelial junctions') with the underlying smooth muscle cells.

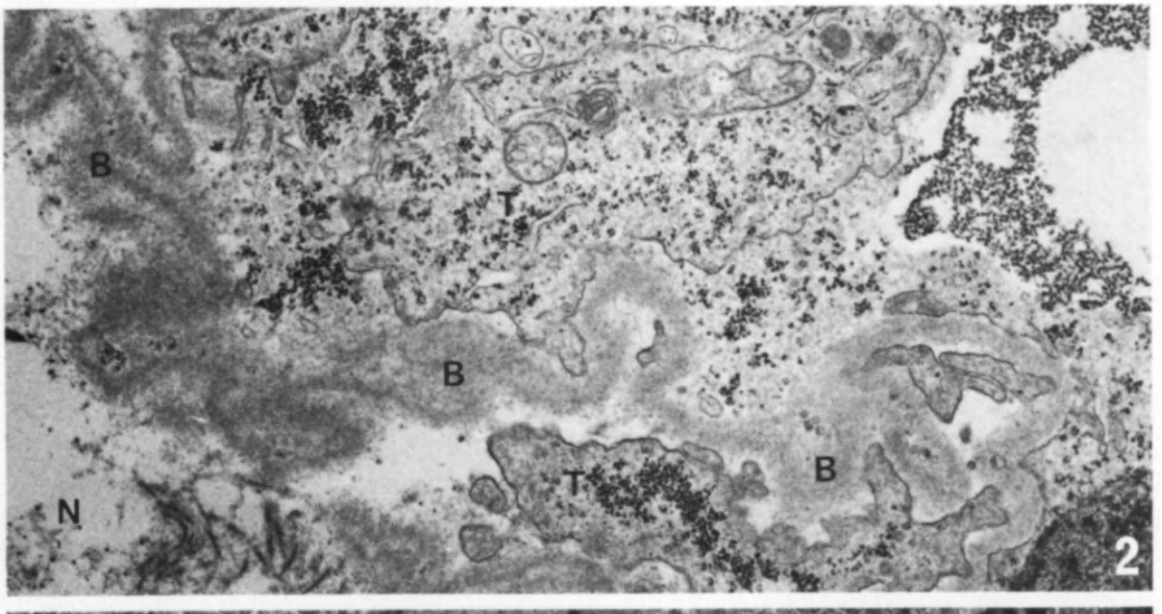
Plate 447

Fig. 1. Adenocarcinoma of the vermiform appendix. A blunt extension or cell process (P) of the tumour cell is seen traversing a break in the basal lamina (arrowheads). ×46 000

Fig. 2. Poorly differentiated carcinoma of the bronchus. A prominent basal lamina (B) is seen at the interface between the tumour (T) and normal tissue (N). ×21 000 (*From Ghadially, 1980*)

Fig. 3. Fibroadenoma of the breast. A reduplicated basal lamina (B) is seen between the myoepithelial cells (M) which lie at the outskirts of the adenomatous elements (not included in the picture) and the fibrous (F) component of the tumour. ×17 500 (*From Ghadially, 1980*)


P
1


B
T
B
T
B
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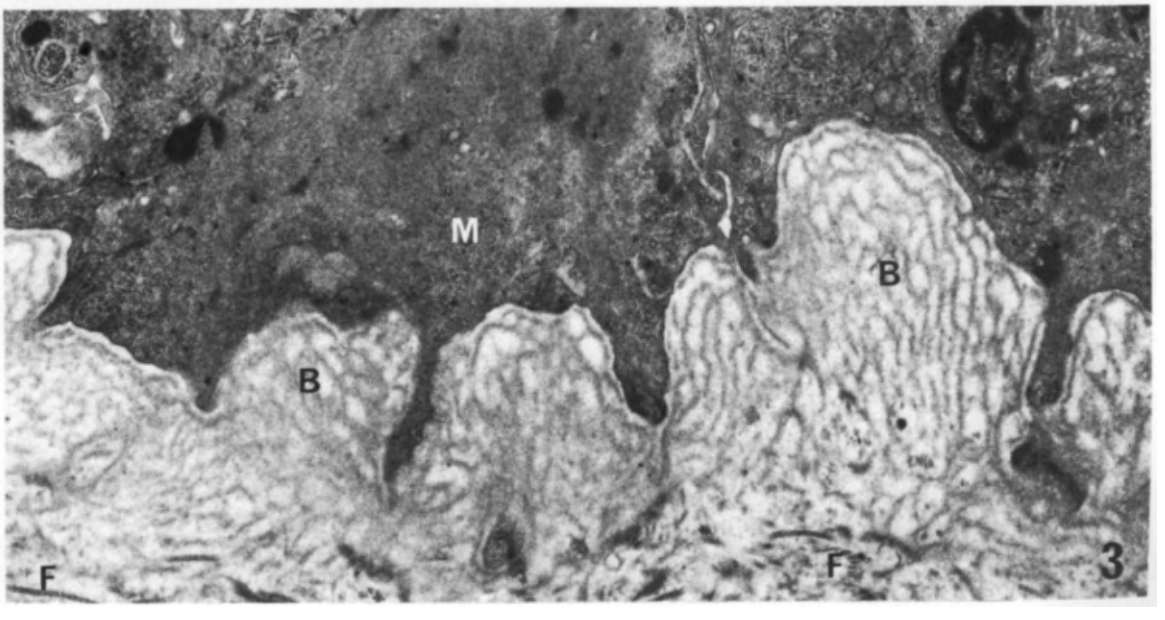
2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frequently encountered. Conversely, the lamina may be thickened and/or reduplicated in some benign or not too aggressive tumours, and it can on rare occasions be quite prominent and well developed near the infiltrating margin of even a highly malignant anaplastic carcinoma. However, it is thought (with some justification) that a reduplicated lamina in a tumour is usually an indicator of innocence and regression or a relatively low level of clinical malignancy.

In passing one may note that attenuation of the basal lamina and/or breaks in the basal lamina of the renal glomerulus have been seen in acute post-streptococcal glomerulonephritis, Goodpasture's syndrome, IgA nephropathy (Berger's disease) and Wegener's granulomatosis. (For references and details *see* Burkholder *et al.*, 1973; Jenis and Lowenthal, 1977.)

A 'pure' thickening of the basal lamina of any magnitude is uncommon, for usually such laminae are split or reduplicated, or contain (and hence are thickened by) dense deposits or other (i.e. formed) deposits (*Plate 448*). Perhaps the best examples of 'pure' thickened laminae are seen when the renal glomerulus shrinks or collapses (e.g. in ischaemia and hypertension). Here, however, the thickening is more apparent than real because the same amount of basal lamina material has to be accommodated in a smaller volume when the glomerulus shrinks. Examples of such thickened laminae are seen in the shrunken glomeruli in the atherosclerotic kidney (*Plate 448*) or in the ischaemic collapsed capillary loops in the glomeruli in progressive systemic sclerosis (Jenis and Lowenthal, 1977).

A thickening of the basal lamina of blood vessels (arterioles and capillaries) in various sites (most studies are on vessels in skin, muscle and kidney) also probably occurs in man and experimental animals with ageing (Bloodworth *et al.*, 1971; Kilo *et al.*, 1972; Pardo *et al.*, 1972) but not all workers accept this (Yodaiken *et al.*, 1969; Siperstein, 1972). Further it is not uncommon to find occasional thickened and moderately reduplicated laminae in normal tissues, particularly the skin.

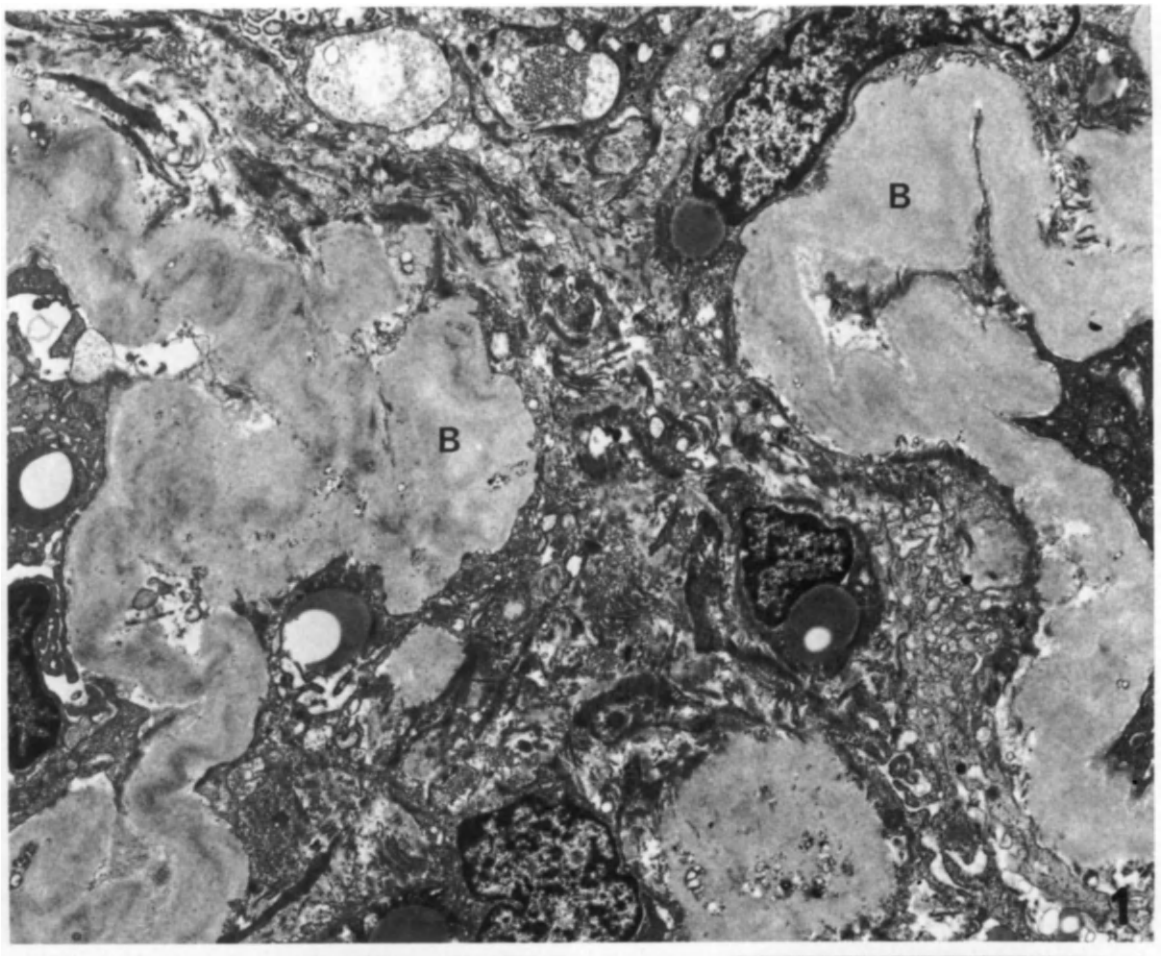
Numerous investigators have shown that a thickening (homogeneous thickening and not infrequently also reduplication) of the capillary basal lamina is a widespread phenomenon occurring in many tissues of diabetics (Osterby and Lundbaek, 1970). This lesion although common among diabetics is not specific for this condition.* First skin biopsies and later muscle biopsies have been extensively used to study this phenomenon. The early changes, if any, are small and in light of technical problems (in assessing the overall thickness of the lamina) not distinguishable from the normal. Hence this method is of no value (Yodaiken *et al.*, 1969; Danowski *et al.*, 1972; Pardo *et al.*, 1972) in diagnosing early diabetes or latent diabetes as claimed by some (Siperstein, 1972). However in the late stages of diabetes this technique provides a means of assessing the state of the capillaries in diabetics. A marked thickening (approx. 400 nm) of the lamina in muscle biopsies of diabetics is said to correlate closely with the presence of retinal lesions and this may at times be helpful when retinal microaneurisms are difficult to detect by ophthalmoscopy (Yodaiken and Pardo, 1975).

*For example, markedly thickened and reduplicated basal laminae have been found in blood vessels in the synovial membrane and skin of patients with rheumatoid arthritis and synovial membrane from cases of villonodular synovitis (Ghadially, 1983).

Plate 448

Fig. 1. From an atherosclerotic kidney. A shrunken glomerulus with a much thickened basal lamina (B). ×5500

Fig. 2. Proliferative glomerulonephritis. Between the endothelium of a glomerular capillary containing an erythrocyte (E) and the podocytes (P) lies a thickened basal lamina containing dense deposits (D) forming subepithelial humps. ×19000


B
B
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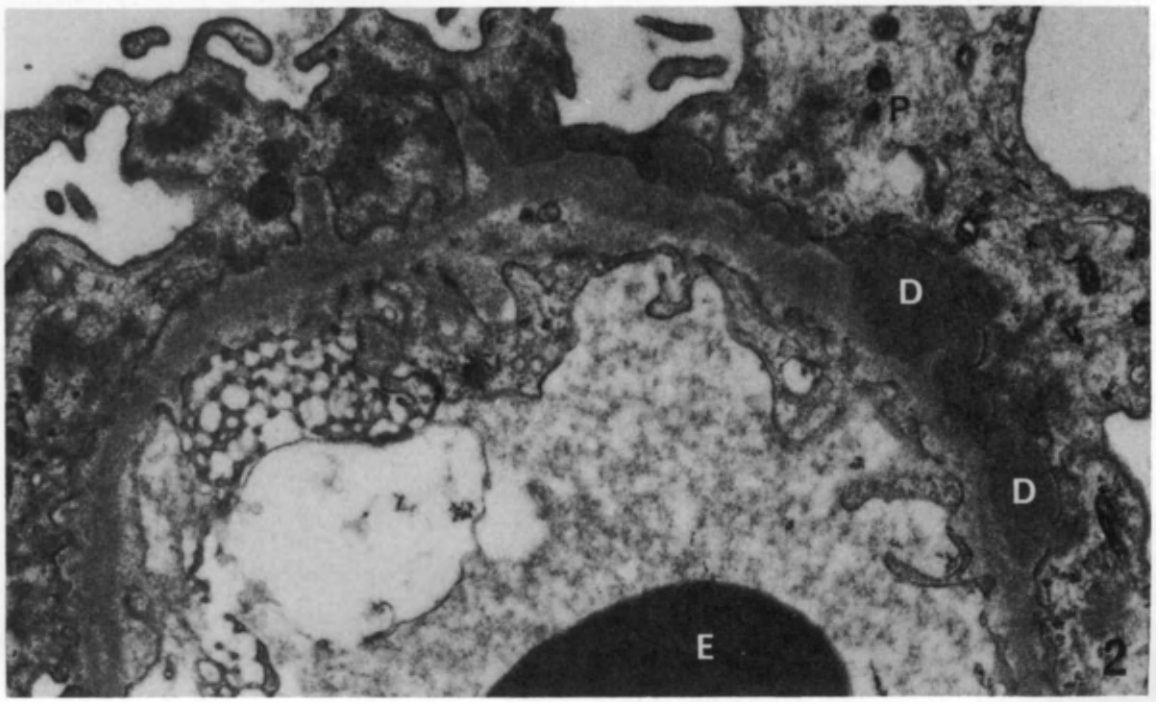
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Basal lamina in Alport's syndrome

Alport's syndrome is a type of hereditary nephritis★ characterized by progressive sensorineural loss of hearing, hematuric nephritis and occasionally also ocular defects and other abnormalities. This disease is transmitted as an autosomal dominant trait. In the early stages the affected children are well except for bouts of macro- or microhaematuria. Renal function tests are normal and histological changes minimal and non-specific. Even at this stage quite characteristic changes in basal lamina (*see below*) are detectable with the electron microscope (Balzar *et al.*, 1976) and this can be of diagnostic value.

Histological examination of renal biopsy is of limited value because various features once thought to be 'typical', or 'distinctive' of this condition are in fact quite non-specific and not always detectable. Such features include the presence of: (1) interstitial and glomerular foam cells; (2) 'fetal' glomeruli; and (3) red cell casts.

In contrast to this are the quite characteristic (but not absolutely diagnostic) and constant (100 per cent of the cases according to some authors, e.g. Zollinger and Mihatsch, 1978) ultrastructural changes found in the basal lamina of glomeruli in Alport's syndrome which develop at quite an early stage of the disease. These changes include: (1) zonal thickening, thinning (attentuation) and fragmentation of the basal lamina; (2) splitting or lamination (also trabeculation) of the lamina densa (i.e. multiple thin electron-dense layers alternating with electron-lucent layers called 'splits'); and (3) presence of spherical microparticles (i.e. small electron-dense granules and vesicles *see* pages 1080–1085) usually in the splits.

Similar changes are seen in the basal lamina of the renal tubules and Bowman's capsule, but they are considered to be not so specific for this condition. The changes in the glomerular basal lamina described above were once thought to be diagnostic of Alport's syndrome†, but this is not absolutely true because similar though less severe and not so extensive alterations of the basal lamina have been seen in: (1) acute poststreptococcal glomerulonephritis; (2) Berger's disease (IgA nephropathy); and (3) benign familial haematuria. Despite these limitations there are now several cases where the diagnosis of Alport's syndrome, suspected purely on the basis of electron microscopy of kidney biopsies, were confirmed by subsequent search and discovery of renal disease in relatives.

Jansen *et al.* (1986) have described a hereditary nephritis in a family of samoyed dogs which resembles human hereditary nephritis (Alport's type). Splitting of glomerular basal lamina was absent at birth. A bilaminar appearance developed within one month and evolved into multilaminar splitting as the disease progressed.

★The group of familial glomerular diseases which are classified under human hereditary nephritis may be divided into: (1) Alport's type in which hearing loss is associated with splitting of glomerular basal lamina with spherical microparticles in the splits; and (2) non-Alport's types in which there is no hearing loss and the glomerular basal lamina may be attenuated, thickened, split or normal-looking. The ultrastructural features of hereditary nephritis have been described by several authors (Hinglais *et al.*, 1972; Churg and Sherman, 1973; Rumpelt, 1980; Gubler *et al.*, 1980, 1981; Piel *et al.*, 1982; Yum and Bergstein, 1983; Grünfeld, 1985; Reznik *et al.*, 1985).

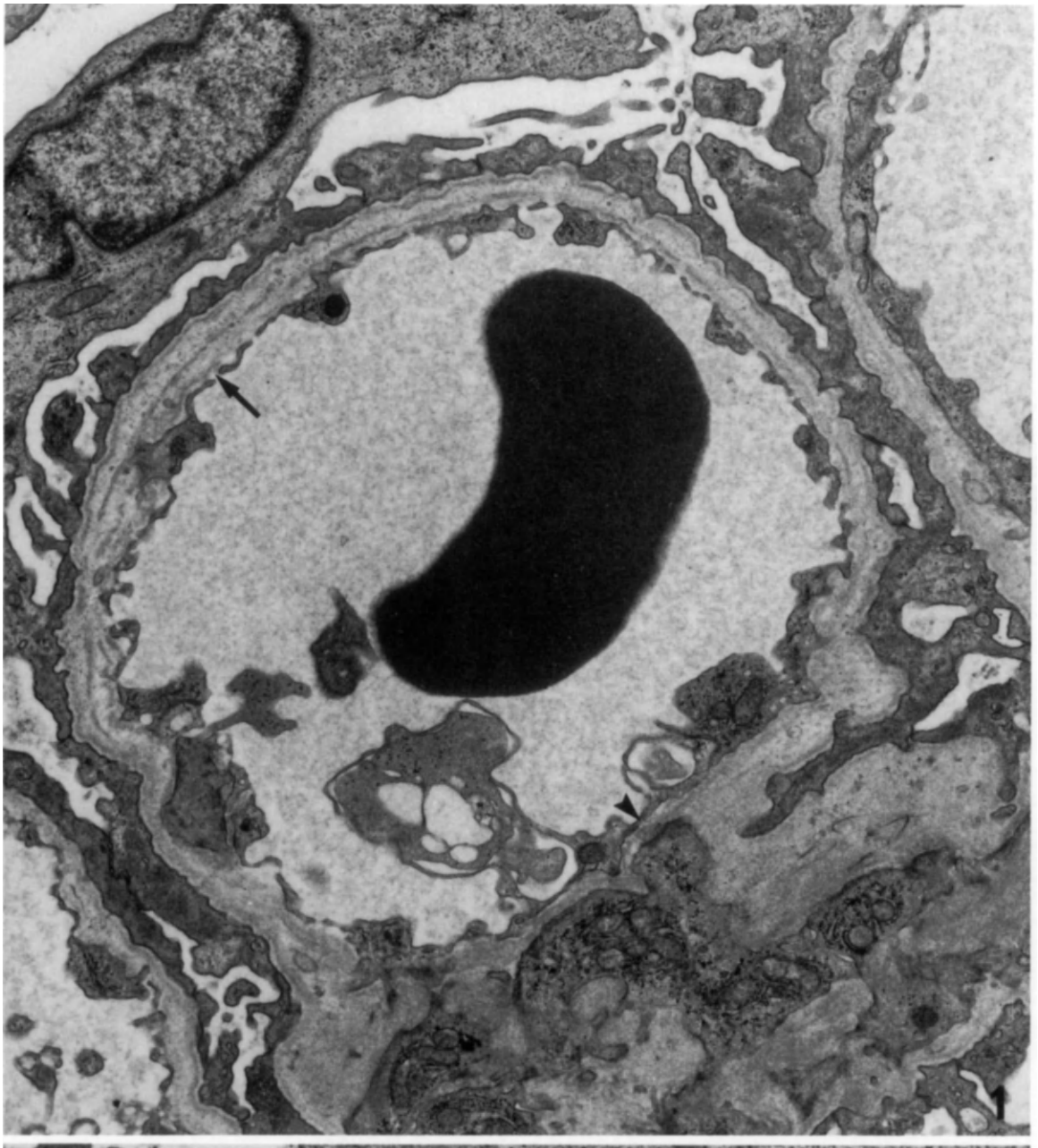
†For details and discussion about the specificity of these lesions see Hill *et al.*, 1974; Kohaut *et al.*, 1976; and Faria and Carvalho, 1984.

Plate 449

Renal glomerulus from a case of Alport's syndrome. (*From a block of tissue supplied by Dr R. Baumal*)

Fig. 1. Focal thickening (arrow) and thinning (arrowhead) of basal lamina. Splitting is not seen too well at this low magnification. ×12 000

Fig. 2. Higher-power view showing splitting of lamina densa. ×25 000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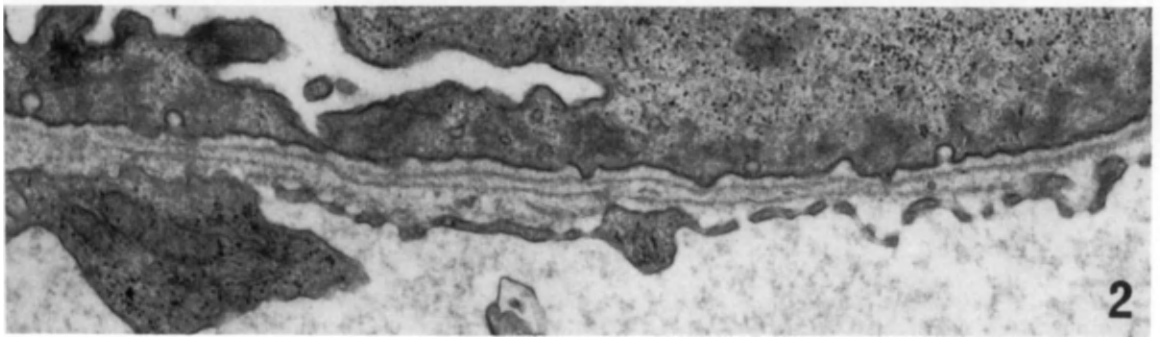

2

Basal lamina in dense deposit disease

Dense deposit disease was characterized as a distinct pathological entity by Galle (1962), Berger and Galle (1963) and Galle and Mahieu (1975) as a 'renal lesion specific of a systemic disease'. This idea is amply vindicated by the fact that dense deposits develop in donor kidneys transplanted into patients with dense deposit disease. Therefore a circulating systemic factor seems to be involved in the production of this disease (Turner *et al.*, 1976; Beaufils *et al.*, 1977).

The principal changes seen with the light microscope in renal biopsy specimens are a generalized increase in mesangial cells and matrix and a ribbon-like thickening of the basal lamina of glomeruli.

The ultrastructural appearances of the basal lamina are both striking and characteristic. In dense deposit disease, the lamina densa of the basal lamina of the glomerulus is thickened (linear or fusiform thickening) and strikingly and homogeneously electron-dense. Similar changes are seen in the basal lamina of Bowman's capsule, renal tubules and arterioles in the kidney. Basal laminae with dense deposits have been found in sinusoids in the spleen, but not in the basal laminae of blood vessels in other organs (Thorner and Baumal, 1982).

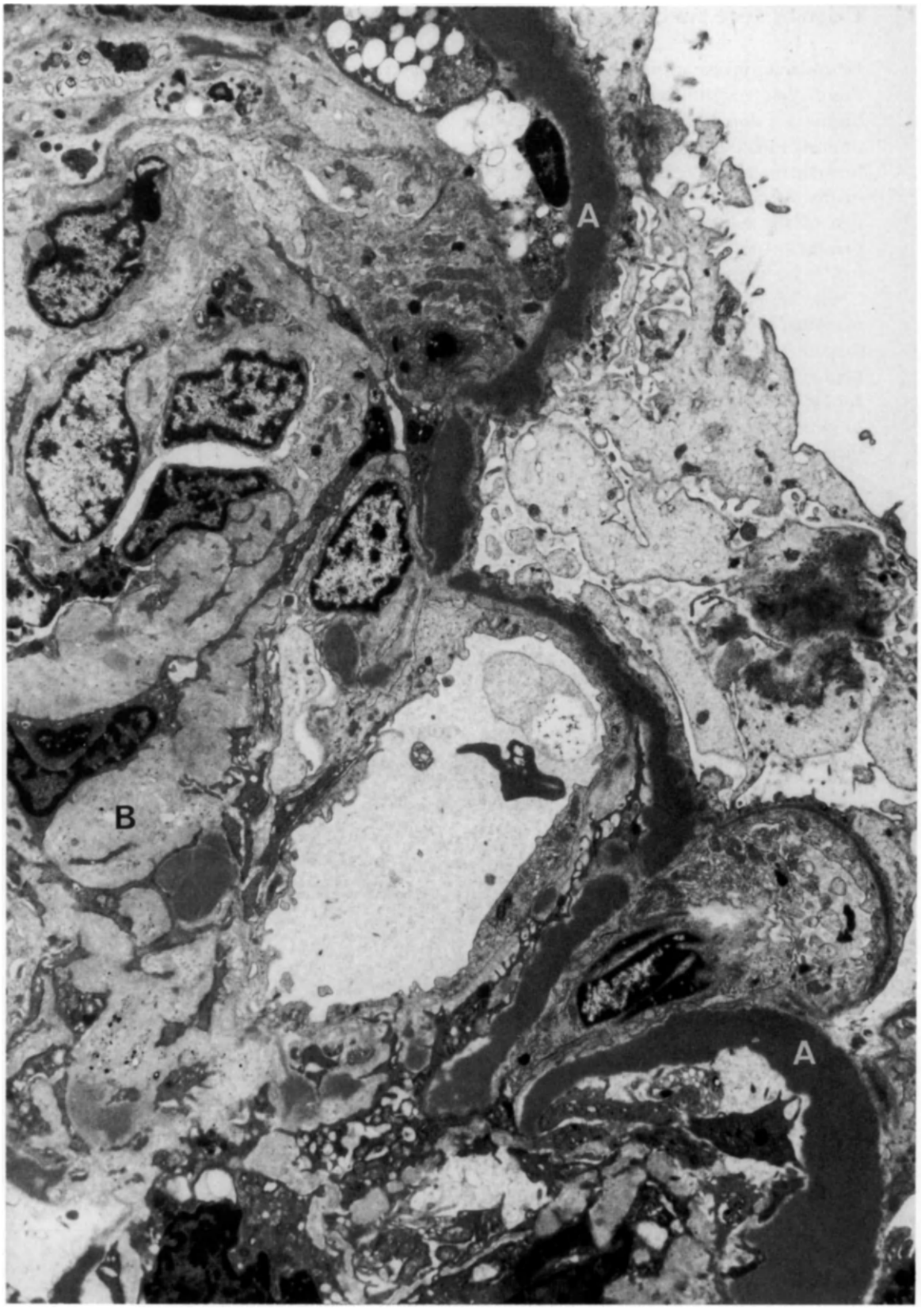
Dense deposit disease is usually a primary or idiopathic entity but it has also been seen secondary to or associated with poststreptococcal infection, lipodystrophy, plasma cell dyscrasias and multiple myeloma (for references *see* Knobler *et al.*, 1983).

Various studies (for references *see* Thorner and Baumal, 1982) have shown that the basal lamina in this condition does not contain immunoglobins or any other protein that is not normally present in the glomerular basal lamina. No differences in amino acid content and carbohydrate content have been detected except for a decrease in the level of cystine and an increase in the level of sialic acid in the lamina from dense deposit disease. Hence it is thought (Thorner and Baumal, 1982) that dense deposits are likely to be altered basal lamina material and not true deposits of exogenous material.

*Apparently the three-dimensional morphology of the acellular glomerular basal lamina (i.e. lamina left behind after removal of cells by sequential treatment with EDTA, Triton X-100, deoxyribonuclease and sodium deoxycholate) as visualized by scanning electron microscopy is also quite distinctive (Weidner and Lorentz, 1986). The lamina is rigid-looking and punctated by crater-like deformities. According to these authors this appearance is 'thus far unique to dense deposit disease'.

Plate 450

Renal glomerulus from a case of dense deposit disease. Homogeneous strikingly electron-dense deposits (A) in the lamina densa have produced discontinuous, linear and fusiform thickening of the glomerular basal lamina. Note also the presence of lamina densa of more or less normal electron density (B). ×5700 (*From a block of tissue supplied by Dr R. Baumal*)


A
B
A

Coat of free surfaces

In routine preparations, the glycocalyx at the surface of many epithelia presents as a more-or-less continuous layer of matted, apparently branching filaments. This layer is most highly developed in the gastrointestinal tract. There are many reasons for believing that this is not just a blanket of mucus poured out by goblet cells. Such reasons include the fact that: (1) the histochemical properties of mucus and the coat are different; (2) the filaments appear to be firmly attached or seem to arise from the plasma membrane, suggesting that they are an integral part of the cell membrane; and (3) in the epithelium of gastric glands the coat is invariably present on the chief cells but lacking on adjacent parietal cells (Fawcett, 1966). This would hardly be likely if the coat was no more than an extraneous substance lying on the free surface.

The best developed and most conspicuous filaments of the free coat arise from the tips of intestinal microvilli. In routine preparations the filaments measure 2–5 nm in diameter and can extend 0.1–0.5 μm beyond the tip of the microvillus. The filaments radiating from the microvilli appear to branch and anastomose to form a network (*see Plate 453*) but such an appearance is probably due to overlapping of filaments in the section thickness, and/or a 'sticking together' of the filaments due to preparative procedures. Such a contention is supported by the fact that in freeze-etched preparations the filaments of the free surface coat (*Plate 451*) pursue a straight parallel course from the tips of the microvilli (Swift and Mukherjee, 1976). Both in routine preparations and in freeze-etched preparations it has been noted that while the thickness of the coat is uniform over an individual cell there are wide variations in the thickness of the coat from cell to cell. In freeze-etched preparations this ranges from no detectable coat on some cells to a coat up to about 0.6 μm in thickness on other cells.

The filaments in the surface coat were first observed by Yamada (1955) on the epithelium of the gall bladder. He called them 'antennulae microvillares'. This term is rarely used now and is not generally applicable because a filamentous coat occurs also on the surface of cells lacking microvilli.

The cell coat of the free epithelial surface of the gut may have many functions but one of these seems to be to filter out large particles, while permitting emulsified lipids and solutes to pass freely for absorption.

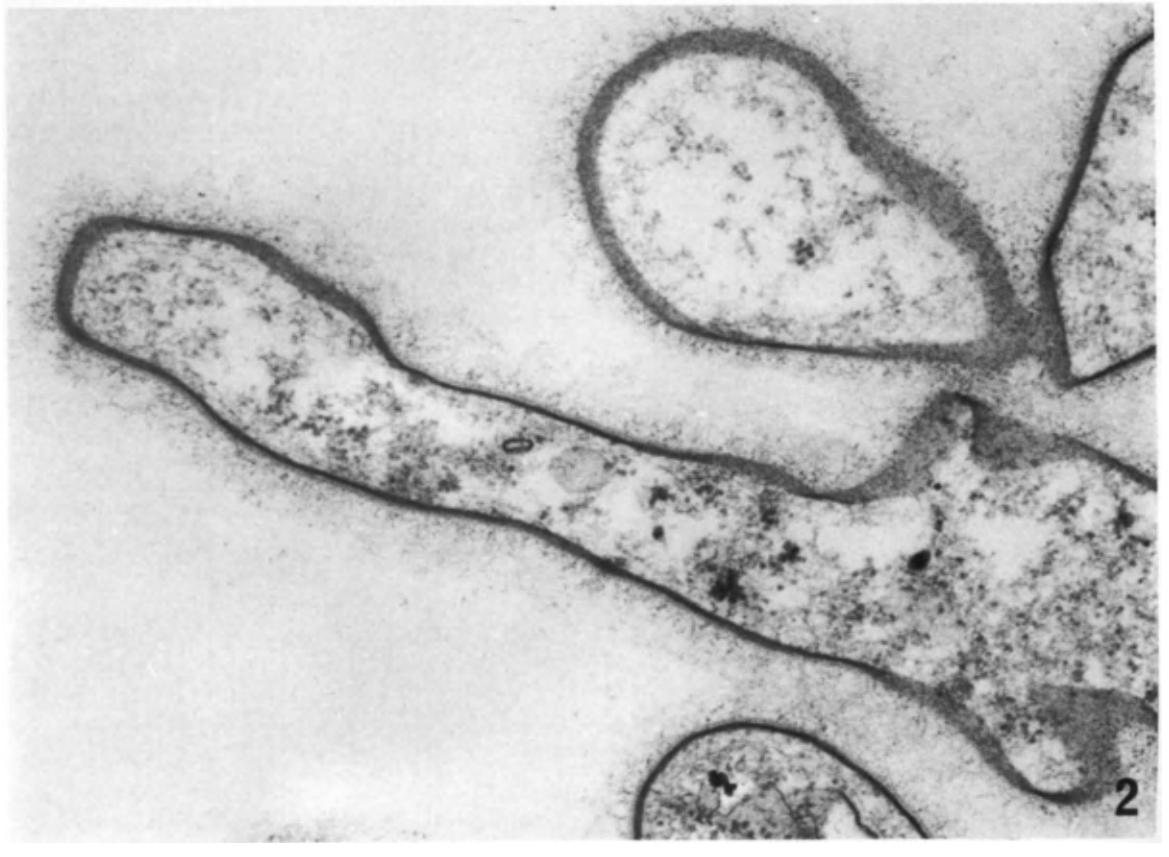
A conspicuous filamentous coat covers the free surface of many unicellular organisms like *Amoeba proteus* (*Plate 451, Fig. 2*). Structurally this coat bears a close resemblance to the free surface coat of epithelia.

Plate 451

Fig. 1. Freeze-etch preparation showing the free surface coat on the luminal surface of the rat rectum. Note parallel filaments which comprise the surface coat. ×56000 (*Mukherjee and Swift, unpublished electron micrograph*)

Fig. 2. A filamentous free surface coat is seen on the pseudopodia of *Amoeba proteus*. ×50000


1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External lamina

Ultrastructural studies show that the basal lamina which constitutes a constant part of the basement membrane occurs not only at the base of epithelia* but also at the base of most endothelia and mesothelia. Further, it has been shown that a similar lamina invests many cells such as muscle cells, Schwann cells and pericytes of capillaries forming an external coat around them, hence it is best referred to as the 'external lamina'. Clearly, terms such as 'basal lamina' or worse still 'basement membrane' are inappropriate for a mantle ensheathing individual cells, but some electron microscopists persist in referring to this layer as the 'basement membrane'. The external lamina is too thin to be resolved by the light microscope, so it cannot be the equivalent of the basement membrane of light microscopy and since it does not have a trilaminar structure it cannot be regarded as a membrane, at least in the electron microscopist's sense of the word.

Various terms such as 'boundary layer', 'glycocalyx' and 'glycoprotein mantle' have been suggested, but the most apt term is the 'external lamina' suggested by Fawcett (1966). The morphology of the external lamina is similar to that of the basal lamina in that here too one can usually discern a lamina lucida and a lamina densa.

The fact that some cells have an external lamina while others do not is a point of some importance in the differential diagnosis of tumours with the electron microscope (Ghadially, 1985). For example, there are times when it is difficult to decide with the light microscope whether a tumour is a schwannoma or a fibroblastic neoplasm. In many such cases the distinction can be made by the presence or absence of an external lamina around the tumour cells. This is because both normal and neoplastic fibroblasts lack an external lamina but an external lamina is readily discerned around normal Schwann cells, and such a lamina usually persists (albeit often in an attenuated and interrupted form) adjacent to the cells of even malignant schwannomas.

Benign schwannomas are as a rule easily diagnosed with the light microscope, but atypical schwannomas or schwannomas in unusual sites are likely to be mistaken for a variety of other tumours. Here electron microscopy is helpful because the external lamina is usually quite well developed (*Plate 452*) and indeed at times excessive so that redundant folds of external lamina† are seen between and around tumour cells. An example of this is seen in the 'ancient schwannoma' where light microscopic features tend to be obliterated by degenerative changes, but where electron microscopic diagnosis is easy because of the abundance of external lamina in the tumour. In passing, one may note that the external lamina seems to be quite a robust structure which persists in autopsy material even when the cells have suffered severe autolytic change.

*The term 'epithelium' (Pl. epithelia) is used by some (e.g. Dorland's dictionary) to cover all examples of tissues composed of cells that cover or line body surfaces and cavities (this includes endothelia and mesothelia). In this book the term 'epithelium' is used in a more restricted fashion as in Butterworth's dictionary where epithelium is defined as 'A closely packed sheet of cells arranged in one or more layers... It covers the external surface of the entire body and lines all hollow structures within the body with the exception of blood vessels and lymphatics, whose lining is properly called an *endothelium*, and serous cavities, whose lining is a *mesothelium*'.

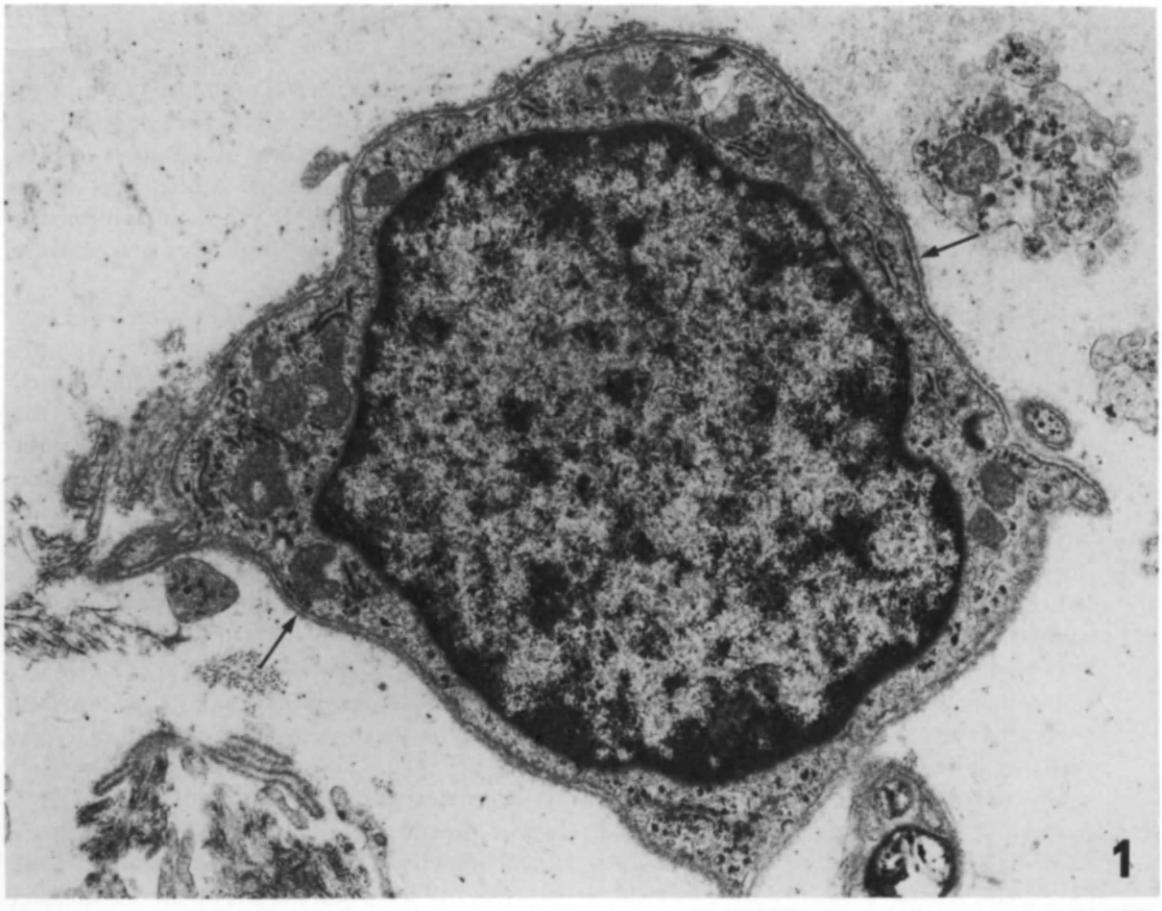
†For illustrations of redundant external lamina in melanotic schwannoma *see Plates 347* and *348*. For illustrations of external lamina in blue naevi *see Plates 346–348*.

Plate 452

From a schwannoma.

Fig. 1. An uninterrupted external lamina (arrows) invests this neoplastic Schwann cell. ×13 000

Fig. 2. Higher-power view of the external lamina. The lamina lucida (L) and lamina densa (D) are easily discerned. ×43 000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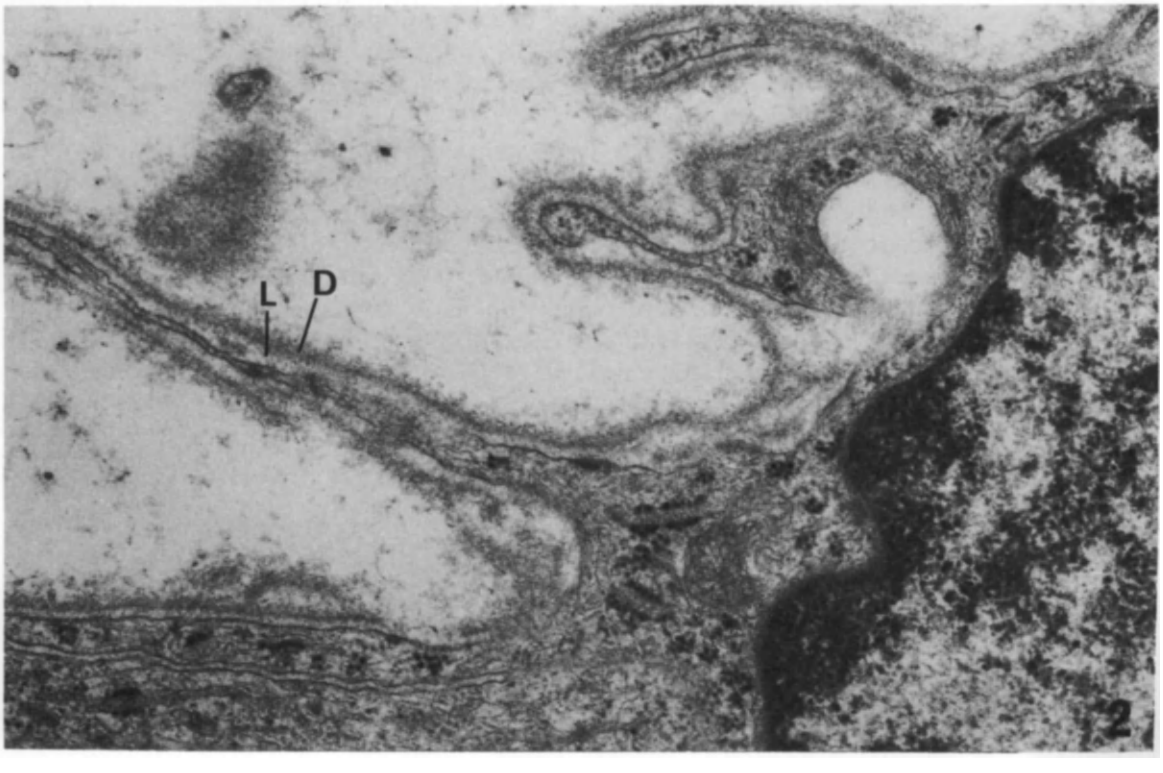
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Glycocalyceal bodies and filamentous core rootlets

Various studies indicate that it is often possible to distinguish colorectal carcinoma from many other adenocarcinomas by the presence of structures called 'filamentous core rootlets' and 'glycocalyceal bodies'. Therefore it behoves us to study the nature and distribution of these structures together in this section of the text, even though filamentous core rootlets do not really belong in a chapter on the cell membrane and its coat. Further, we will have to reconsider R-bodies (described and illustrated on pages 602–605) because it is thought that glycocalyceal bodies probably derive from R-bodies.

Most microvilli have a few filaments in their core, but in microvilli from some sites (e.g. colon) the filamentous core is very prominent and it extends into the cell cytoplasm as a rootlet (*Plate 453, Fig. 1*). It is convenient to collectively refer to this as the 'filamentous core rootlet'. The occurrence of small spherical bodies enmeshed in the glycocalyx on the surface of the microvilli of the colonic mucosa was first noted by Shnitka (1964) in a case of ulcerative colitis. He regarded them as clusters of small 'clavate fimbriae' projecting from the tips of microvilli. Later students of the subject have referred to them as 'C-bodies', 'coccoid bodies' and 'glycocalyceal bodies'. Stone *et al.* (1977) report that the glycocalyceal bodies of the normal and diseased human rectum 'measure 20–80 nm in diameter and are bounded by a 7 nm wide trilaminar membrane'. They found fewer glycocalyceal bodies in ulcerative colitis than in the normal mucosa.

A review of the published electron micrographs shows that these bodies are essentially single-membrane-bound vesicles and that most of the 'solid-looking' bodies probably represent tangential cuts through such vesicles, but a few may be particulate or granular rather than vesicular in nature (*Plate 453, Fig. 1*).

Glycocalyceal bodies have been seen in: (1) normal and pathological colonic and rectal mucosa of man (ulcerative colitis, Crohn's disease, pseudomembranous colitis and radiation colitis) (Rifaat *et al.*, 1965; Gonzalez-Licea and Yardley, 1966; Pittman and Pittman, 1966; Nagle and Kurtz, 1967; O'Connor, 1972; Stone *et al.*, 1977) (*Plate 453*); (2) colon of monkey (*Cynamolgus irus*) (Schofield, 1970) but not rat or mouse (Stone *et al.*, 1977); (3) metaplastic intestinal-type epithelium occurring in various sites (Marcus *et al.*, 1979); (4) respiratory epithelium of human airways* (Afzelius, 1984; Baert, 1986; Ellinger *et al.*, 1987); and (5) certain varieties of adenocarcinomas (*see below*).

Marcus *et al.* (1979) state that their 'study and a review of the literature shows that glycocalyceal bodies have a considerably wider distribution' and that they are not just restricted to the colonic and rectal mucosa†. The highest point in the digestive tract where both filamentous core rootlets and glycocalyceal bodies have been recorded to occur is the distal oesophagus, in patients with gastrointestinal reflux where metaplastic columnar epithelium had developed (Ozzello *et al.*, 1977). The highest point in the respiratory tract where both glycocalyceal bodies and microvilli with prominent filamentous core rootlets have been found is the nasal mucosa (*see Fig. 4d* in Ellinger *et al.*, 1987).

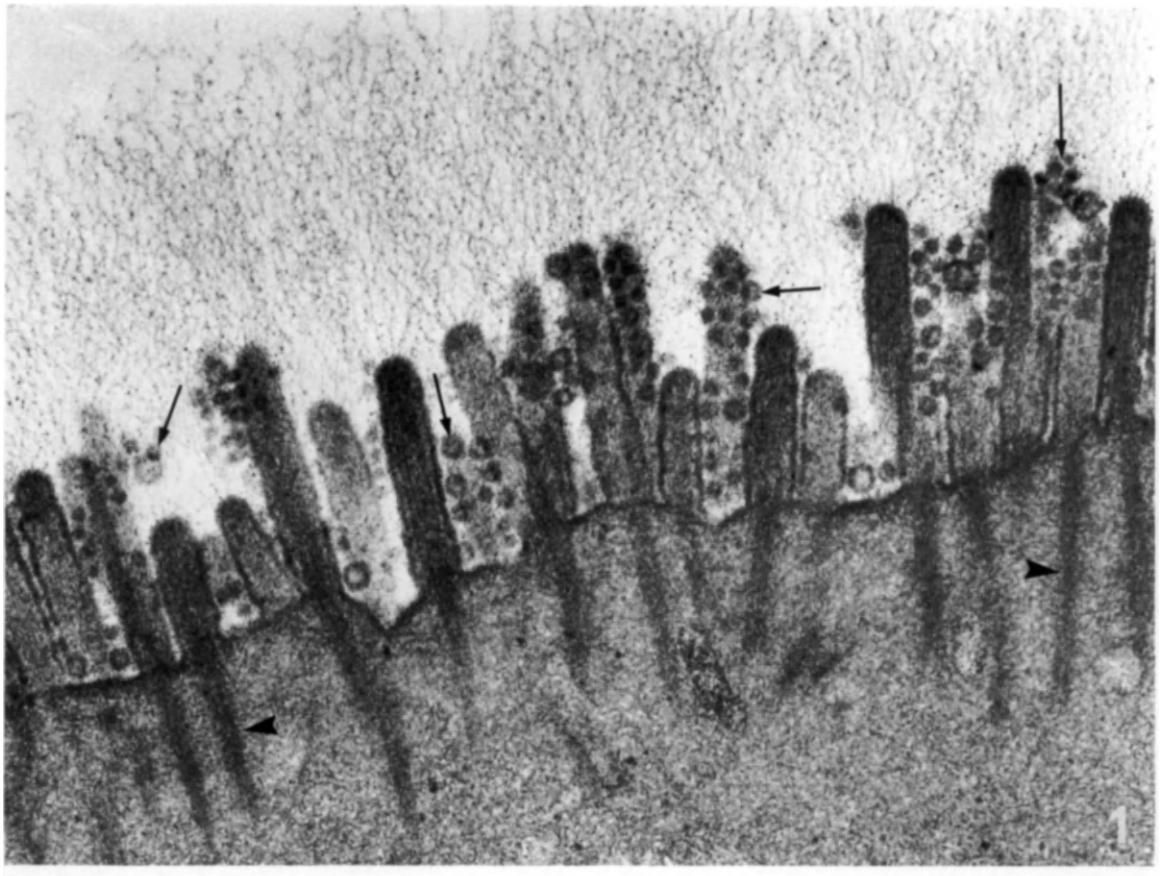
*Apparently glycocalyceal bodies are not found in association with ciliated cells. Afzelius (1984) and Baert (1986) found them on the surface of goblet cells only, but Ellinger *et al.* (1987) found them also on the surface of undifferentiated cells and metaplastic squamous cells. Afzelius (1984) has found a few glycocalyceal bodies on the surface of brush cells of the rat and goblet cells of the dog.

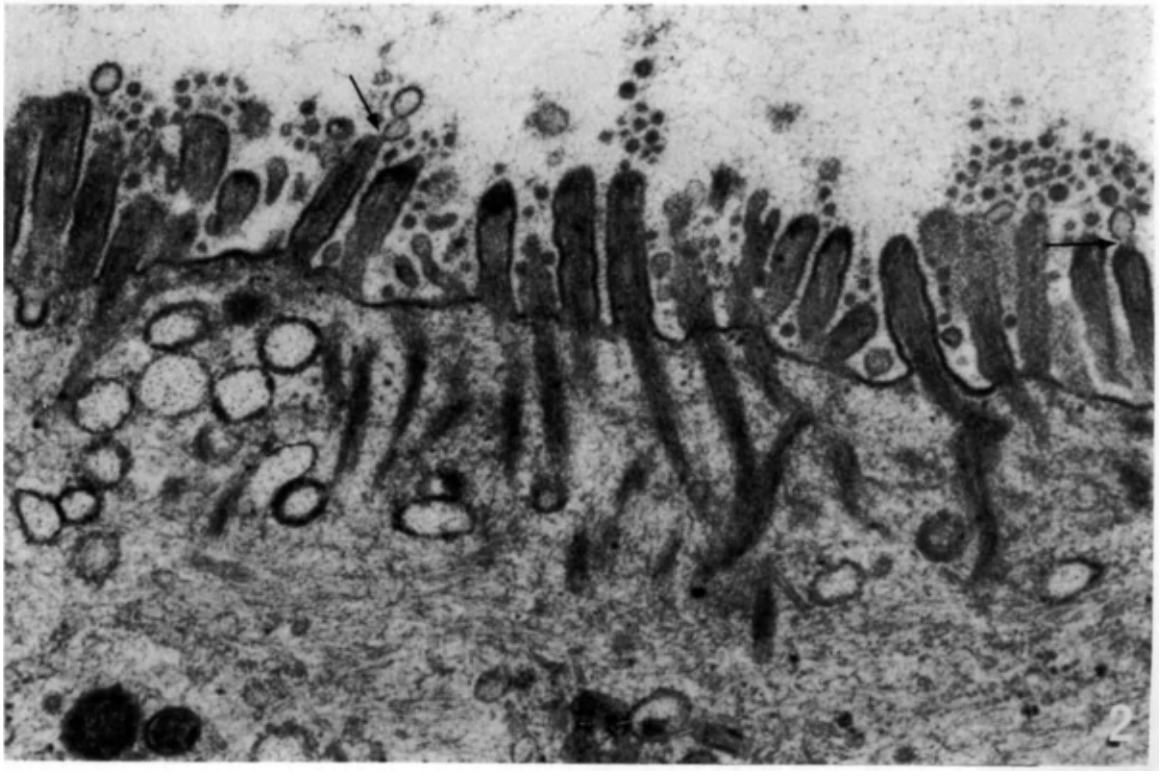
†On the other hand, Mukherjee (personal communication) reports that he has not seen glycocalyceal bodies in the hundreds of jejunal biopsies he has examined.

Plate 453

Fig. 1. Biopsy of colon from a case of pseudomembranous colitis. Glycocalyceal bodies (arrows) are seen on and between the microvilli. Note also the filamentous glycocalyx radiating from the microvilli and the prominent core rootlets (arrowheads). ×46 000

Fig. 2. Carcinoma of colon, showing glycocalyceal bodies and prominent filamentous core rootlets. Appearances suggesting budding (arrows) are seen but this is likely to be an illusion created by the plane of sectioning. ×41 000





Various opinions have been expressed regarding the mode of formation, nature and significance of glycocalyceal bodies in the colonic and rectal mucosa. Steer (1975) thought that they were virus particles and a unique feature of pseudomembranous colitis. This is unacceptable because glycocalyceal bodies have been seen in normal and various pathological states so one can hardly regard them as unique or pathognomonic for pseudomembranous colitis.

Rifaat *et al.* (1965) regarded glycocalyceal bodies as finger-like processes extending from the microvillus or as artefacts. Such views are not attractive because: (1) profiles acceptable as longitudinal sections through the alleged finger-like extensions are rarely if ever seen; (2) some of these vesicular profiles are seen lying quite a distance from microvilli; and (3) such bodies have been shown in replicas of freeze-etched unfixed rectal mucosa (Stone *et al.*, 1977), so they can hardly be fixation artefacts.

The possibility that glycocalyceal bodies arise either by a process of budding, or by degeneration and breakdown of microvilli (Pittman and Pittman, 1966) cannot be completely excluded, but there is no compelling evidence to support such hypotheses. Indeed, Marcus (1981) elegantly demonstrates with the aid of a line drawing that such appearances (i.e. budding) can be created by fortuitous sections through glycocalyceal bodies closely applied to microvilli. Further, he has failed to find a correlation between degenerating microvilli and the presence of glycocalyceal bodies. Biempica *et al.* (1976) found 'distinctive globular bodies ranging in diameter from 0.2–1.5 μm' in the epithelial cells of the human rectal mucosa which they call 'R-bodies'. These single-membrane-bound bodies (i.e. R-bodies) contain round, oval, elongated or rod-shaped vesicles (they call them 'rods') with an electron-dense content. Such bodies were first described by us (Ghadially and Parry, 1966a) as a special type of multivesicular body (*see Plate 259*). They have long been regarded as probably lysosomal in nature, but Biempica *et al.* (1976) could not find acid phosphatase activity in these bodies. They consider, but reject, the possibility that R-bodies are derived by endocytosis of glycocalyceal bodies, while Stone *et al.* (1977) suggest that glycocalyceal bodies derive from R-bodies by a discharge of their contents into the gut lumen. However, Marcus *et al.* (1979) state that 'R-bodies were an inconsistent finding' in the adenocarcinomas which they studied. This seems to suggest that glycocalyceal bodies can form even when R-bodies are absent. However, Marcus (1981) has succeeded in finding a diminutive version of the 'R-body' which may in some cases be the source of glycocalyceal bodies.

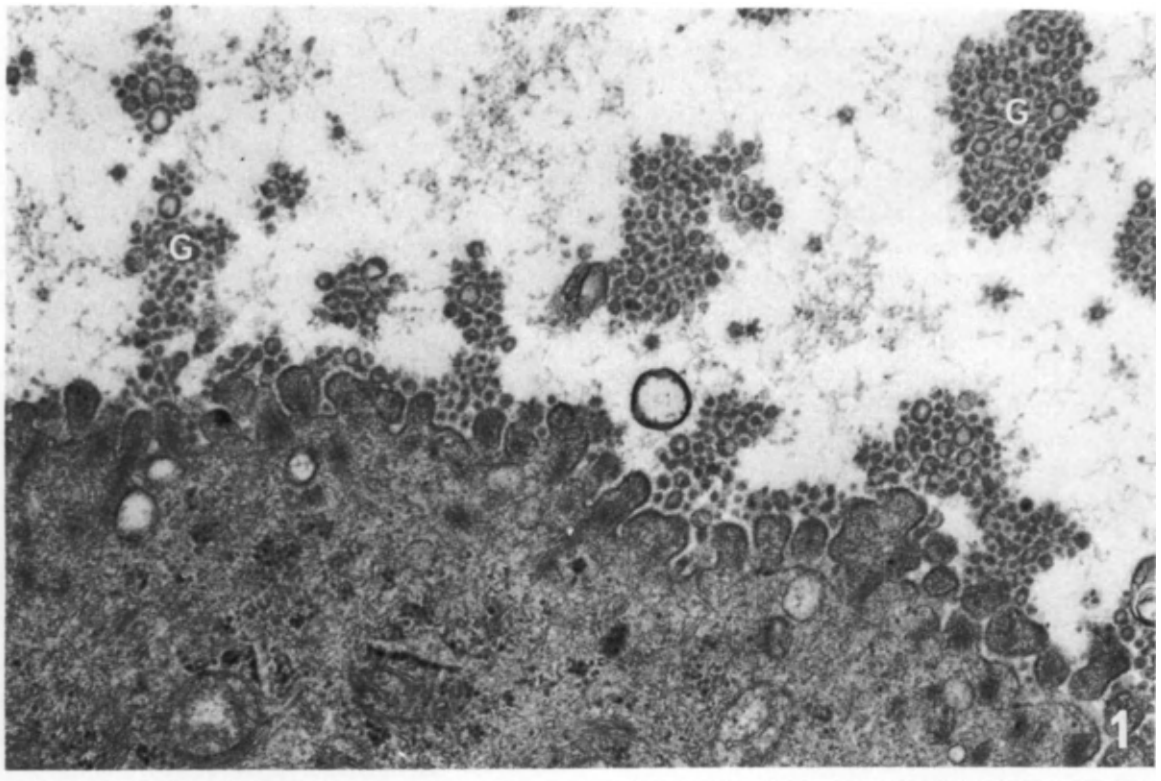
It has been suggested (Baert, 1986) that in human respiratory epithelium, glycocalyceal bodies may derive from excess membrane shedding during exocytosis of mucous granules from goblet cells. This is an attractive idea, but as Ellinger *et al.* (1987) point out, the occurrence of glycocalyceal bodies on the surface of metaplastic squamous epithelial cells argues against this idea. The exact manner in which glycocalyceal bodies form is not known. The formation of glycocalyceal bodies does not appear to be a degenerative phenomenon witnessed by a breakdown of microvilli, but a physiological process whose significance is obscure.

Plate 454

Adenocarcinoma of the vermiform appendix.

Fig. 1. Clusters of glycocalyceal bodies (G) are seen above a tumour cell (T). The short stumpy appearance of microvilli stems from oblique sectioning. ×35 000

Fig. 2. Higher-power view of glycocalyceal bodies lying in the mucus produced by the tumour which contains electron-dense particles (arrowheads) with associated filaments similar to the proteoglycan particles described in the matrix of connective tissues (*see Plate 538*). ×49 000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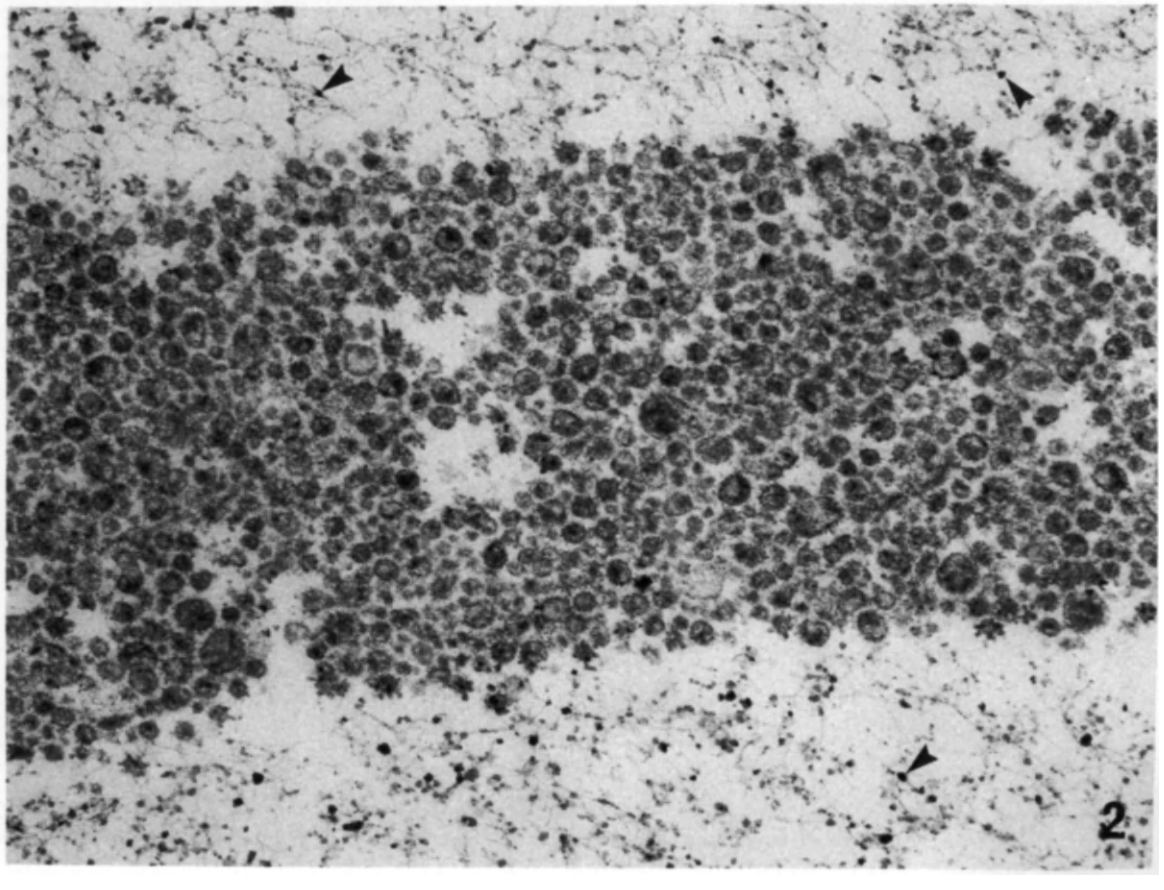
G
1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It will be apparent from the foregoing text that the combination of microvilli with prominent core rootlets and glycocalyceal bodies is seen principally in the mucosa of the colon (and its appendix) and rectum (*Plate 453, Fig. 1*). Therefore, as one would expect, it is principally in colorectal carcinomas (including carcinoma of vermiform appendix) that one finds these structures (*Plate 453, Fig. 2* and *Plate 454*). Thus they are of value in identifying these tumours. The fact that these features persist in secondary deposits (*Plate 455*) further enhances their value as a diagnostic aid (Mukherjee, 1982; Posalaky *et al.*, 1983). These features are seen even in quite poorly differentiated tumours, but then they are considerably more difficult to demonstrate. It seems likely that highly anaplastic colorectal carcinomas may lose the ability to produce these structures, hence the failure to detect glycocalyceal bodies and filamentous core rootlets does not totally exclude the diagnosis of colorectal carcinoma.

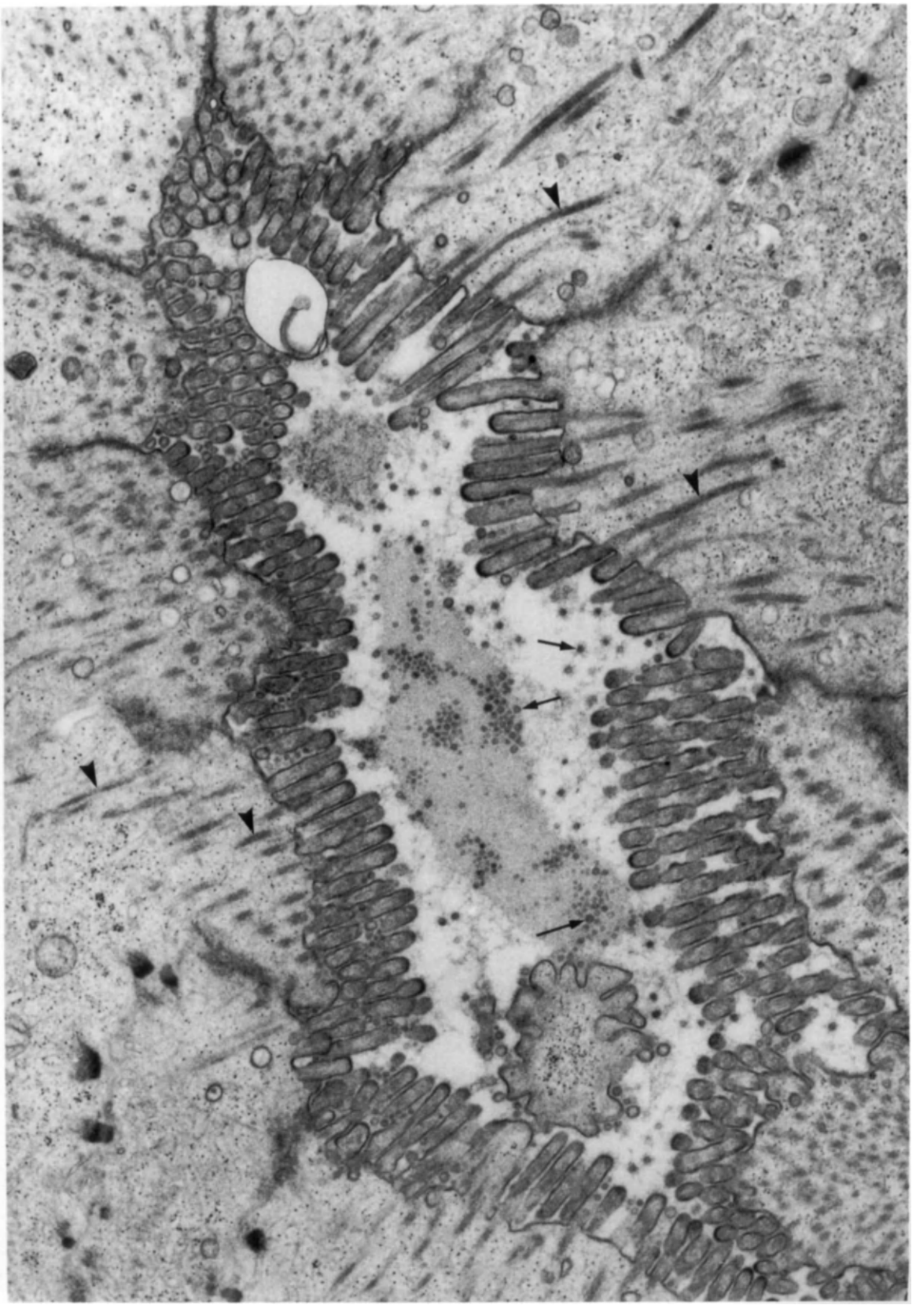
Some caution is needed in diagnosing colorectal cancer on the basis of prominent filamentous core rootlets and glycocalyceal bodies because these features have at times been seen in other tumours. According to Marcus (1981) glycocalyceal bodies plus microvilli bearing filamentous core rootlets have been seen in (besides large intestinal polyps and adenocarcinomas): (1) oesophageal adenocarcinoma; (2) intestinal type gastric adenocarcinoma; (3) small intestinal adenocarcinomas; (4) large pancreatic ductal adenocarcinomas; (5) intestinal type gall-bladder carcinoma; (6) pulmonary adenocarcinomas; (7) urachal type bladder carcinoma; (8) strumal ovarian carcinoids; and (9) carcinomas of anal glands (Marcus, personal communication).

Various other tumours, mucin-producing or otherwise, have not to date shown these features (i.e. glycocalyceal bodies plus filamentous core rootlets). These include tumours of the breast, thyroid, renal tubular epithelium, liver parenchyma, prostate and mesothelium (Marcus *et al.*, 1979; Marcus, 1981). This negative point is of considerable diagnostic value, for in the case of a metastatic carcinoma from an unknown primary site, the finding of these 'intestinal features' (i.e. glycocalyceal bodies plus filamentous core rootlets) would rule out the tumours mentioned in this paragraph.

There has been much debate as to whether pulmonary adenocarcinoma can be distinguished from intestinal and intestinal-type adenocarcinoma on the basis of presence or absence of glycocalyceal bodies and filamentous core rootlets. Seiler and Hickey (1979) and Hickey and Seiler (1981) report that they were able to distinguish these on the basis of 'size, shape and substructure of microvilli, and quantity of glycocalyx and/or glycocalyceal bodies'. However, it seems unlikely that an unequivocal distinction can be made because there are at least two reports where glycocalyceal bodies and microvilli with filamentous core rootlets have been seen

Plate 455

Secondary deposit in a mesenteric lymph node from a carcinoma of the colon. The intercellular lumen is lined by microvilli bearing remarkably long filamentous core rootlets (arrowheads). In the lumen lie glycocalyceal bodies (arrows) admixed with mucus. ×21 000 (*From Ghadially, 1985*)



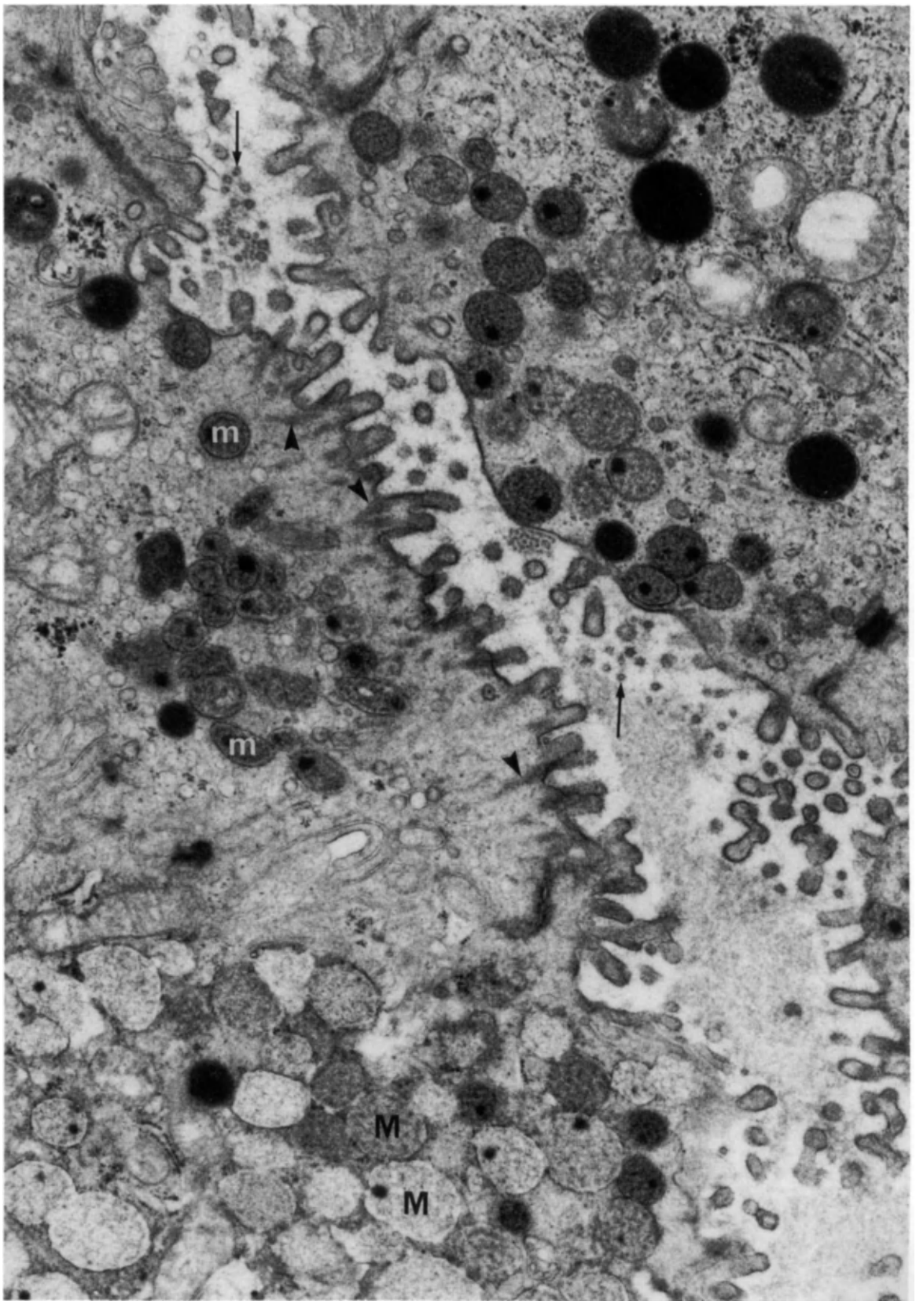
in mucus-secreting bronchiolo-alveolar carcinomas (Kuhn, 1972; Greenberg *et al.*, 1975) and I too have seen this phenomenon (*Plate 456*). Thus from the available data one may conclude that the combination of glycocalyceal bodies and filamentous core rootlets is a very strong, but not infallible marker of colorectal cancer.

One of the problems faced by the diagnostic electron microscopist is that there are some structures which may be confused with glycocalyceal bodies. Clearly, it is important to learn to recognize them if errors in diagnosis are to be avoided. The structures which may cause confusion include: (1) particles of inspissated glycocalyx or mucus. These present as medium density tear-drop-shaped structures with the tip of the tear-drop touching the microvillus surface (*see Fig. 9* in Marcus, 1981). Since these are not membrane-bound vesicular structures like glycocalyceal bodies, they are unlikely to cause confusion if one is aware of the existence of these structures; (2) membrane-bound vesicles occur amongst the microvilli in ducts of sweat glands (particularly the eccrine type) and in tumours of sweat glands. These vesicular structures which are larger and more pleomorphic than glycocalyceal bodies arise mainly from microvilli by a process of 'pinching off' (Hashimoto *et al.*, 1966) or as a 'microapocrine secretion' (Kurosumi, 1977). The microvilli are short, plump, at times V-shaped (branched) and they lack filamentous core rootlets; (3) membrane-bound vesicles derived from disintegration of microvilli (and at times also cell fragments). The vesicular debris formed in this manner varies much in size and shape. The diameter of the larger vesicles equals or exceeds the diameter of microvilli; but the smaller ones resemble glycocalyceal bodies.

Finally, it is worth mentioning that in several tumours prominent filamentous core rootlets have been noted but in which glycocalyceal bodies were not demonstrated. These include: (1) enteric nasal adenocarcinomas (Friedmann and Bird, 1971; Schmid *et al.*, 1979); (2) gastrointestinal tract carcinoids (Warner and Seo, 1979); (3) yolk sac tumours (Nogales *et al.*, 1978); (4) hepatomas (Ghadially and Parry, 1966b; Lapis and Johannessen, 1979); (5) extrahepatic bile duct carcinomas (Marcus, 1981); (6) glandular schwannoma (Uri *et al.*, 1984); and (7) synovial sarcoma (Ghadially, 1985).

Plate 456

Bronchiolo-alveolar carcinoma. A goblet cell containing dark and light mucous granules (M) is seen adjacent to another which contains rather small dark mucous granules (m). The microvilli on this cell bear fairly prominent filamentous core rootlets (arrowheads). Some glycocalyceal bodies (arrows) are present in the intercellular lumen. ×23 000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Spherical microparticles

The term 'spherical microparticles' is used to describe moderately to markedly electron-dense particles and membrane-bound vesicles, with a lucent to electron-dense content, which are found in certain extracellular locations and situations. These particles which measure about 40–100 nm in diameter, have been seen *in vitro* and *in vivo*. *In vivo*, these particles generally occur in small clusters or quite massive accumulations of many thousands of particles, in or adjacent to the basal or external lamina (which may be thickened, displaced or disrupted by these particles), and the connective tissue matrix.

In 1965 we (Ghadially *et al.*, 1965) saw osmiophilic granular and membranous (vesicles, myelinoid membranes and figures) material in the matrix of human and rabbit articular cartilage. We called this material 'matrical lipidic debris' and postulated that it derived from extruded cell processes of chondrocytes (i.e. tips of cell processes breaking off and drifting into the matrix). Matrical lipidic debris is the same as spherical microparticles*. However, quite a large literature has developed about matrical lipidic debris and calcification so this is dealt with separately (pages 1278–1289).

Spherical microparticles were noted by de Tkaczevski (1968) in tissue culture fluids in company with virus particles and in animal sera used for preparing culture media. It was suggested that spherical microparticles may be a variety of lipoprotein. Next it became apparent (Dalton, 1975; Moses *et al.*, 1968) that such particles are found in close association with the surface of many cell types in culture and that some of them are limited by a trilaminar membrane. It was hence suggested that these are not viral particles, but particles derived by budding from the cell membrane and/or degeneration and disintegration of cell processes and cells (i.e. by a process similar to that described by us (Ghadially *et al.*, 1965) in articular cartilage).

Spherical microparticles have been found quite often in the kidney (*Plates 457* and *458*), usually in the glomerular basal lamina, sometimes also in the basal lamina of the tubules and mesangial matrix. Spherical microparticles have been found in the kidney: (1) of normal rats (Johnston *et al.*, 1973); (2) of rats after unilateral nephrectomy and protein overload nephropathy (Lalich *et al.*, 1975); (3) of rats after halothane adminstration (Chang *et al.*, 1975); (4) in nephrotic syndrome (Deodhar *et al.*, 1973; Hyman *et al.*, 1973; Mandal *et al.*, 1974); (5) in hereditary nephritis (Chiricosta *et al.*, 1970; Hinglais *et al.*, 1972; Spear and Slusser, 1972, Churg and Sherman, 1973; Sherman *et al.*, 1974; Hill *et al.*, 1974); (6) allografts (Rowlands *et al.*, 1970; Busch *et al.*, 1971; Zollinger *et al.*, 1973; Olsen *et al.*, 1974); (7) in membranous glomerulonephritis (MacDonald, 1973; Burkholder *et al.*, 1973); and (8) in various other conditions such as diabetic nephropathy, lupus nephropathy, essential hypertension, pyelonephritis, amyloidosis, rheumatoid arthritis and sarcoidosis (Bariety and Callard, 1972; Györkey *et al.*, 1972; Burkholder *et al.*, 1973).

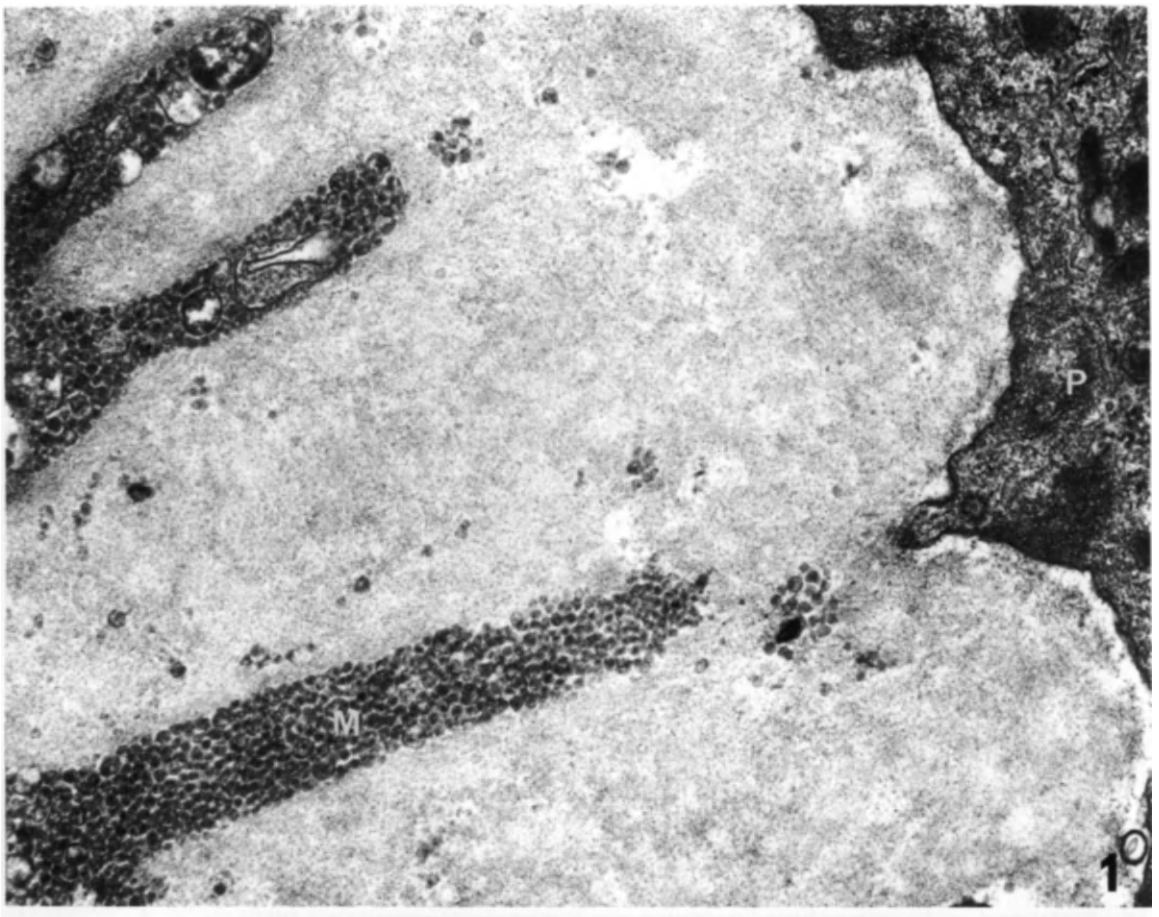
It will be noted from the list presented above that spherical microparticles are seen quite often in hereditary nephritis (Alport's syndrome) (*see* page 1064). Burkholder *et al.* (1973) found such particles in renal biopsies from 55 out of 476 patients. They state that 'the highest incidence was in membranous glomerulonephropathy, lupus nephropathy and focal sclerosing glomerulonephropathy'. Although a few workers have speculated that these might be viral particles most

*Spherical microparticles or matrical lipidic debris contain lipid-rich materials shed from cells and given the correct milieu they will calcify.

Plate 457

Fig. 1. Glomerulosclerosis. Radiating streams of spherical microparticles (M) are seen in a much thickened basal lamina under a parietal cell (P) of Bowman's capsule. ×40 000

Fig. 2. Atherosclerotic kidney. A collection of spherical microparticles is seen in the glomerular basal lamina. ×48 000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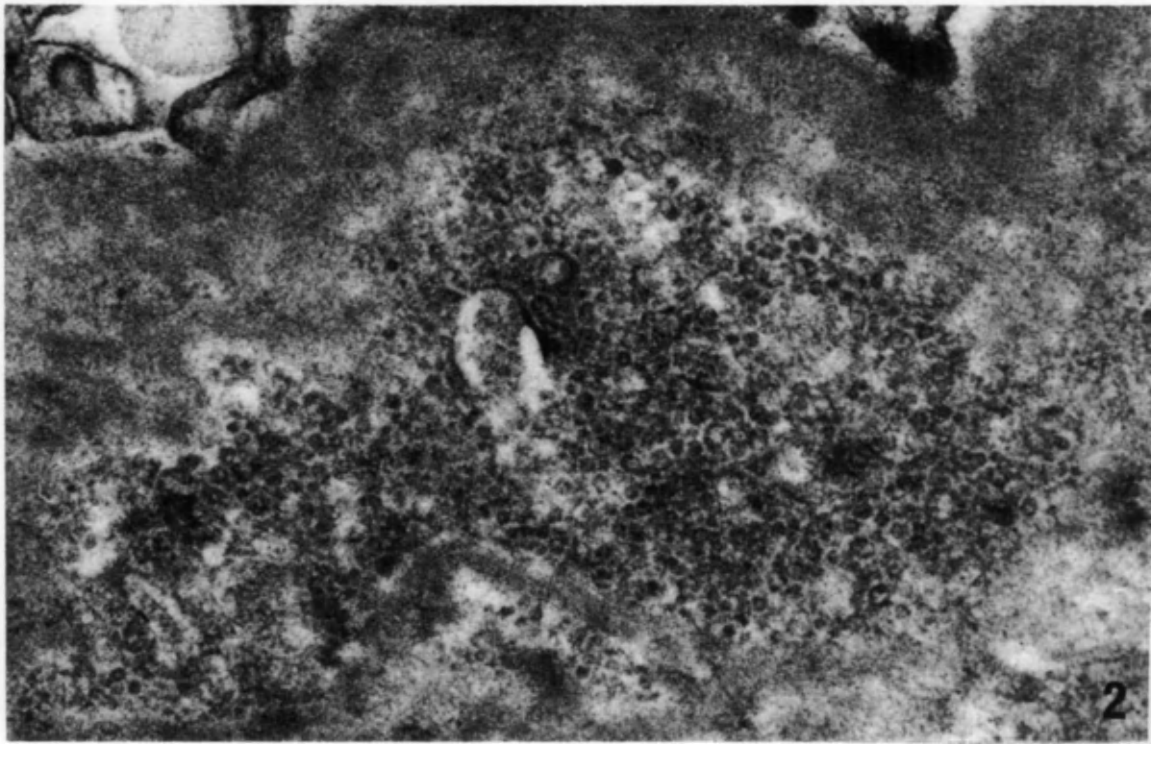
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others have rejected the idea. It is now thought that spherical microparticles represent cell debris or a cellular reaction to injury manifested by a discharge of these particles from the cell. There is also little doubt now that calcified spherules at times seen in the renal basal lamina develop from spherical microparticles and larger fragments cast off from cells. (*Plate 458, Fig. 1*).

Another set of publications focuses our attention on the frequent occurrence of spherical microparticles in arteries. Deposits of such particles occur in and adjacent to the subendothelial basal lamina and between smooth muscle cells (i.e. in and adjacent to the external lamina surrounding these cells). In quite a few instances these particles have also been seen in phagocytic vacuoles. Examples of spherical microparticles in arteries include: (1) normal chicken and rat aorta (Moss and Benditt, 1970; Schwartz and Benditt, 1972a, b); (2) aorta of ageing rats (Cliff, 1970; Gerrity and Cliff, 1972; Kojimahara *et al.*, 1973); (3) coronary arteries of normal, young and ageing rats (Joris and Majno, 1974); (4) human atherosclerotic intracranial arteries (Hoff, 1972); (5) canine intracranial arteries in experimental atherosclerosis (Suzuki, 1972); (6) hypertensive arteries (mesenteric, cerebral, retinal, and coronary) in the rat (Kojimahara and Ooneda, 1970; Takebayashi, 1970; Giacomelli *et al.*, 1972; Wiener and Giacomelli, 1973).

The impression gained from these studies is that while a few spherical microparticles are of normal occurrence, they increase in numbers with age and in pathological situations. Once again it would appear that we are witnessing an accumulation of cellular debris. In hypertension particularly, the accumulation of such particles is associated with focal necrosis of smooth muscle cells.

A few reports deal with spherical microparticles in the heart (atrial and ventricular myocardium). Here they occur: (1) near the surface of muscle cells displacing the external lamina; (2) in widened spaces between intercellular junctions; and (3) within cytoplasmic vacuoles which may be phagosomes.

Spherical microparticles have been seen in the normal heart of chicken, mouse, rat, cat and sheep (Page, 1967; Meddoff and Page, 1968; Page *et al.*, 1969; Grillo, 1970; Sommer and Johnson, 1970); and (2) human heart in various pathological states (valvular disease, congenital heart disease, hypertrophy, degeneration and fibrosis (Ferrans *et al.*, 1973, 1976).

Here again the picture emerges that abundant spherical microparticles are an indicator of cell injury and that these particles may be looked upon as debris cast off by injured cells. Ferrans *et al.* (1976) look upon shedding of small numbers of spherical microparticles as a normal biological phenomenon involved in remodelling of cell surfaces and junctions whereby obsolescent areas of membrane are cast off by budding. The occurrence of much larger numbers of spherical microparticles in diseased hearts probably represents an exaggeration of this phenomenon.

Finally a few sporadic sightings of spherical microparticles in various states is worth recording. Such studies include: (1) human placenta in congenital diabetes mellitus where spherical microparticles were seen near trophoblastic epithelium (Liebhart and Janczewska, 1973); (2) brain and ganglia of a normal embryo insect. (*Oncopeltus fasciatus*) where spherical microparticles were seen near the cell membrane and were thought to have a neurosecretory

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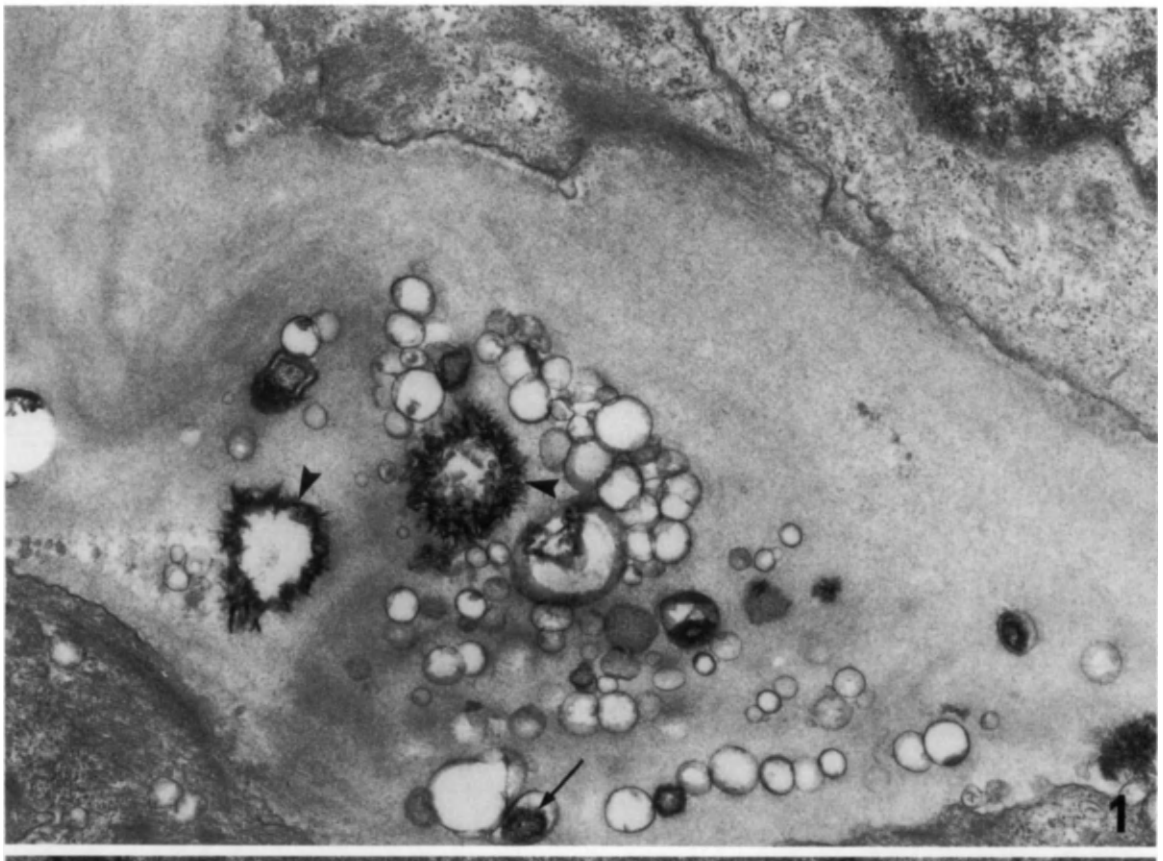
Plate 458

Fig. 1. Glomerulosclerosis (same case as *Plate 457*). Within a much thickened basal lamina lie vesicular structures (i.e. spherical microparticles or cell debris); some of which are calcified. Crystalline (note the needle-shaped calcium apatite crystals) and amorphous calcium salts seen to precipitate either on the surface (arrowheads) or within (arrow) the vesicles. ×23 000

Fig. 2. Adenocarcinoma of vermiform appendix (same case as *Plate 459*). Adjacent to cell processes (P) extending into the matrix lie vesicular (thin arrow) and granular (thick arrow) spherical microparticles and what could be interpreted as larger cast-off cell fragments (arrowhead). ×52 000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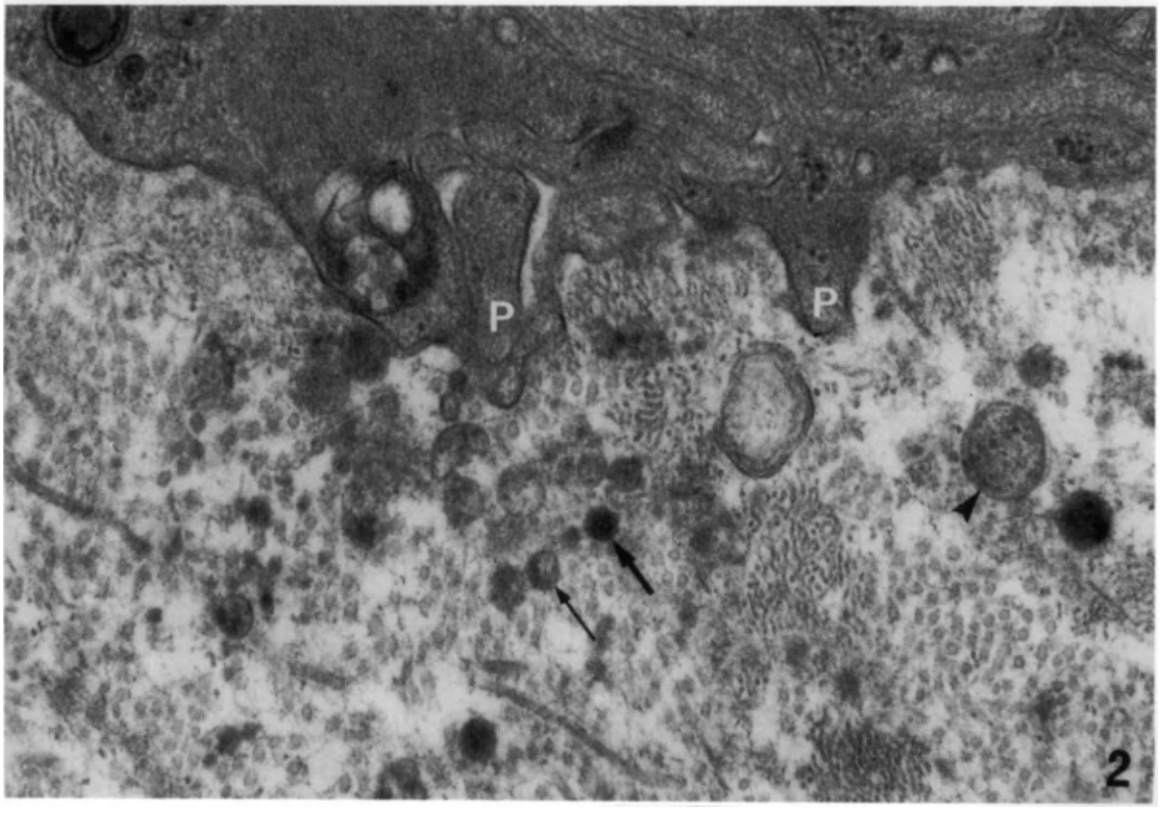
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function (Dorn, 1975); and (3) an adenocarcinoma of the appendix producing a condition of pseudomyxoma peritonei (Ghadially and Lalonde, unpublished observations) (*Plate 458, Fig. 2* and *Plate 459*).

The biopsy specimen of the adenocarcinoma showed areas of matrix containing focal and diffuse collections of innumerable spherical microparticles adjacent to the base of the malignant cells. The basal aspect of the cell membrane of the tumour cells frequently showed small protrusions or cell processes extending into the matrix and it appeared that spherical microparticles were derived by a process of pinching off, or casting off of small vesicles and particles from such cell processes.

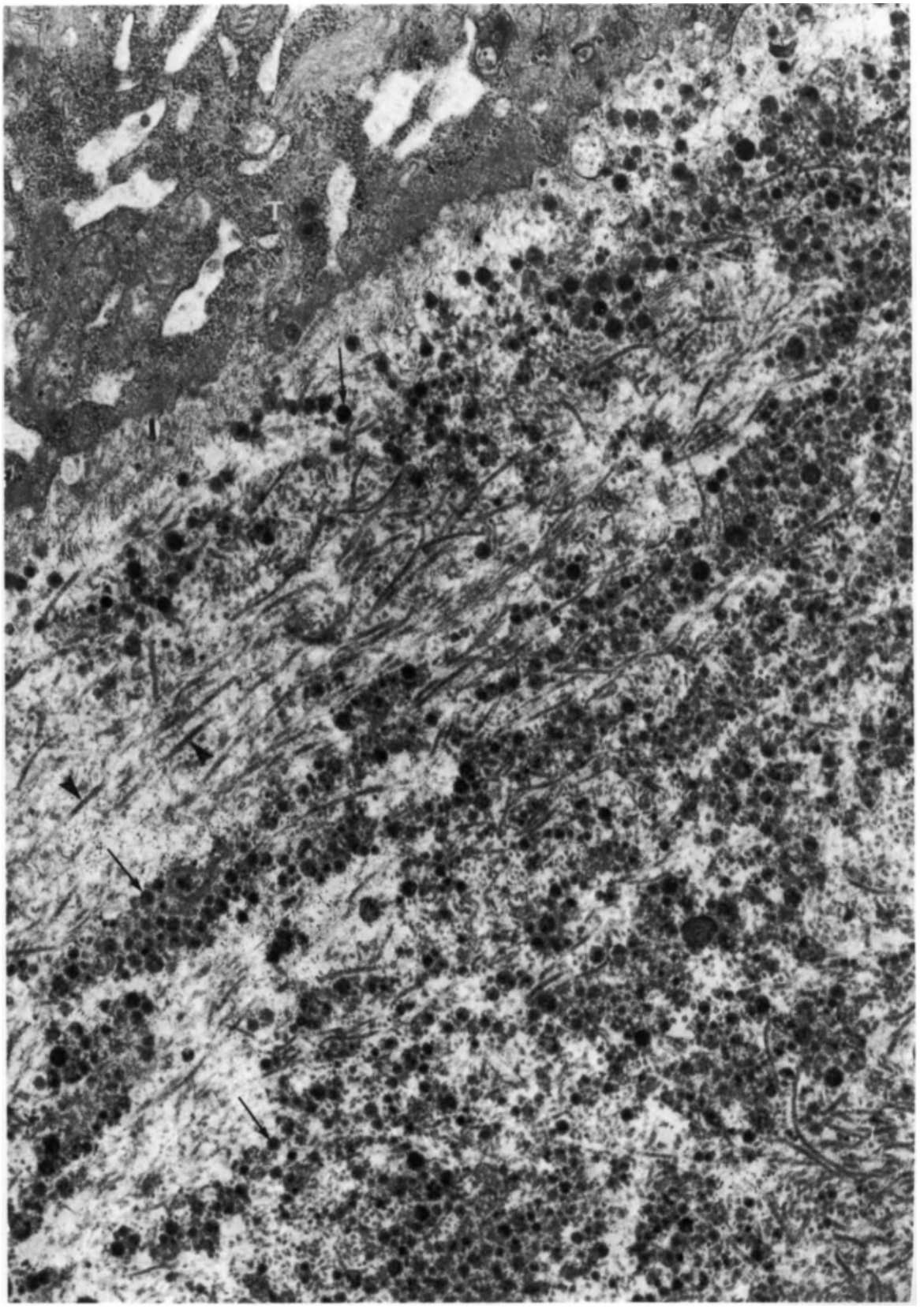
Regarding the nature of spherical microparticles (found in all sites and situations mentioned above) there is now general agreement that they commence as membrane-bound cell fragments which as they degenerate and disintegrate turn into electron-dense particles. Their osmiophilia (as seen in unstained sections) and their origin (i.e. from cell membrane) show that they are lipid-rich structures deserving to be called 'intramatrical lipidic debris' just like the similar or identical structures found in articular cartilage (*see* pages 1278–1289).

Spherical microparticles identical in appearance to those illustrated in *Plate 459* were found in colorectal carcinomas by Carr *et al.* (1986) and illustrated by them in their *Fig. 11*. Apparently unaware of the existence of an entity called 'spherical microparticles' (despite the fact that there are well over 100 papers on this topic) they conclude that these structures are 'lysosomal granules'★, which are 'presumably released from the cells'. This is absurd because: (1) acid phosphatase has not been demonstrated in these structures (but alkaline phosphate has; *see* page 1282) despite several attempts to do so by students of cartilage; (2) destruction (lysis) of matrix components is not evident adjacent to these structures, as would be expected if hydrolytic enzymes were released at this site; (3) lysosomes are intracellular vesicular structures, while the spherical microparticles are extracellular structures which can be granular or vesicular. The granular ones resemble osmiophilic lipid droplets while the vesicular ones usually contain cytoplasmic matrix and some ribosomes and not heterogeneous electron-dense contents which one expects to see in lysosomes; and (4) if the spherical microparticles are lysosomes, one would expect the tumour cells to contain many such 'lysosomes' but they do not. In fact electron-dense bodies acceptable as lysosomes were rarely encountered in the tumour illustrated in *Plate 459*.

★Because of reports of increased proteolytic enzymes in some tumours, it has become fashionable to assume that tumour infiltration is engendered by enzymic (presumably lysosomal) destruction of normal tissue adjacent to the infiltrating tumour, despite the fact that at least seven ultrastructural studies have failed to visualize such a destruction (for references *see* Gabbert *et al.*, 1987). The appearances seen are more in keeping with the idea that a gradual atrophy of normal tissue occurs and that it is probably engendered by a competitive withdrawal or 'starving' of normal cells of nutrients by tumour cells as proposed by us (Ghadially and Wiseman, 1957; Wiseman and Ghadially, 1958) and now reiterated by others (Gabbert *et al.*, 1987). The ludicrousness of the proposition that spherical microparticles are lysosomes responsible for tumour infiltration is also evidenced by the facts that spherical microparticles are seen much more frequently in non-neoplastic than neoplastic tissues and that tumours as a class (i.e. barring rare exceptions like granular cell myoblastoma) are poorly endowed with lysosomes. All this does not preclude the possibility that enzymes (e.g. collagenases) also play a role in tumour infiltration, but if enzymes are involved they would most likely be non-lysosomal enzymes which operate at pH values found in tissues and not lysosomal enzymes which operate in a markedly acidic milieu.

Plate 459

Adenocarcinoma of vermiform appendix (same case as *Plate 458, Fig. 2*). Innumerable spherical microparticles (arrows) intermingled with collagen fibrils (arrowheads) are seen in the matrix adjacent to a tumour cell (T). ×24 000


T

Crystals in basal lamina (striated lamellar structures, fibrin and others)

Crystalline structures at times found in the basal lamina may be divided into three groups: (1) striated lamellar structures; (2) crystalline fibrin deposits; and (3) other crystalline structures.

The term 'striated membranous structures' has been adopted by many workers to describe certain sheet-like extracellular structures which have been found in the basal lamina and on rare occasions in the connective tissue matrix. In sections they present as ribbon-like formations (profiles) with a striated pattern of about 10 nm periodicity (*Plate 460*). Usually these structures range in thickness from about 12–25 nm, but there are instances where thicknesses of 30 nm (Bariety and Callard, 1972, 1975), 40 nm (Stekhoven and van Haelst, 1973) and 55 nm (Osterby, 1972) have been reported. These structures do not show the characteristic trilaminar structure of cytomembranes. Hence, it would be preferable to call them 'striated lamellar structures', or more fully as 'extracellular striated lamellar structures' if needed, because as we have seen (page 1002) banded or striated structures have also been found in the cytoplasmic matrix.

Striated lamellar structures have been found in: (1) basal lamina of renal glomeruli and tubules (also at times in the mesangial matrix and occluded capillaries) in diverse nephropathies (Nagle *et al.*, 1969; Osterby, 1972; Bariety and Callard, 1972, 1975; MacDonald, 1973; Stekhoven and van Haelst, 1973; Olsen *et al.*, 1974); (2) Bruch's membrane in the eyes of patients with intraorbital tumours, diabetic retinopathy, glaucoma and perforating injuries of the globe (Garron, 1963; Lerche, 1967); (3) basal lamina and connective tissue in myocardium, endocardium and valves of patients with various heart diseases (Renteria *et al.*, 1976).

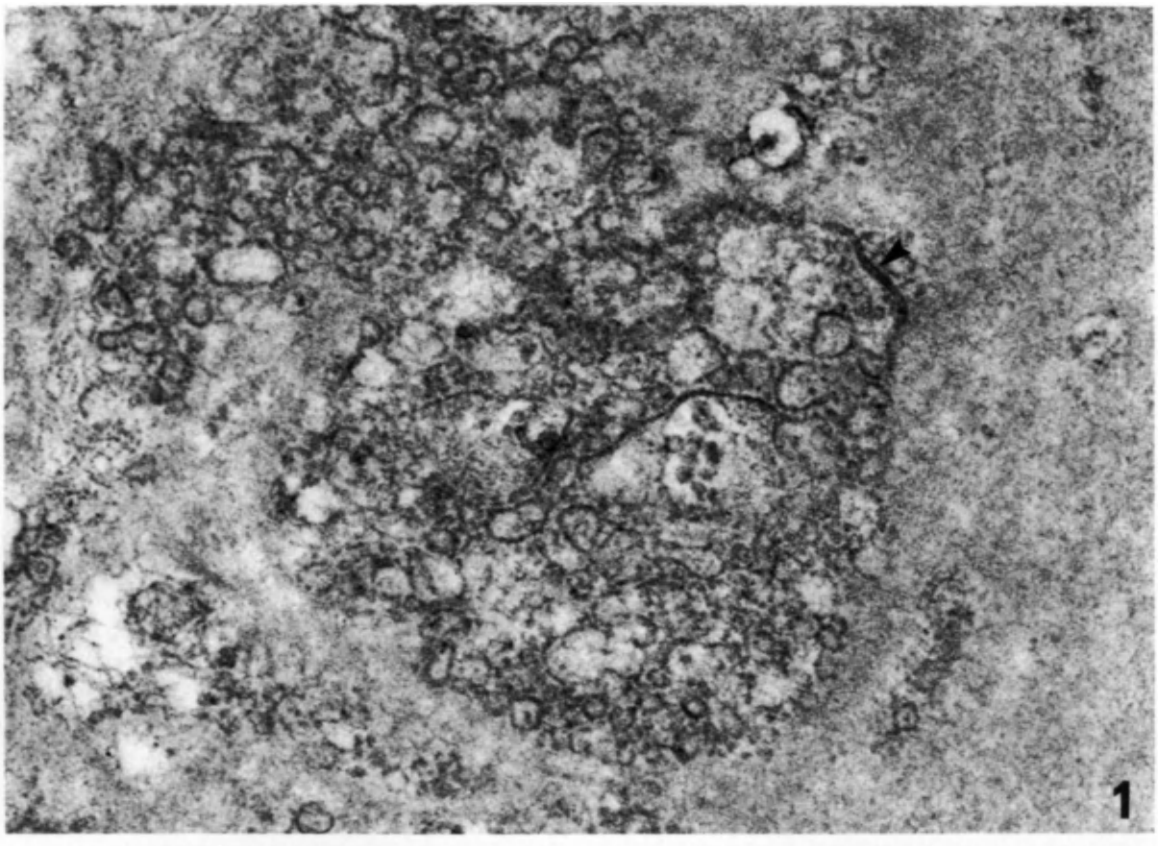
The mode of origin of striated lamellar structures is obscure but several hypotheses have been proposed. They have been thought to originate from: (1) atypical polymerization of the basement membrane glycoproteins; (2) material synthesized and secreted by cells; (3) protein subunits of viral origin; (4) altered spherical microparticles (described on pages 1080–1085); (5) modified Type IV collagen.

Plate 460

Human kidney. Focal fibrosis.

Fig. 1. Within a thickened glomerular basal lamina lie several striated lamellar structures. They present several configurations—linear, C-shaped and ring-shaped. Note also the variations in thickness. The one indicated by an arrowhead is about 23 nm thick. ×42 000

Fig. 2. High-power view of striated lamellar structures of about the same thickness as that indicated by arrowhead in *Fig. 1.* ×86 000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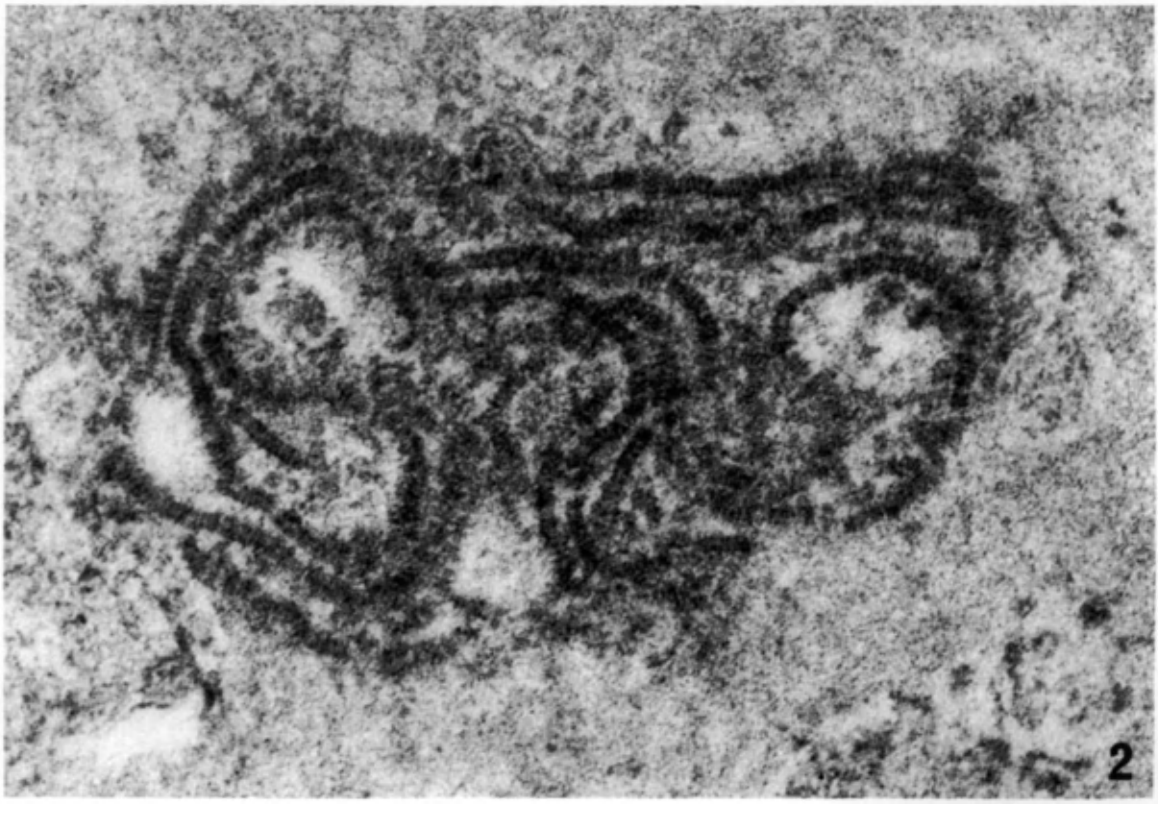
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The only attractive hypothesis is the last one. Type IV collagen is a normal constituent of the basal lamina but normally it does not form collagen fibrils. It can, however, be induced to form segment-long-spacing collagen aggregates *in vitro* (Kefalides, 1973). It is, therefore, possible that alterations engendered by some pathological states could alter Type IV collagen and/or its milieu and permit polymerization to occur *in vivo*. This combined with the fact that under certain circumstances tropocollagen can polymerize to form collagen sheets (O'Hara *et al.*, 1970; Hay and Dodson, 1973) rather than thread-like structures (i.e. fibrils and fibres) lends credence to the idea that the striated lamellar structures are probably collagen sheets derived by polymerization of altered Type IV collagen.

Crystalline fibrin deposits represent another variety of banded or striated structure seen at times in basal lamina of renal glomeruli and tubules. Fibrin deposits may present as: (1) pale mottled deposits; (2) medium density deposits; (3) electron-dense fibrils or fibres which may or may not show the characteristic banding pattern of fibrin (periodicity about 24 nm). These deposits (i.e. items 1–3) have been seen in the kidney in a variety of conditions (Jenis and Lowenthal, 1977). This includes: (1) Goodpasture's syndrome; (2) Schönlein–Henoch syndrome; (3) systemic lupus erythematosus; (4) acute post-streptococcal glomerulonephritis; (5) diabetic nephropathy; (6) haemolytic uraemic syndrome; (7) polyarteritis nodosa; (8) Wegener's granulomatosis; (9) Berger's disease; and (10) mesangiocapillary glomerulonephritis.

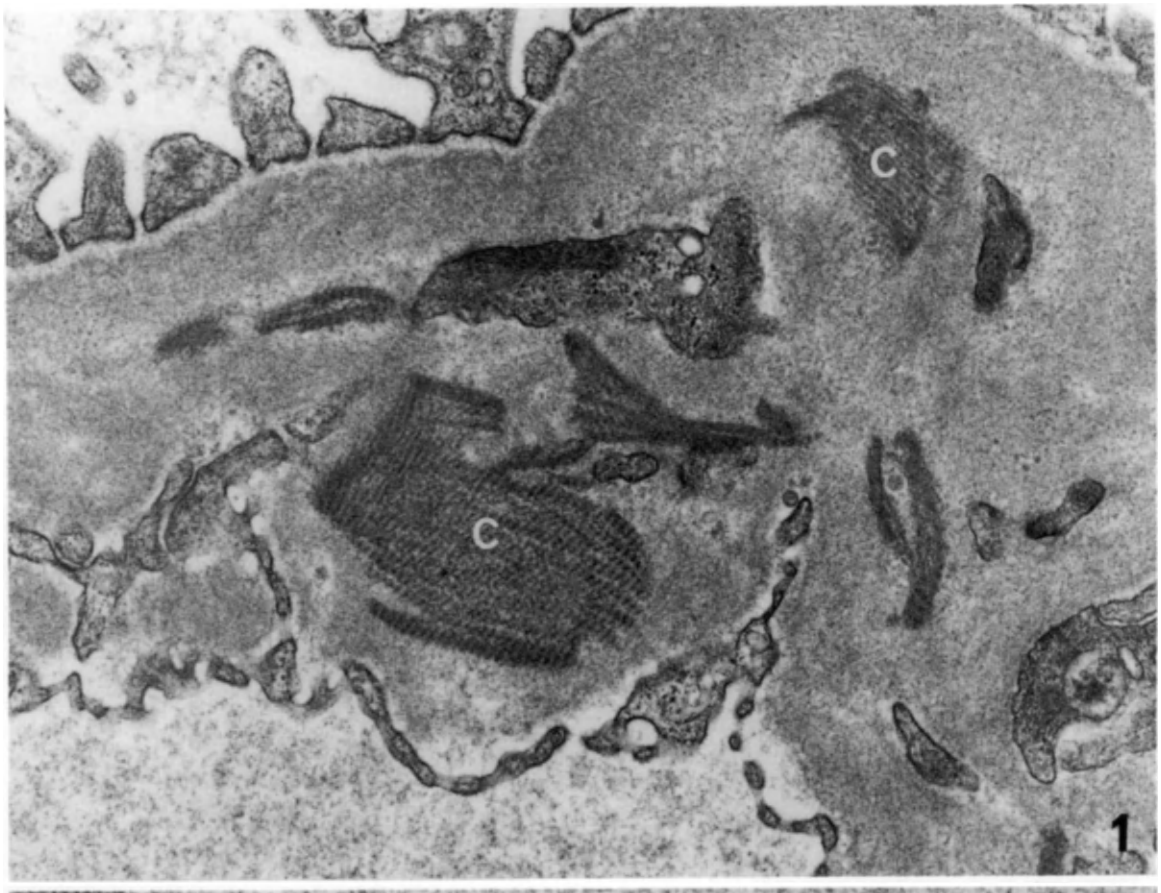
The periodically striated form of fibrin qualifies as a crystal (*see* pages 1268–1271), and so does the periodically banded striated lamellar structure. Except for these, crystalline deposits in renal basal lamina are quite rare. In fact I am aware of only one case referred to me by Dr S. Hammar where quite large accumulations of crystals (periodicity 14 nm) were found within the lamina dena and in the subendothelial and mesangial regions of the glomeruli of a patient with diabetes mellitus. The chemical composition of these crystals is obscure.

Plate 461

Kidney biopsy from a patient with a history of diabetes mellitus. (*Electron micrographs supplied by Dr S. Hammar*)

Fig. 1. Several crystalline deposits (arrows) are seen in a markedly thickened glomerular lamina densa. ×82 000

Fig. 2. This subendothelial crystalline deposit in a glomerulus is composed of several crystals with a periodicity of about 14 nm. ×106 000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15

Cell junctions

Introduction

The narrow intercellular space which separates the apposed lateral surfaces of epithelial*, endothelial and mesothelial cells contains a material which in the past was often referred to as the cement substance. It is now thought that this mucopolysaccharide-rich layer is a part of the cell coat (Chapter 14).

The relatively constant interval (15–20 nm wide) and the parallel orientation of the apposed lateral surfaces of such cells suggest the presence of a cohesive force operating over these surfaces. Often such apposed surfaces are amplified by the formation of a few or many complex interdigitating folds which probably play an additional role in cell-to-cell adhesion. Besides evidence of such a generalized mechanism, one also finds focal areas of specialization of the cell surface which are thought to represent zones of firmer attachment.

Such specialized areas of cell surface serving to bind that surface to another cell surface or non-cellular structure are referred to as junctions. Many morphological and functional varieties of junctions have been described. While the sole function of some seems to be the provision of sites of firm attachment, others also act as watertight seals or as areas of low electrical resistance across which ions can flow. The structure of such junctions and their variations in normal and pathological states form the topic of this chapter.

*As used in this book, the term 'epithelial cells' excludes cells lining blood vessels and lymphatics which are better designated as 'endothelial cells' and cells lining serous cavities which are best referred to as 'mesothelial cells' (*see also* footnote on page 1070).

Structure and function of cell junctions

Although our knowledge about cell junctions stems largely from ultrastructural studies, it is worth recalling that the presence of some junctions can be appreciated with the light microscope.

Perhaps the best-known example of this is the so-called 'intercellular bridge' found between the prickle cells of the epidermis. Such bridges present as fine processes or fibrils traversing a clear space between adjacent cells, and it is this which gives the prickly appearance to prickle cells. The belief that such bridges represent tonofibrils crossing over from the cytoplasm of one cell into the next one, or that the cytoplasm of neighbouring cells is continuous at such sites is not borne out by electron microscopy. It would appear that this phenomenon is largely a shrinkage artefact, the bridges being no more than plaques or zones of firm attachment (in the past referred to as 'granules of Ranvier' or 'nodes of Bizzozero', and now as desmosomes) which become drawn out as the cells shrink away from each other during fixation (*see Plate 468*).

However, bridges establishing continuity between the cytoplasm of neighbouring cells do occur between developing male germ cells (primary spermatocyte to late spermatid stage) and female germ cells and between certain cells in *Daphnia* and *Hydra* (Fawcett, 1961; Nagano, 1961; Zamboni and Gondos, 1968). More slender connections called 'plasmadesmata' are known to occur between the cells of higher plants and some algae (e.g. *Nitella*) (Spanswick and Costerton, 1967). However, the commonest type of intracellular connections or bridges are the numerous extremely fine (1.5–2 nm in diameter) aqueous channels linking the cytoplasm of adjacent cells which occur in gap junctions (page 1104) found in a wide variety of tissues.

Another example of junctions seen at light microscopy are the terminal bars demonstrable by classic histological methods near the surface of various epithelia, particularly the small intestine. In ultrathin sections the terminal bar is seen to be a junctional complex, comprising three morphologically distinct types of junctions. Starting from the lumen of the gut one finds a tight junction followed by an intermediate junction and a desmosome or desmosomes (*Plate 462*). Similar junction complexes have been described in various epithelia (Farquhar and Palade, 1963,

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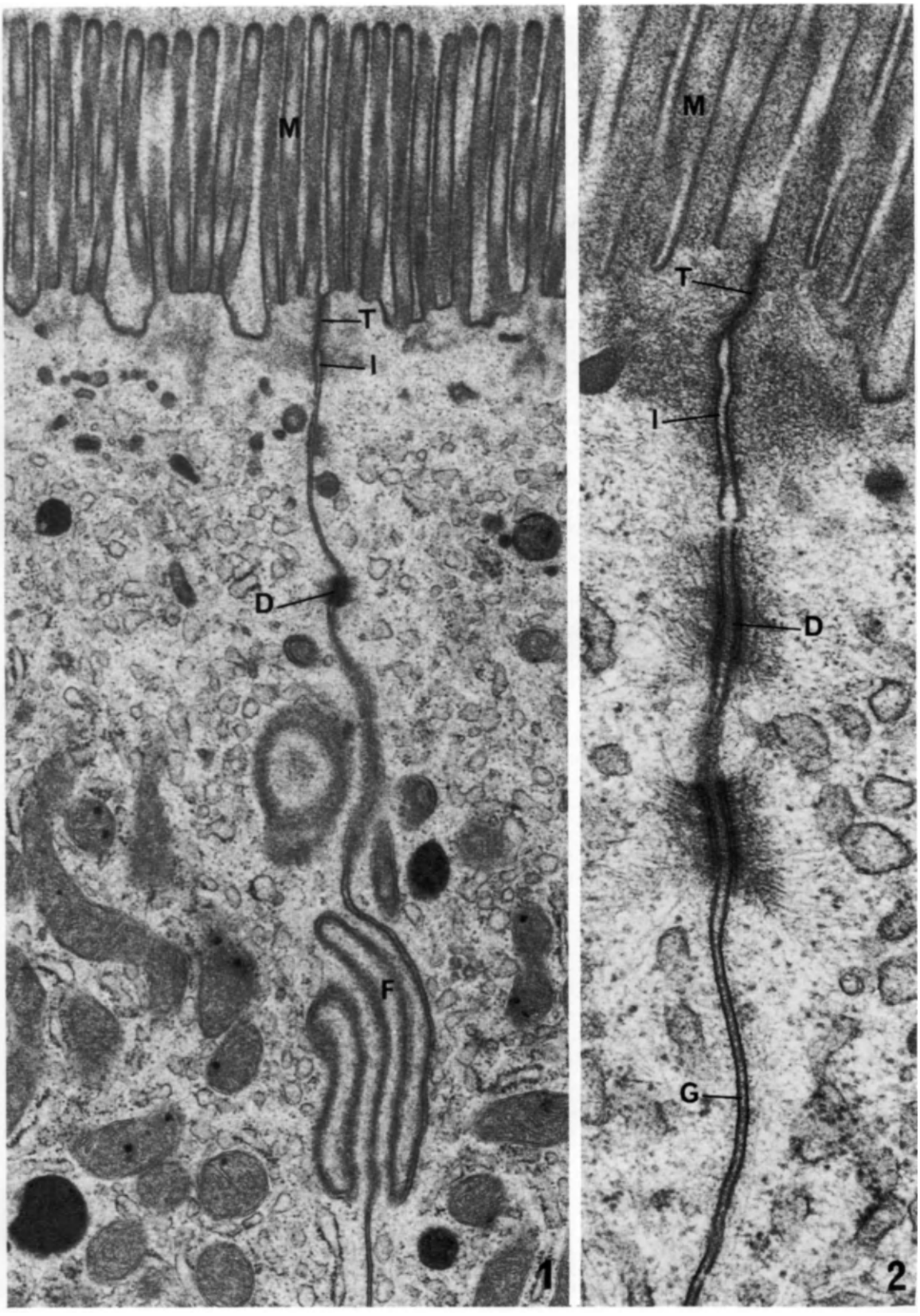
Plate 462

The terminal bar or junction complex between adjacent human intestinal epithelial cells is illustrated here. The tight junction (T) is seen near the lumen of the gut lined by microvilli (M). It presents as a dense line both in the low (*Fig. 1*) and in the high (*Fig. 2*) power views shown here. The fusion of the outer leaflets of the plasma membrane characteristic of this type of junction is not revealed in these electron micrographs. Below the tight junction lie an intermediate junction (I) and some desmosomes (D). The desmosomes show dense plaques with converging filaments on their cytoplasmic faces. Less well oriented filamentous material is seen adjacent to the intermediate junction and some cytoplasmic 'fuzz' is also seen near the tight junction. In the desmosomes and intermediate junction the intercellular gap is widened. The parallel alignment of the cell membranes and the constancy of the intercellular gap (G) is clearly demonstrated in *Fig. 2*. The complex interdigitating folds (F) which are also thought to help to hold cells together are seen in *Fig. 1*. ×31 000; ×63 000 (*Ghadially and Ailsby, unpublished electron micrographs*)


M
T
I
D
F
1
M
T
I
D
G
2

1965). However, the constant feature of the terminal bar is the tight junction which seals the intercellular space from the external environment (e.g. contents of gut, duct, acinus, etc.). The arrangement, number and type of the junctions which follow are quite variable*.

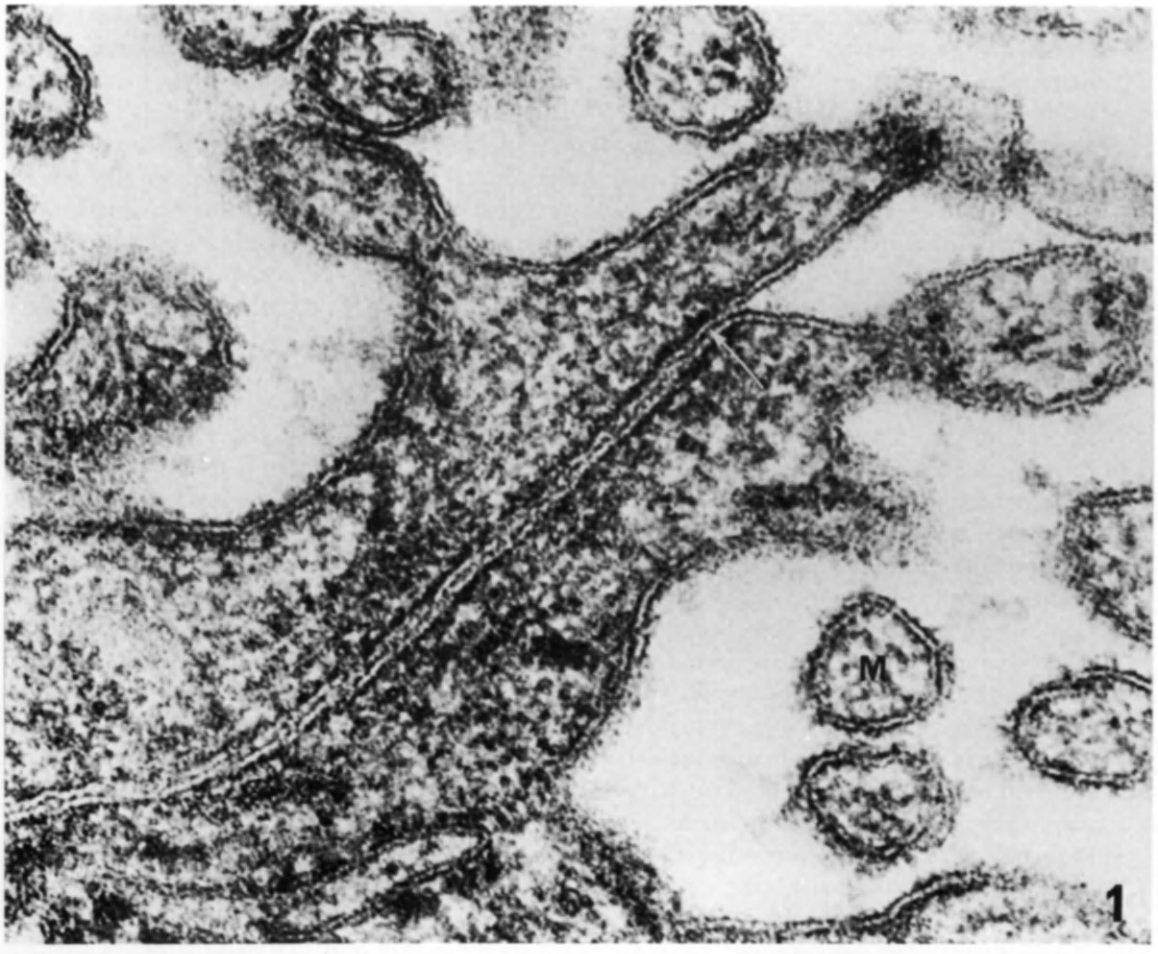
The three types of junctions mentioned above and also other types have now been seen in a variety of tissues, and junctions have also been seen between cells in culture (Locke, 1965; Bullivant and Loewenstein, 1968; Brightman and Reese, 1969; Cobb and Bennett, 1969; Flaxman *et al.*, 1969; Schatzki, 1969; Goodenough and Revel, 1970; Martinez-Palomo, 1970; Sanel and Serpick, 1970; Nunez, 1971; Scaletta and MacCallum, 1972). It is important to note, however, that some of the literature that has accumulated is confusing and misleading. It is now known that examination of tissues prepared in the routine manner is not adequate to demonstrate and distinguish certain types of junctions. Studies using tissues stained *en bloc* with uranyl acetate (which gives better visualization of the structure of certain junctions), and studies using various tracers such as lanthanum hydroxide (which permeates the intercellular space and allows one to determine the degree and extent of obliteration of this space achieved in junctions), have helped to clarify the situation (*Plate 463*). In the light of such studies, one may briefly summarize what is currently thought about the structure and function of some junctions. In ultrathin sections of tissues stained *en bloc* with uranium, the tight junctions of the junction complex of epithelia are characterized by punctate and linear fusion of the outer leaflets of the cell membrane of adjacent cells. (The fusion of the outer leaflets of the two apposing trilaminar membranes results in a pentalaminar structure, hence these junctions are at times referred to as 'pentalaminar junctions'.) Lanthanum, which readily permeates the intercellular gap, may also invade part of the zone of punctate fusion, but it does not traverse the fusion 'line'. Thus the collective evidence indicates that in this region the intercellular gap is obliterated. Since in epithelia such junctions occur as a continuous band around cells (but not in all endothelia, *see below*), it follows that tight junctions act as an effective seal which isolates the epithelial surfaces from the intercellular spaces. The term 'zonula occludens', used to describe these structures, is therefore quite appropriate.

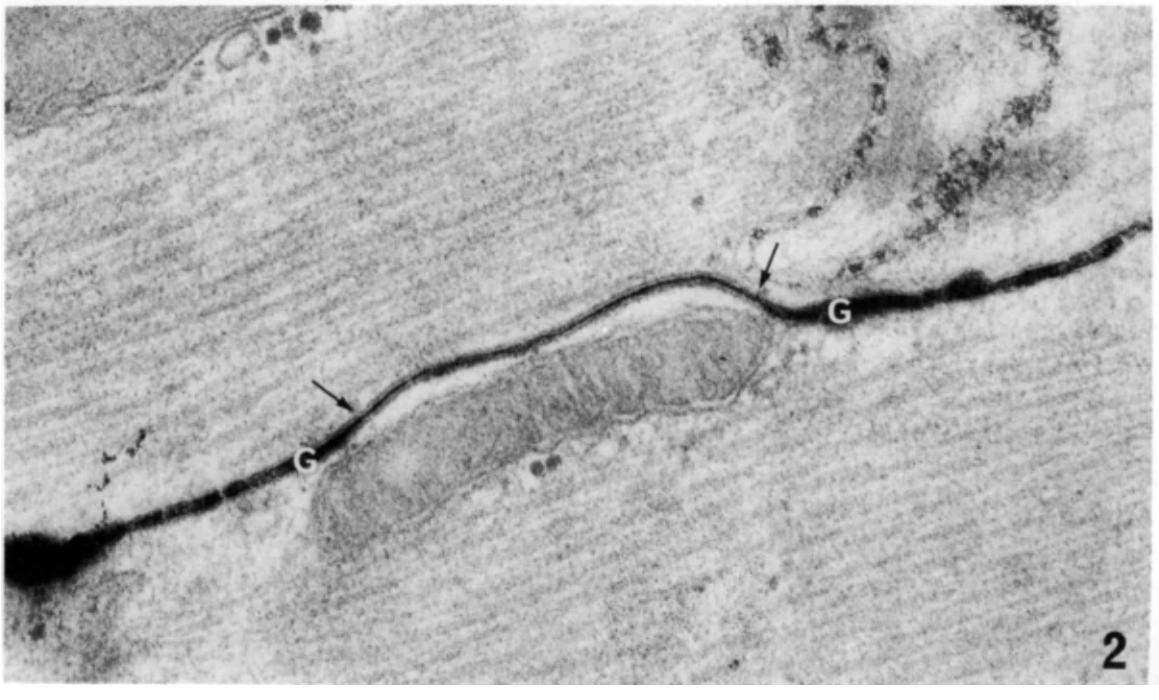
*For example, intermediate junctions are prominent in the gut, but as a rule they are small or absent in ducts and acini. Similarly, the number of desmosomes also decreases as the size of the lumen sealed off by tight junctions decreases.

Plate 463

Fig. 1. A tight junction between acinar cells of mouse mammary gland, showing the characteristic pentalaminar structure (*see also Plate 342*). The line of fusion of the outer leaflets of adjoining cell membranes is indicated by an arrow. Sections of microvilli (M), showing the characteristic trilaminar structure of the cell membrane, are seen within the lumen of the acinus. Tissue stained *en bloc* with uranium. ×120 000 (*Martinez-Palomo, unpublished electron micrograph*)

Fig. 2. Rat myocardium, showing a gap junction (between arrows) permeated by lanthanum. The intercellular gap (G) between the muscle fibres is abruptly narrowed in the junction which extends over quite a distance. ×84 000 (*Martinez-Palomo, unpublished electron micrograph*)


M
1


G
G
2

In routine preparations, structures morphologically acceptable as tight junctions have been seen in a wide variety of tissues. In such preparations, however, and also in permanganate-fixed material it is difficult to distinguish a tight junction where the intercellular gap is obliterated from what is now known as a gap junction where the intercellular gap is reduced to a width of about 2–3 nm. Yet another problem is distinguishing what are known as 'labile or simple appositions of the cell membrane' from true tight junctions. Labile appositions are thought to be either transient associations of the cell membrane or an artefact producing a pentalaminar structure. Their incidence varies with preparative techniques and such structures, comprising apposed rather than fused membranes, do not impede the passage of tracers. They lack the zone of increased cytoplasmic density (cytoplasmic fuzz) seen adjacent to true tight junctions and their overall width is not less than the combined width of the two membranes forming the junction, as is the case with true tight junctions.

The points made above are clearly brought out by the meticulous studies of Brightman and Reese (1969) on junctions found in the vertebrate brain. They show that tight junctions which retain their pentalaminar appearance in tissues stained *en bloc* with uranium are restricted to the endothelium of capillaries and the epithelium of the choroid plexus. Such junctions are neither circumvented* nor penetrated by tracers such as lanthanum or horse-radish peroxidase, but various other junctions between astrocytes and between some neurons which, in the past, had been regarded as tight junctions are in fact gap junctions which are readily permeated by tracers. Gap junctions seem to be very widely distributed and have been seen in many tissues. In many studies a polygonal lattice has been demonstrated within the gap of the gap junction. There is much evidence that the gap junction represents a site of electrical coupling between cells. This has been shown to be so for gap junctions found in mammalian heart, smooth muscle, liver and Mauthner cells in goldfish brains (for details and references, *see* Goodenough and Revel, 1970).

It will be noted from the above discussion that confident identification of tight junctions and gap junctions in routine ultrathin sections can at times be quite difficult or even impossible. However, in freeze-fractured tissues, tight junctions and gap junctions have characteristic morphologies which permit their confident identification.

In the case of tight junctions what appears (in sectioned material) as punctate or linear fusion of cell membranes corresponds with a meshwork of ridges and matching grooves on the fracture faces exposed by freeze-cleavage (*Plate 464*); while in the case of gap junctions what appears (in sectioned material) as a polygonal lattice in the gap of the gap junction corresponds

*Junctions in many capillaries outside the brain are known to be circumvented by tracers for they do not extend as a continuous band around the cells.

Plate 464

Details of tight junction as seen in a freeze-fractured specimen of mouse intestine. Note the meshwork of ridges (arrows) and grooves (arrowheads) on the membrane fracture faces. ×60 000 (*Mukherjee and Swift, unpublished electron micrograph*)

to numerous particles (about 7 nm in diameter) and matching pits on the fracture faces of freeze-cleaved material (*Plate 465*) (Chalcroft and Bullivant, 1970; Staehelin, 1974; Bullivant, 1978). Each particle penetrates the cell membrane and projects into the 2 nm wide gap in the gap junction. At the centre of each particle lies a pore or channel. End-to-end alignment and bonding of these particles produces units called 'connexons', through which runs an aqueous channel 1.5–2 nm in diameter. Small molecules (molecular weight up to about 1200) can pass through these channels. The passage of fluorescent dyes, amino acids, sugars, cyclic AMP and other nucleotides has been demonstrated. Thus the gap junction is a permeable structure which facilitates diffusion of ions and small molecules from cell to cell through low resistance pathways.

The arrangement and number of the strands or ridges vary in the tight junctions from different epithelia (Friend and Gilula, 1972). How effectively a tight junction acts as a seal depends on the number of strands within the width of the tight junction band. On the basis of this, tight junctions have been categorized from 'very leaky' to 'very tight'. An example of very leaky tight junctions are those found in the proximal tubule of the mouse kidney (average 1.9 strands), while examples of tight or very tight junctions are those seen between cells of toad urinary bladder (average 8.1 strands) and between the Sertoli cells of seminiferous tubules of the rat (average 36 strands) (Claude and Goodenough, 1973; McGinley *et al.*, 1977). Further, it has been shown that in the tight junctions of thyroid epithelium, the number of tight junction strands varies with different functional states of the organ (Tice *et al.*, 1975) and they were found to be completely absent in highly malignant anaplastic carcinoma of the thyroid (Kerjaschki *et al.*, 1979).

Intermediate junctions and desmosomes are fundamentally different from tight junctions and gap junctions in that the width of the intercellular gap is widened not narrowed. Further, while gap junctions provide channels of communication, and tight junctions provide more or less impermeable seals, intermediate junctions and desmosomes provide firm mechanical union between cells*. The desmosome is a button-like structure, hence it is at times referred to as 'macula adherens', while the intermediate junction is at times referred to as a 'zonula adherens' or 'belt desmosome' because it forms a continuous belt around the cell (like the tight junction which is called 'zonula occludens').

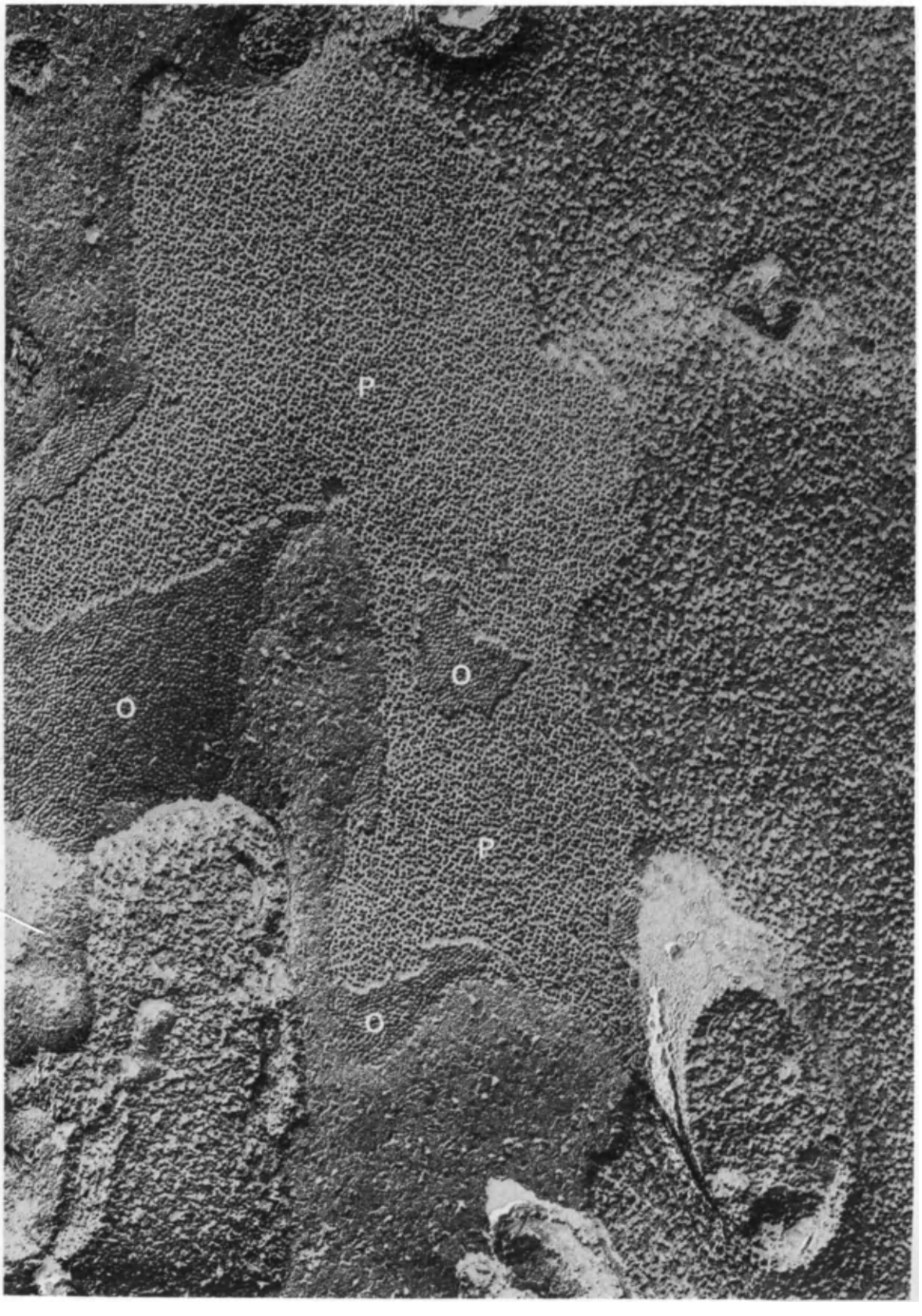
Little is known about intermediate junctions† except that they are more prominent in cells with a well developed brush border, and that the filaments associated with them are the filaments of the terminal web, which are mainly actin filaments. It is thought that intermediate junctions provide anchorage for the web and also serve as a band of firm attachment of neighbouring epithelial cells.

In contrast to this are the much studied, widely distributed, desmosomes which are found in epithelia, mesothelia, meninges and myocardium. They are also found in the endothelia of some species (Fawcett, 1981) but not in human endothelia. The morphology and size of desmosomes are quite variable but they are characterized by the presence of: (1) attachment plaques (also called 'dense plaques') on their cytoplasmic faces; (2) associated intermediate filaments (often called 'tonofilaments') or bundles of filaments ('tonofibrils'); and (3) an intermediate line in a widened intercellular gap (about 30 nm wide).

*One need hardly point out that all junctions provide some degree of mechanical union between adjacent cells.
†For example, in the 70-page long masterly chapter on cell junctions in Fawcett (1981) only about half a page of the text and one illustration are devoted to intermediate junctions.

Plate 465

Details of gap junction as seen in a freeze-fractured specimen of mouse liver. Note the particles (P) and pits (O) on the membrane fracture faces. ×100 000 (*Mukherjee and Swift, unpublished electron micrograph*)


P
O
O
P
O

The attachment plaques are usually about 20 nm thick and 500 nm in diameter. The converging intermediate filaments form loops near or within the plaques (Kelly, 1966), they do not appear to be immovably attached to the plaques. Quite different very fine filaments (called 'transmembrane linkers') arising within the plaque traverse the intercellular gap. These transmembrane linkers are impossible to visualize* as such in routine preparation. It is thought that superimposition of densities of the lattice produced by these filaments presents as a central dense line (usually called 'intermediate line') in sectioned material (Staehelin and Hull, 1978).

Immunohistochemical studies (for references *see* Schmelz *et al.*, 1986) have shown that desmoplakin I is a general component of the desmosomal plaques from all sites, but the intermediate filaments are different. In epithelia, cytokeratin filaments are associated with the desmosomal plaques. They are quite long and prominent in squamous epithelia where they have for long been referred to as 'tonofilaments' and 'tonofibrils'. However, vimentin filaments are associated with the plaques of desmosomes in the meninges, and desmin filaments are associated with the plaques of desmosomes in cardiac myocytes and Purkinje fibres.

As mentioned earlier, desmosomes are button-like structures which bind cells together. Their prominence in the epidermis and also in the intercalated disc of cardiac muscle indicate that this is the mechanically strongest type of junction. Not all desmosomes, however, bind adjacent cells together. On rare occasions a desmosome is seen binding an area of cell surface to another area on the surface of the same cell. This is referred to as an 'autodesmosome'. The term 'hemidesmosomes' is used for the 'half desmosomes' that bind an area of cell surface to some other non-cellular structure such as a basement membrane, an area of tooth surface, or a plastic membrane such as that used in tissue culture (Kelly, 1966; Flaxman *et al.*, 1968). Hemidesmosomes are of common occurrence along the dermo-epidermal junction.

A common type of cell junction seen in the epithelia of invertebrates was called the 'septate desmosome'. It bears little resemblance to desmosomes (described above), so the term 'septate junction' is now used. The septate junction occurs as a circumferential band around the apex of cells. In sections normal to the apposing cell surfaces it presents a ladder-like appearance. This is because the 17–18 nm intermembrane gap is traversed at regular intervals by structures called 'septa' or 'bridges'. However, tangential sections through the gap reveal that the 'septa' are in fact profiles stemming from sections through a crystalline structure with hexagonal subunits demarcating symmetrical intercellular compartments. It is thought that septate desmosomes are involved in cell-to-cell electronic coupling (Locke, 1965; Bullivant and Loewenstein, 1968).

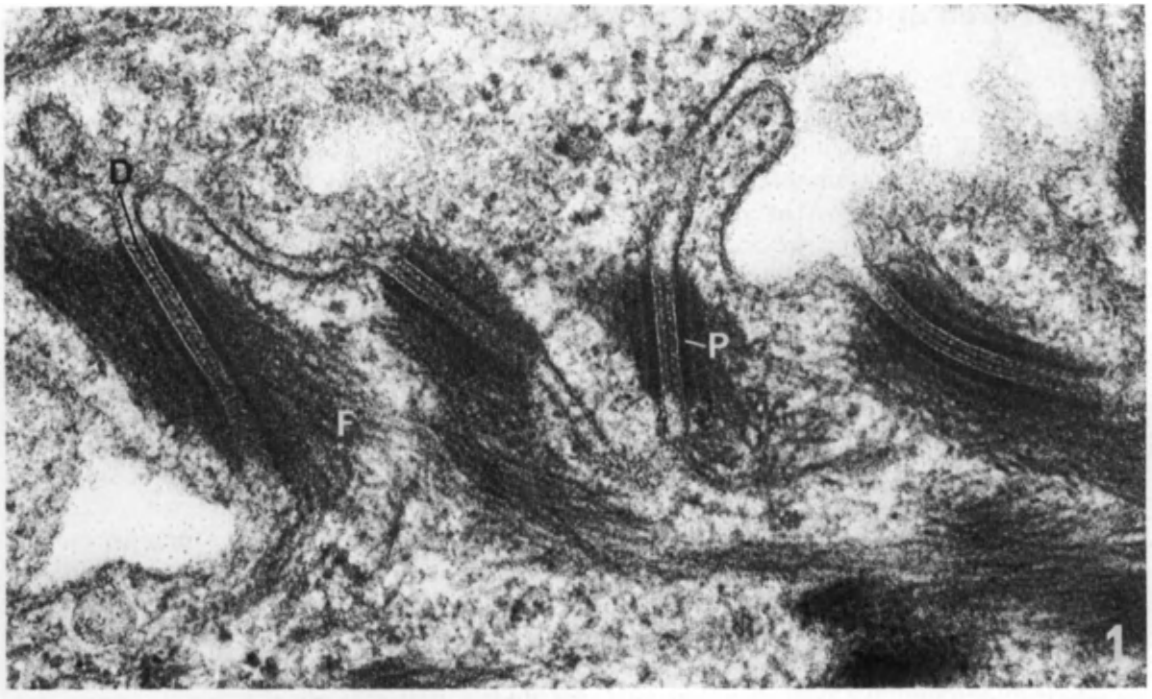
*Perhaps the most persuasive evidence regarding the existence of transmembrane linkers comes from freeze-fracture studies which show irregularly shaped elevations which appear to be derived from breaking off of the linkers during fracturing (Fawcett, 1981). While it is true that in sections of normal desmosomes it is virtually impossible to see transmembrane linkers, I have found that they can at times be quite clearly seen in the poorly differentiated desmosomes found in anaplastic carcinomas, presumably because there is a marked reduction in the number of transmembrane linkers. It stands to reason that when there are only a few linking filaments they are more likely to be visualized as such than when there are many filaments superimposed on each other.

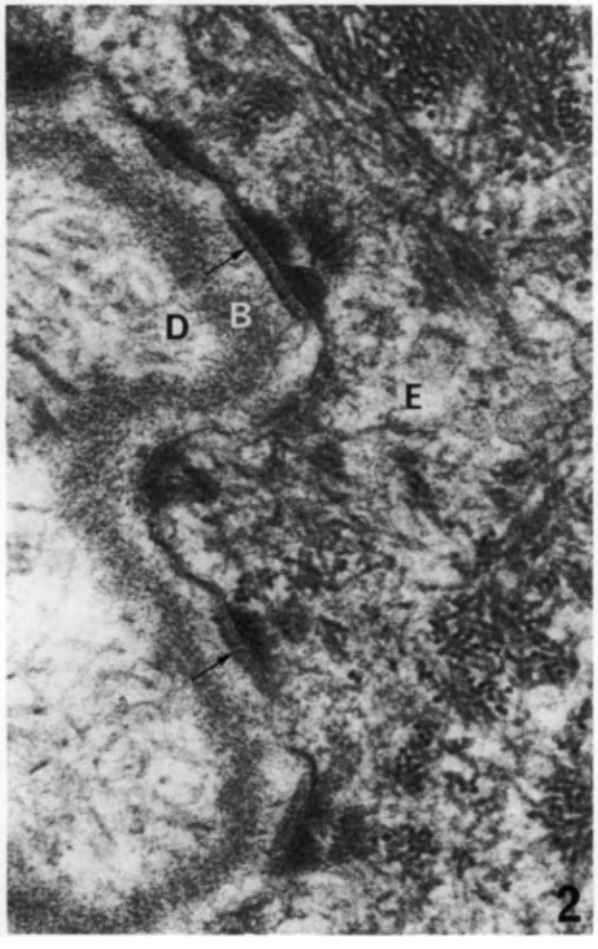
Plate 466

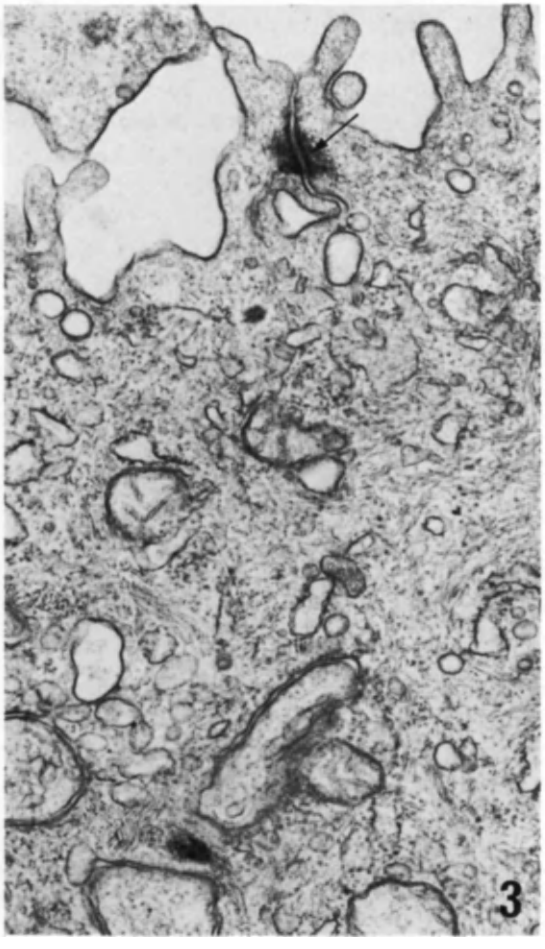
Fig. 1. Desmosomes from human skin. Note the dense plaque (P), filaments (F) and dense line (D) in the intercellular space. ×96 000

Fig. 2. Hemidesmosomes (arrows) are seen at the junction of the epidermis (E) and dermis (D). Also seen is the basal lamina (B), composed of a lamina densa and lamina lucida. From the same specimen as *Fig. 1.* ×94 000

Fig. 3. Autodesmosome (arrow) from placental amnion of a 23-week-old human fetus. ×30 000 (*Parry, unpublished electron micrograph*)


D
P
F
1


D
B
E
2


3

Alteration of cell junctions in neoplasia

A fair-sized literature has now accumulated on this topic, and it is now evident that there is a reduction in the number of various types of junctions and a widening of the intercellular spaces in malignant tumours and during carcinogenesis (Easty and Mercer, 1960; Bruni *et al.*, 1961; Friedmann, 1961; Vandrewalla and Sirsat, 1963; Hruban *et al.*, 1965; Ghadially and Parry, 1966; Ma and Webber, 1966; Mao *et al.*, 1966; Benedetti and Emmelot, 1967; Murad and Scarpelli, 1967; Usui, 1967; Shingleton *et al.*, 1968; Sugar, 1968; Sirsat and Shanbhag, 1969; Watanabe and Essner, 1969; Martinez-Palomo, 1970; Harris *et al.*, 1971).

It is said that, while there is a marked reduction in the number of desmosomes and a loss of structural cohesion in squamous carcinoma of the skin, in basal cell carcinomas a close mutual adhesion is maintained between adjacent cells (Vandrewalla and Sirsat, 1963). In human warts an increase in the number of desmosomes has been reported to occur (Chapman *et al.*, 1963). In keratoacanthomas desmosomes are plentiful (Fisher *et al.*, 1972) and, according to Takaki *et al.* (1971), increased in numbers. A direct correlation has been noted between the number of desmosomes and the degree of differentiation in several carcinomas in humans, and cell culture studies show that N,N-dimethylformamide, which induces differentiation of human colonic carcinoma, leads to a sixfold increase in the number of desmosomes (Christensen *et al.*, 1985).

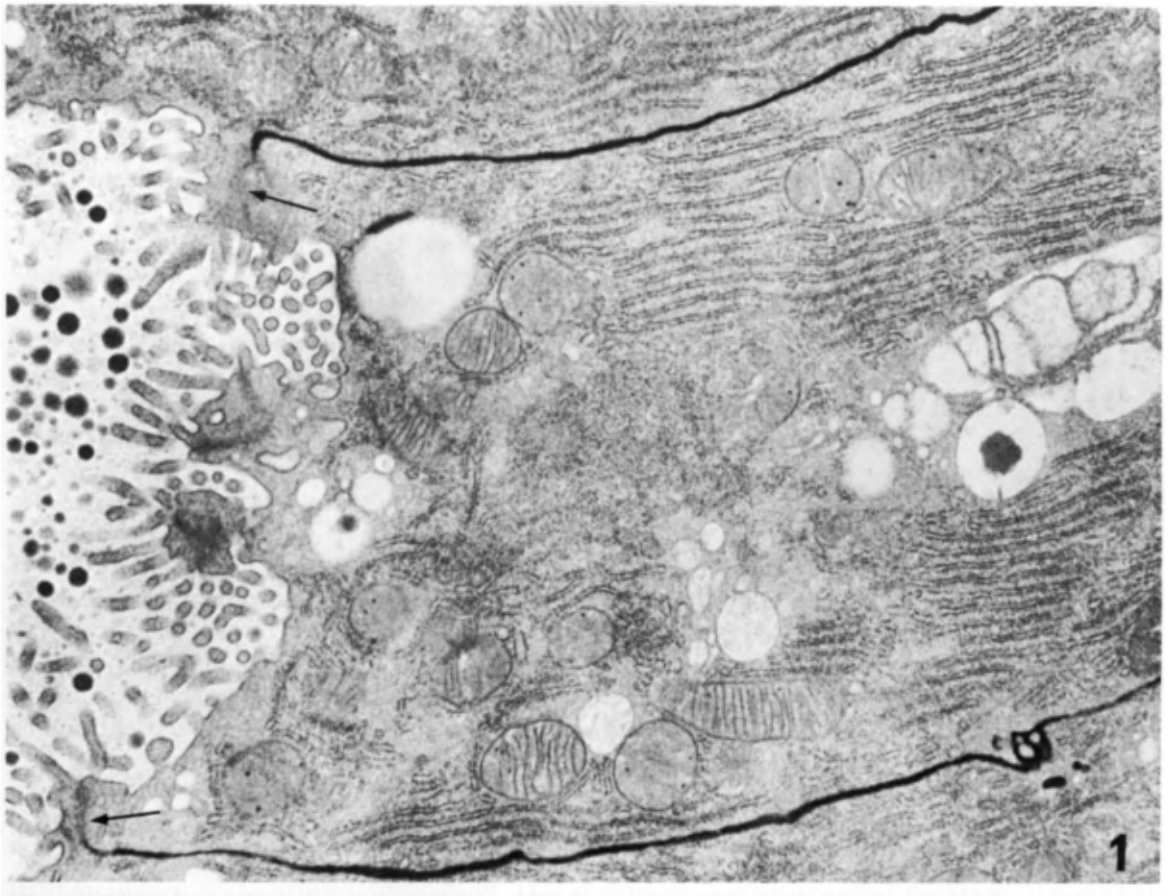
A reduction in the number and size of tight junctions and gap junctions has been noted in several tumours. It is thought that alterations in such junctions might result in faulty coordination of cellular activity (Loewenstein and Kanno, 1966, 1967; Potter *et al.*, 1966). Electrophysiological studies have revealed that some cancer cells are electrically coupled but that others are not (Johnson and Sheridan, 1971). The elegant studies of Martinez-Palomo (1970) show that in many epithelial tumours tight junctions are absent between tumour cells, and gap junctions tend to be sparse (*Plate 467*). Similarly freeze-fracture studies have shown an absence of tight junctions in anaplastic carcinoma of the thyroid (Kerjaschki *et al.*, 1979).

Plate 467

Normal acinus from a mouse mammary gland (*Fig. 1*) and acinus from a mouse mammary carcinoma (*Fig. 2*) are compared here. Tissues were treated with lanthanum hydroxide, which presents here as a highly electron-dense linear deposit in the intercellular spaces. (*From Martinez-Palomo, 1970*)

Fig. 1. The lateral intercellular space is permeated with lanthanum except in the apical region, where the tight junctions (arrows) have occluded the intercellular space. ×13 500

Fig. 2. The absence of tight junctions in the tumoral acinus is evidenced by the fact that the lanthanum has permeated the intercellular spaces up to the lumen of the acinus (arrows). ×23 000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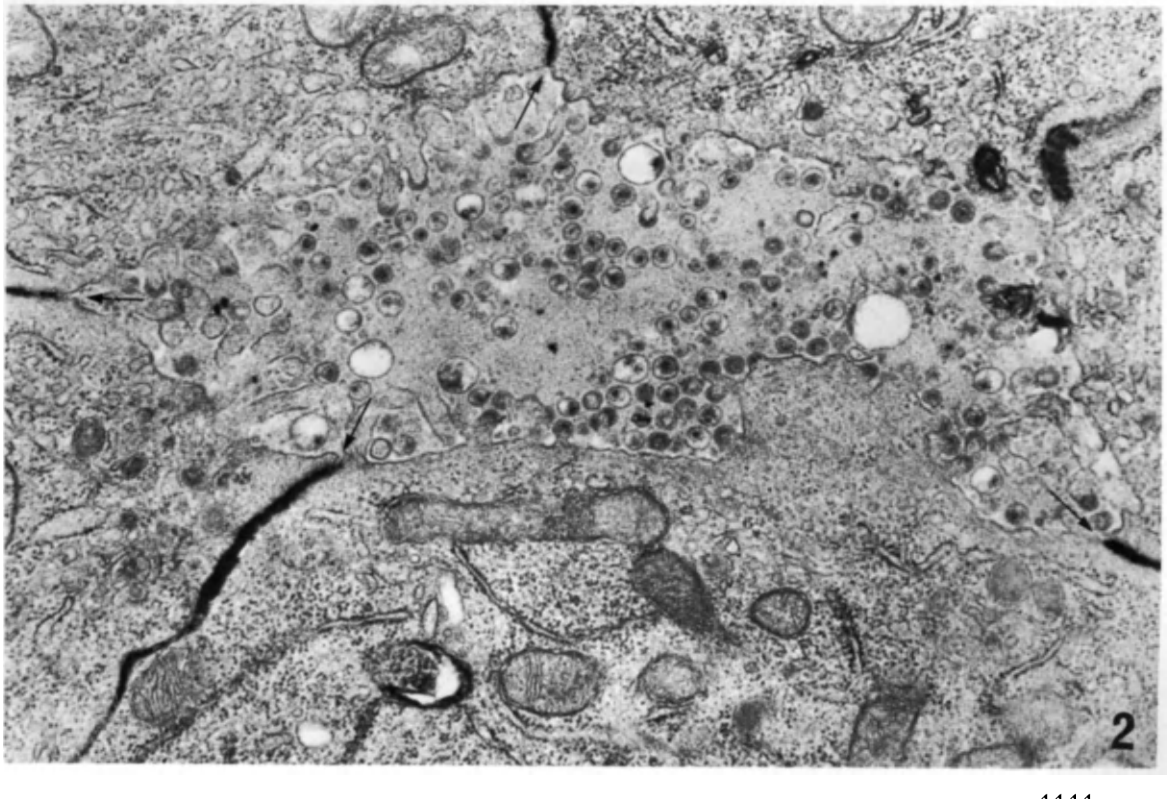
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Diagnostic value of cell junctions in tumours

The accurate evaluation of cell junctions is an important factor in deciding whether a tumour is a carcinoma or a sarcoma (*Plates 468–472*). The theory behind this is that, since cell junctions such as desmosomes and tight junctions are characteristic of epithelia, it follows that demonstration of such junctions in an anaplastic tumour should be diagnostic of carcinoma, while the absence of junctions would argue strongly in the favour of a sarcoma. By and large, this is correct, but the situation is somewhat complicated because: (1) as noted before (page 1110) there is a marked reduction in the number of junctions in malignant tumours as compared with the normal tissue of origin; (2) morphological changes occur in junctions—the desmosomes lose some of their characteristic features and become what one might call 'poorly differentiated desmosomes' or 'attentuated desmosomes', while tight junctions open up (leaky junctions) and hence become difficult to identify as such; (3) desmosome-like structures are at times seen between some normal mesenchymal cells and they have been seen in quite a few tumours including sarcomas. Such desmosome-like structures are at times difficult to distinguish from the much altered attenuated desmosomes in carcinomas.

These desmosome-like structures are of little diagnostic value but they constitute a potential hazard in that they may erroneously lead one to believe that the tumour is a carcinoma. Hence it behoves us to examine the differences between desmosomes and desmosome-like structures (*Plate 468, Fig. 1*). What one might call the 'perfect' desmosome, 'classic' desmosome or 'well-differentiated' desmosome shows (*Plate 466, Fig. 1*) the following features: (1) a widened or 'unwidened' but not narrowed intercellular gap filled by dense material, which shows a line or a pattern of lines; (2) dense plaques on the cytoplasmic faces; and (3) tonofilaments from the cytoplasm converging upon plaques.

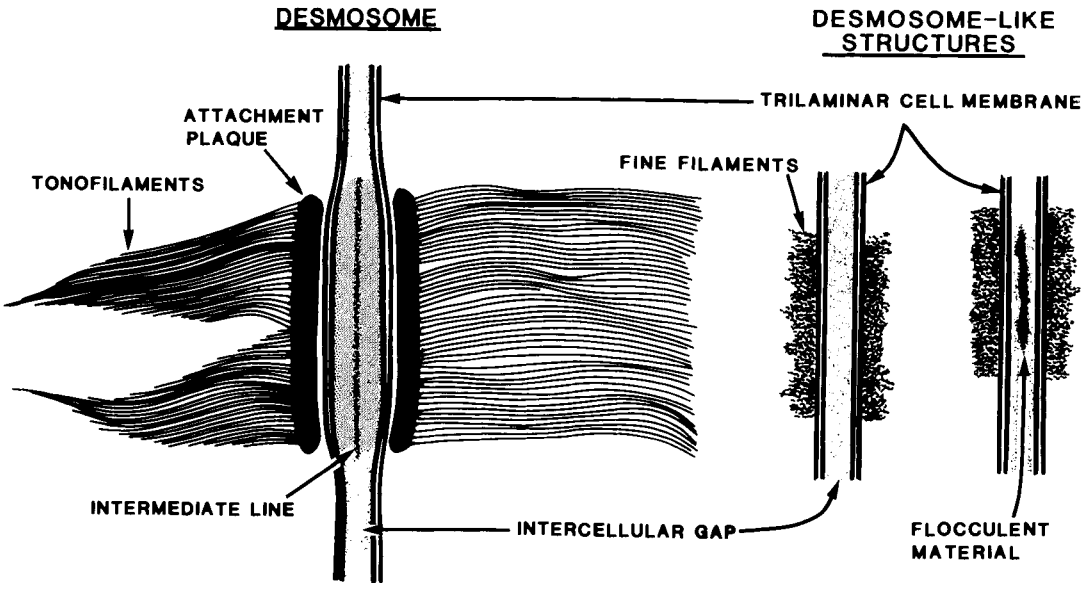
In human material the presence of such desmosomes virtually guarantees that one is looking at epithelium, mesothelium (but not endothelium), meninges* or their tumours (*Plate 468, Fig. 2* and *Plates 469* and *470*). The so-called synovial sarcoma also contains true desmosomes and other features (e.g. basal lamina, microvilli with prominent filamentous core and rootlets) which are not seen in normal or diseased human synovium (Ghadially, 1983). Hence, it has been suggested that this tumour which clearly does not show a synovial type of differentiation should be renamed 'carcinosarcoma of connective tissue' (Ghadially, 1985, 1987).

*The term 'meningothelium' is not accepted even though meningothelial meningiomas are a recognized entity and a layer of squamous cells does line the surface of the dura and the surface of the arachnoid and its trabeculae.

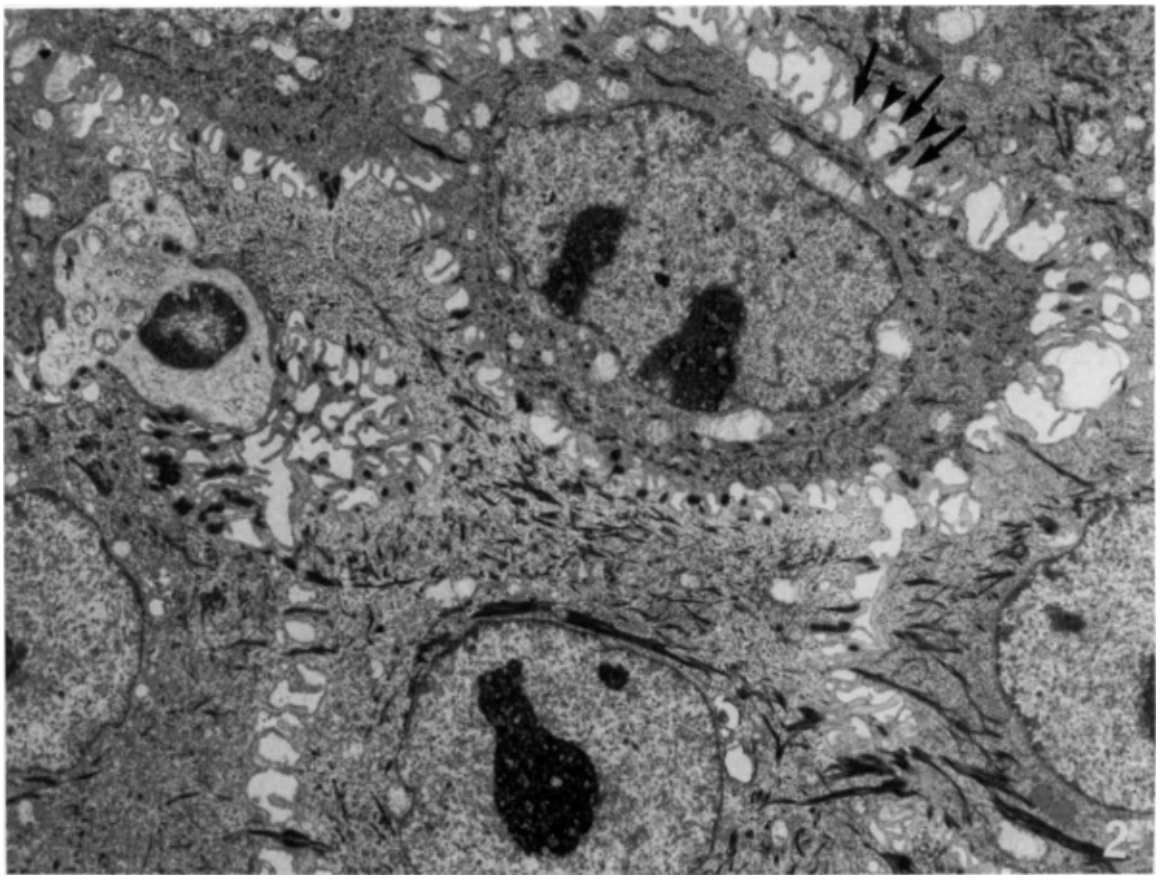
Plate 468

Fig. 1. This diagram schematizes the differences between desmosomes and desmosome-like structures which are fully explained in the text.

Fig. 2. Squamous cell carcinoma of lip. Shrinkage of cells has produced intercellular spaces (arrows) and bridges (arrowheads). As the cells shrink away from each other the points where the cells are firmly bound together by desmosomes are pulled out and present as bridges. ×7000


DESMOSOME
DESMOSOME-LIKE STRUCTURES
TRILAMINAR CELL MEMBRANE
ATTACHMENT PLAQUE
TONOFILAMENTS
FINE FILAMENTS
INTERMEDIATE LINE
INTERCELLULAR GAP
FLOCCULENT MATERIAL

1



In meningiomas one finds not only true desmosomes but also desmosome-like structures (paired subplasmalemmal densities) and hemidesmosome-like structures (subplasmalemmal densities) (*Plate 470*). This unusual situation is of common or perhaps constant occurrence in meningiomas (Copeland *et al.*, 1978; Mirra and Miles, 1982; Ghadially, unpublished observations) and may hence be of value in distinguishing this class of neoplasm from others.

Although easily identifiable true desmosomes are often seen in carcinomas (often even in quite poorly differentiated carcinomas), this is not invariably so. Indeed in poorly differentiated or anaplastic carcinomas the desmosomes can, at times, be quite small and difficult to confidently identify as such. In such 'attenuated desmosomes' or 'poorly developed desmosomes' (*Plate 471, Fig. 1*) the details in the intercellular gap are usually lacking, but slender dense plaques may be present and at least a few intermediate filaments are seen converging on the plaques. However, it is possible that at times these structures may be so poorly developed that it may not be possible to identify them as attenuated desmosomes, and then one might confuse them with small desmosome-like structures.

Be that as it may, the important point that emerges is that if in an anaplastic, difficult-to-diagnose tumour one finds desmosomes or attenuated desmosomes, then the diagnostic possibilities are narrowed down to tumours such as carcinoma, mesothelioma, meningioma and chordoma, and one can with some confidence discard a whole host of other diagnoses such as lymphoma, melanoma and various sarcomas, except of course the so-called 'synovial sarcoma' where true desmosomes and cytokeratin are found.

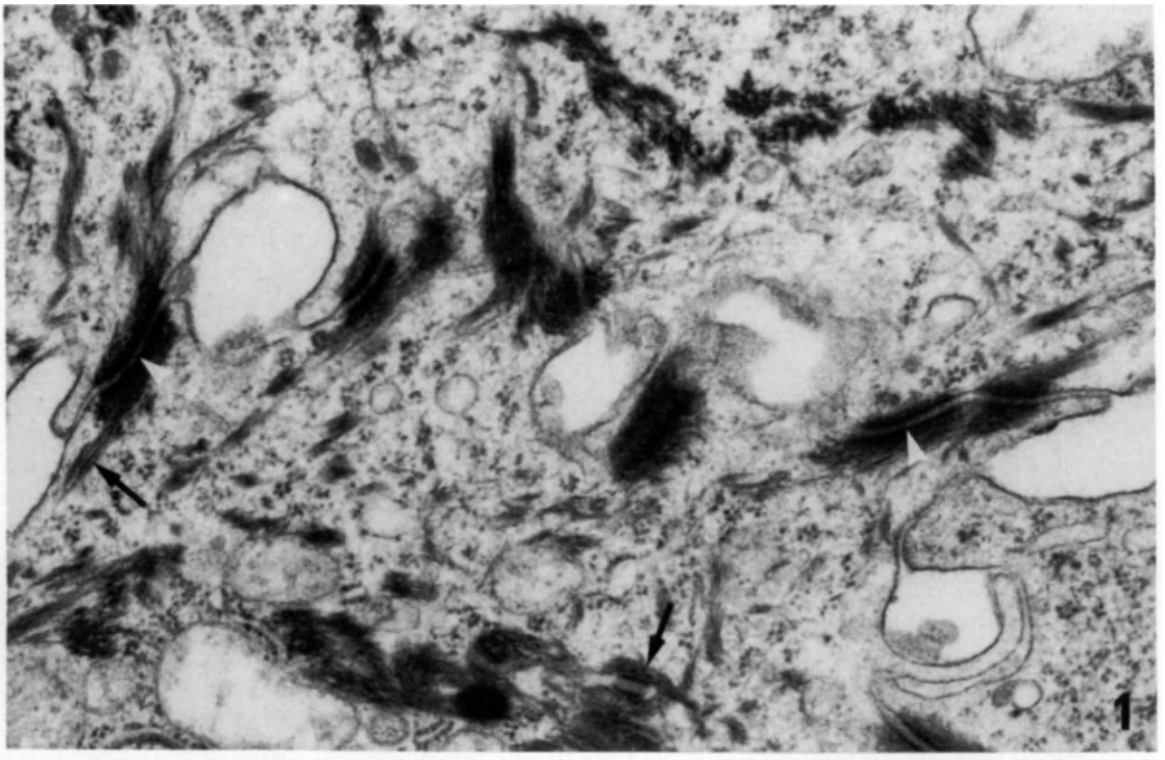
In the text above, we have repeatedly spoken about desmosome-like structures. These structures are, in fact, quite different from true desmosomes although at a casual glance they bear some resemblance to them. Learning to distinguish these two (*Plate 468, Fig. 1* and *Plate 471, Figs. 2–4*) is of paramount importance to the diagnostic electron microscopist, because true desmosomes and hemidesmosomes are principally markers for epithelia (also mesothelium and meninges) and their tumours; while the desmosome-like structures (which are as a rule little more than paired subplasmalemmal densities) and the hemidesmosome-like structures (subplasmalemmal densities), are markers for many mesenchymal cells and their tumours. Therefore some comments about these structures will now be presented.

Plate 469

Fig. 1. Metastatic deposit in a lymph node from a squamous cell carcinoma of the mouth. Note the well-differentiated desmosomes (arrowheads) and tonofibrils (arrows). ×34 000

Fig. 2. Thymoma. Note the tonofibrils (arrows) and well-differentiated desmosomes, one of which (arrowhead) is quite long. Such long desmosomes are rather characteristic of thymomas and mesotheliomas. ×60 000

Fig. 3. Chordoma. Note the well-differentiated desmosome (arrowhead), a tangentially cut hemidesmosome (arrow) and tonofilaments (F). ×62 000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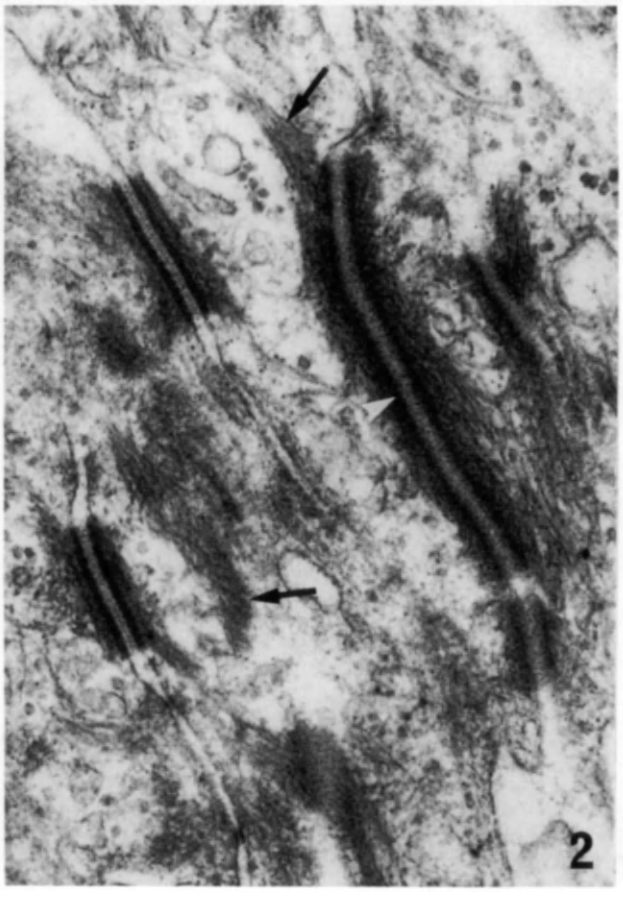
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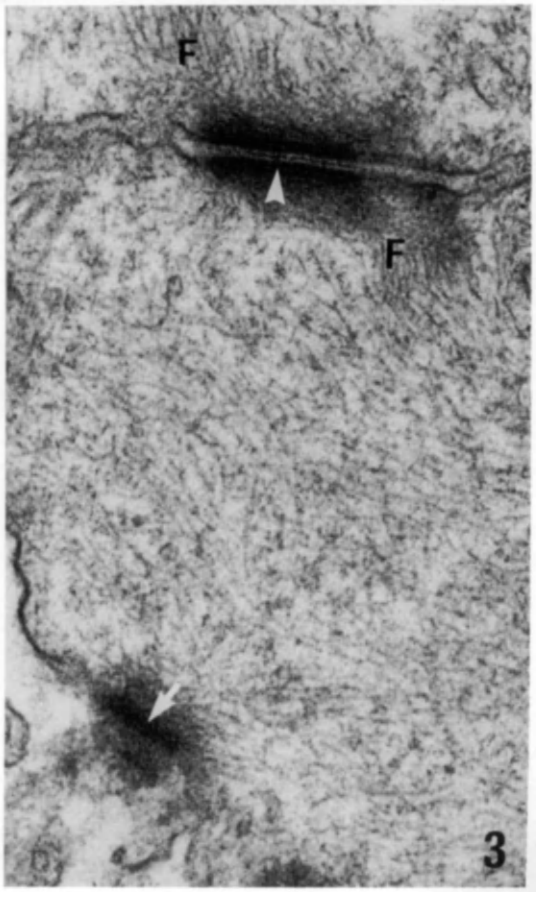
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Subplasmalemmal linear densities are regions of increased electron density found on the inner or cytoplasmic face of the cell membrane of many mesenchymal cells and their tumours (Mirra and Miles, 1982). For example, such densities are seen in normal and neoplastic smooth muscle cells (*Plate 367*), myofibroblasts (*Plate 375*), synovial intimal cells (*Plate 473*) and at times also macrophages or histiocytes. Small or poorly developed subplasmalemmal densities present as little more than a fuzzy cobweb of very fine short filaments adjacent to the cell membrane. In larger, better developed versions, a narrow dense irregular band of presumably compacted filaments is seen. Thus, in contrast to true desmosomes, one does not have an indubitable well defined electron-dense plaque and a less dense zone of radiating intermediate filaments.

In smooth muscle cells, subplasmalemmal densities form anchoring sites for some of the thin filaments (i.e. actin filaments) and also a few intermediate filaments (i.e. desmin filaments), but the latter are rarely detectable in the kind of preparations commonly employed by diagnostic electron microscopists. Another variety of subplasmalemmal density is that which forms when proteinaceous material comes to lie close to the cell membrane of certain cells, and in some, but not all instances, it is a prelude to the formation of a coated vesicle or vacuole, which impounds proteinaceous material from the extracellular compartment. Thus, when fibrin approaches the cell membrane of a macrophage or synovial intimal cell (*Plate 477*), a subplasmalemmal density may develop which bears some resemblance to the hemidesmosome found at the base of epithelial cells. Paired subplasmalemmal densities on cell membranes of adjacent cells can create a desmosome-like structure which may be mistaken for a real desmosome.

The most important feature distinguishing true desmosomes from desmosome-like structures and genuine hemidesmosomes (subplasmalemmal densities) from hemidesmosome-like structures (paired subplasmalemmal densities) is the presence of intermediate filaments* which converge on classic desmosomes and hemidesmosomes, but such filaments are not seen to converge on desmosome-like or hemidesmosome-like structures†. In or adjacent to desmosome-like and hemidesmosome-like structures one can discern only a fuzzy cobweb-like accumulation of thin filaments, or quite a dense compact accumulation of such filaments which are difficult to resolve‡.

Yet another distinguishing point is that in a true desmosome the intercellular gap is widened and there is an intermediate line in this gap. In the desmosome-like structure, the gap is very variable, but usually it is quite narrow. No sharp intermediate line is seen but a small irregular, or linear flocculent mass of proteinaceous material is at times present.

*As a rule the intermediate filaments converging on the plaques of desmosomes are cytokeratin filaments, but in meningiomas, granulosa cell tumours and blastema cells of nephroblastomas they are vimentin filaments (Kartenbeck *et al.*, 1984; Czernobilsky *et al.*, 1987).

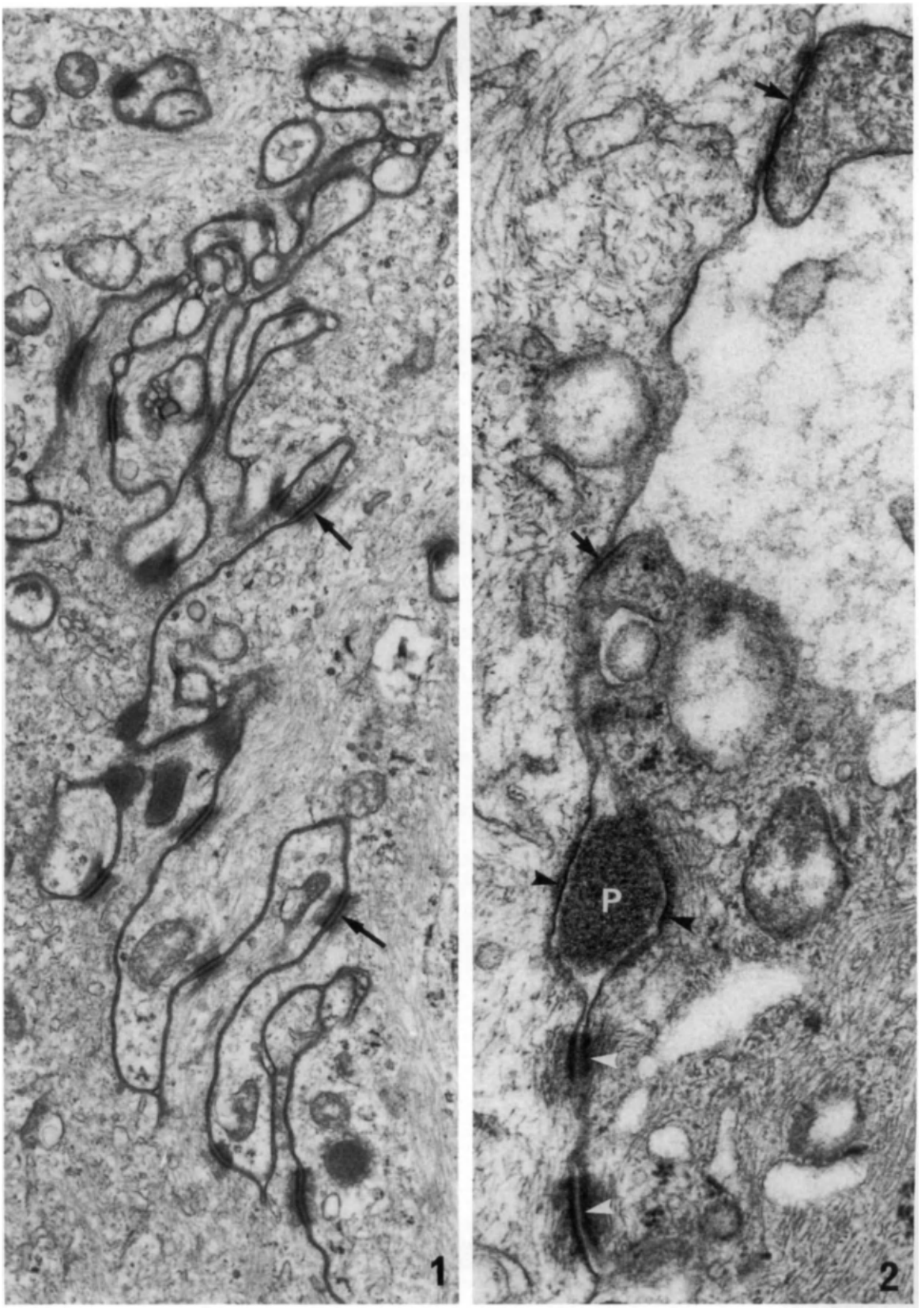
†This is true of all subplasmalemmal densities, but it should be noted that in smooth muscle, desmin and actin filaments are attached to the subplasmalemmal densities. However, desmin filaments are few and difficult to demonstrate in routine preparations, hence here also all that one sees are masses of thin filaments.

‡The essentially filamentous composition of these densities is surmised from the fact that the borders which are not so dense are filamentous, and also the fact that small not so dense densities of this kind clearly show a filamentous substructure.

Plate 470

Fig. 1. Meningioma. The interdigitating cell membranes or cell processes of the tumour cell are linked by several desmosomes (arrows). ×22 000

Fig. 2. Meningioma. Same specimen as *Fig. 1.* Seen here are structures interpreted as tight junctions (arrows), hemidesmosome-like structures (black arrowheads) engendered by proteinaceous material (P) between cells and true desmosomes (white arrowheads). The filaments converging on these desmosomes and elsewhere in the cell are now known to be vimentin filaments (Kartenbeck *et al.*, 1984) and not the usual cytokeratin filaments. ×47 000


P
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Finally, a not too constant difference is that a basal lamina is seen adjacent to true hemidesmosomes (after all a hemidesmosome is the agent which anchors the epithelial cell to the basal lamina), but this is usually absent in the case of many hemidesmosome-like structures (i.e. subplasmalemmal densities); but some caution is needed because some proteinaceous material or a collagen fibril lying in this region may create the impression that a basal lamina is present. Further, in the case of muscle cells, where an external lamina is present one does see this structure adjacent to the subplasmalemmal densities in these cells. Thus the presence or absence of a basal or external lamina is of limited value in distinguishing hemidesmosomes from subplasmalemmal densities.

It will be apparent from the above that desmosome-like structures are quite different from desmosomes and that they do not arise by a process of attenuation or modification of a true desmosome. Desmosome-like structures are seen not only in a wide variety of benign and malignant mesenchymal tumours but also in tumours such as neuroblastoma (*Plate 471, Fig. 4*) and schwannoma (*see Fig. 204* in Ghadially, 1985) and very rarely also melanoma. Hence, these desmosome-like structures are of little diagnostic value, but they constitute a potential hazard, in that they may be mistaken for true desmosomes and thus lead to an erroneous diagnosis of carcinoma.

Recent immunocytochemical studies support the concept presented here (and briefly enunciated in Ghadially, 1975) namely, that the desmosome-like structure is not just a poorly differentiated desmosome as was once imagined but that it is in fact an entirely different entity. These studies (Franke *et al.*, 1983) have shown that antibodies to desmoplakins (peptides found in the plaques of desmosomes) which bind specifically to the desmosomal plaques are found in a large number and variety of well differentiated and poorly differentiated epithelial tumours (e.g. squamous cell carcinoma of skin, tongue, epiglottis, oesophagus, cervix and rectum; adenocarcinomas of stomach and colon; hepatocellular carcinoma; cholangiocarcinoma; carcinomas of lung, parotid gland and breast) which as we know contain desmosomes and attenuated desmosomes, but not in a large variety of non-epithelial tumours (e.g. leiomyoma and leiomyosarcoma, endometrial stromal sarcoma, rhabdomyosarcoma, haemangioma, malignant fibrous histiocytoma, melanoma and neuroblastoma) which as discussed previously contain only desmosome-like structures (paired subplasmalemmal densities) and not genuine desmosomes or attentuated desmosomes. This study showed that lymphoma also does not contain desmoplakin. My ultrastructural studies lead me to believe that lymphomas contain neither desmosomes nor desmosome-like structures. However, some claim that on rare occasions a few small desmosome-like structures are seen (discussed more fully on pages 1122–1125). My present view is that if several even moderately well-developed desmosome-like structures are present then the tumour is highly unlikely to be a lymphoma.

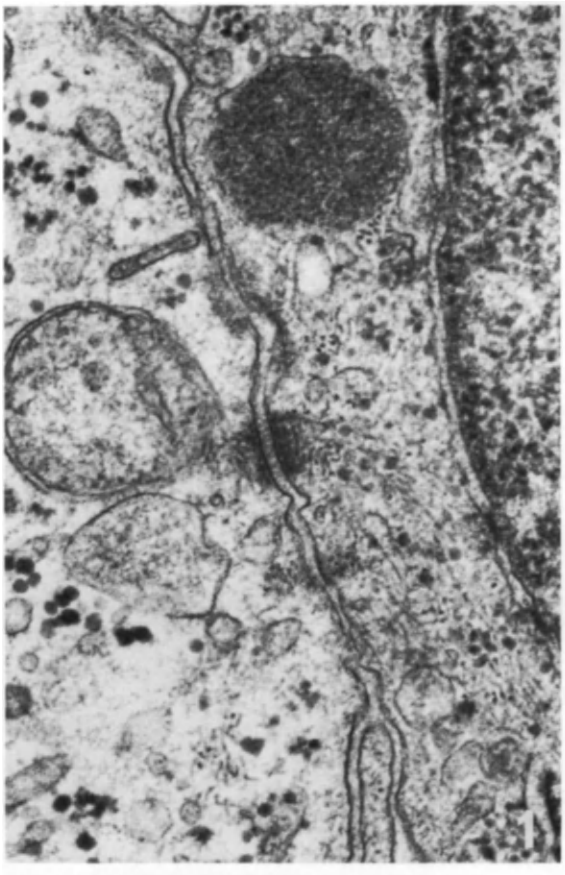
Plate 471

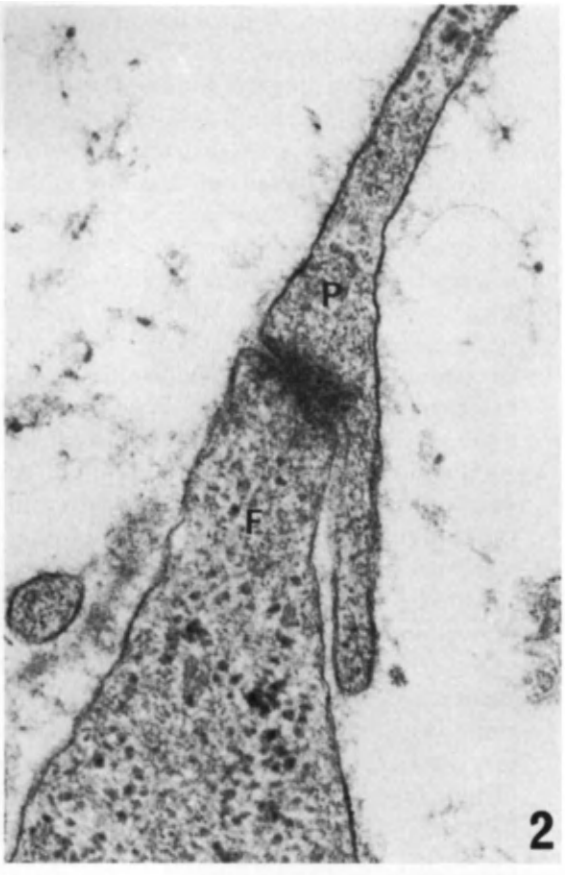
Fig. 1. An attenuated desmosome from a carcinoma of the breast. ×27 000 (*From Ghadially, 1980*)

Fig. 2. From a fibroadenoma of breast. A desmosome-like structure is seen between a fibroblast (F) and the cell process (P) of another fibroblast. ×66 000 (*From Ghadially, 1980*)

Fig. 3. Desmosome-like structures found in an Ewing's sarcoma. ×40 000

Fig. 4. Desmosome-like structures found in a neuroblastoma. ×103 000





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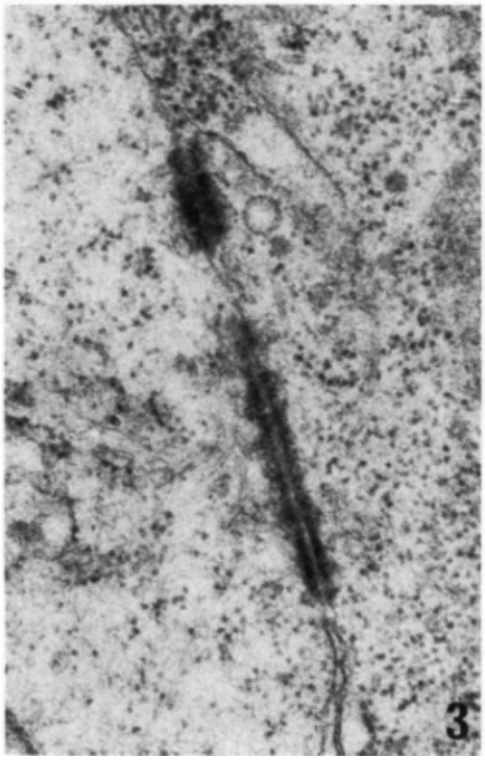

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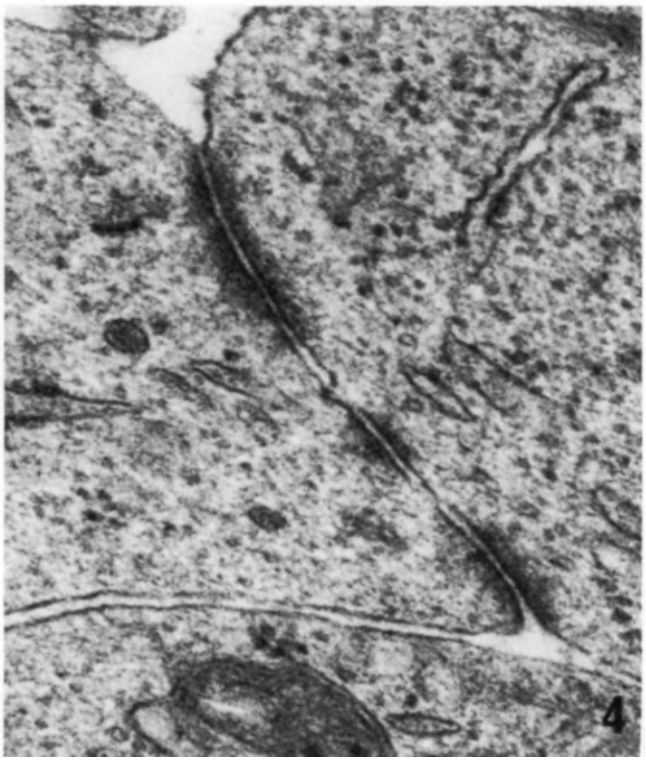


4

Let us now look at the value of tight junctions and terminal bars in tumour diagnosis. As mentioned before (page 1102) the tight junctions of the junction complex of epithelia are characterized by punctate and linear fusion of the outer leaflets of the cell membrane of adjacent cells. A zone of increased density (cytoplasmic fuzz) is seen adjacent to true tight junctions and their overall width is less than the combined width of the two membranes forming the junction.

Unfortunately, details of structure as described above are rarely demonstrable in routinely prepared tissues. Therefore, in the kind of preparations used for diagnostic electron microscopy, one has little choice but to use the term 'tight junction' in a loose fashion when adjacent membranes appear to be fused (i.e. when there is a dense line and/or dense dots uniting adjacent cell membranes) and there is an electron-dense fuzz in the region. Since tight junctions occur almost invariably adjacent to intercellular lumina, the position of the structure is of paramount importance in its identification. The leaky tight junction is identified by its position, cytoplasmic fuzz, absence of dense line and dots suggesting fusion and presence of a lucent gap between the apposed membranes (overall width more than the thickness of two membranes). As mentioned before (page 1102), tight junctions form belts or bands around cells. Thus the length of the junction (i.e. profile) seen in sections is quite variable. Markedly oblique sections through the belt produce quite long profiles which are at times mistakenly interpreted as 'long tight junctions'.

Modified terminal bars (comprising tight junctions or altered tight junctions followed by desmosomes or attentuated desmosomes) and also interdigitations of adjacent cell membranes together comprise highly characteristic features which frequently assist in the diagnosis of adenocarcinoma. However, the common adenomas and adenocarcinomas are not the only tumours in which tight junctions and desmosomes (i.e. junction complexes) occur, for these junctions are seen also in: (1) transitional cell papilloma and carcinoma (*Plate 472, Fig. 3*) (Tannenbaum *et al.*, 1978); (2) some neuroendocrine tumours (Ghadially, 1985); (3) synovial sarcoma (Gabbiani *et al.*, 1971; Klein and Huth, 1974; Kubo, 1974; Dische *et al.*, 1978; Ghadially, 1985); (4) meningioma (*Plate 470*); and (5) mesothelioma (*Plate 472, Fig. 2*).

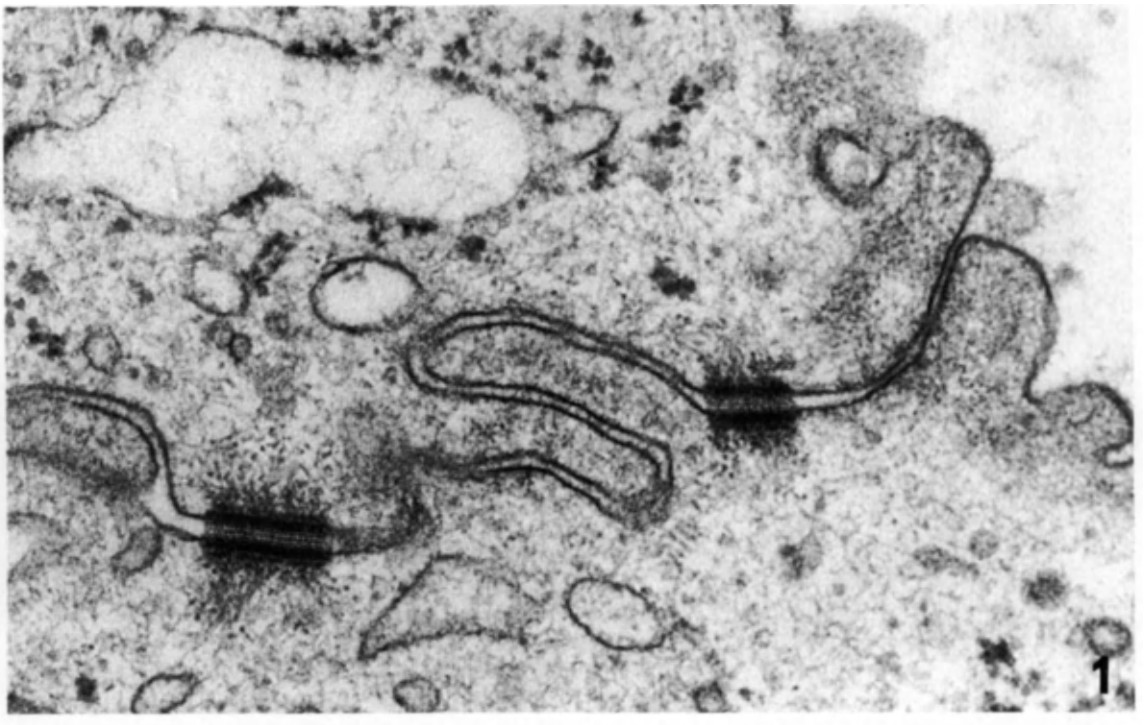
It will be apparent from the above that by studying the junctions in a difficult to diagnose tumour one can narrow down the possibilities if one remembers that the presence of: (1) desmosomes plus tight junctions characterize adenocarcinoma (all types including some neuroendocrinomas), transitional cell carcinoma, mesothelioma and synovial sarcoma; (2) desmosomes alone characterize squamous cell carcinoma, thymoma and chordoma; (3) tight junctions alone (i.e. no desmosomes) characterize vasoformative tumours; (4) desmosomes plus desmosome-like structures and very poor or small tight junctions characterize meningioma; (5) desmosome-like structures characterize sarcomas; and (6) absence of cell junctions characterizes lymphomas.

Plate 472

Fig. 1. Adenocarcinoma in ascitic fluid; primary probably in the stomach or colon. Two tumour cells are seen linked by a terminal bar comprising two well-formed desmosomes and a tight junction (arrow) which is probably leaky. ×78 000 (*From Ghadially, 1980*)

Fig. 2. Mesothelioma. The tumour cells are linked by a terminal bar comprising a tight junction followed by desmosomes. ×46 000 (*From Ghadially, 1980*)

Fig. 3. Transitional cell carcinoma of urinary bladder. The tumour cells are linked by a tight junction and a desmosome. ×55 000 (*From Ghadially, 1980*)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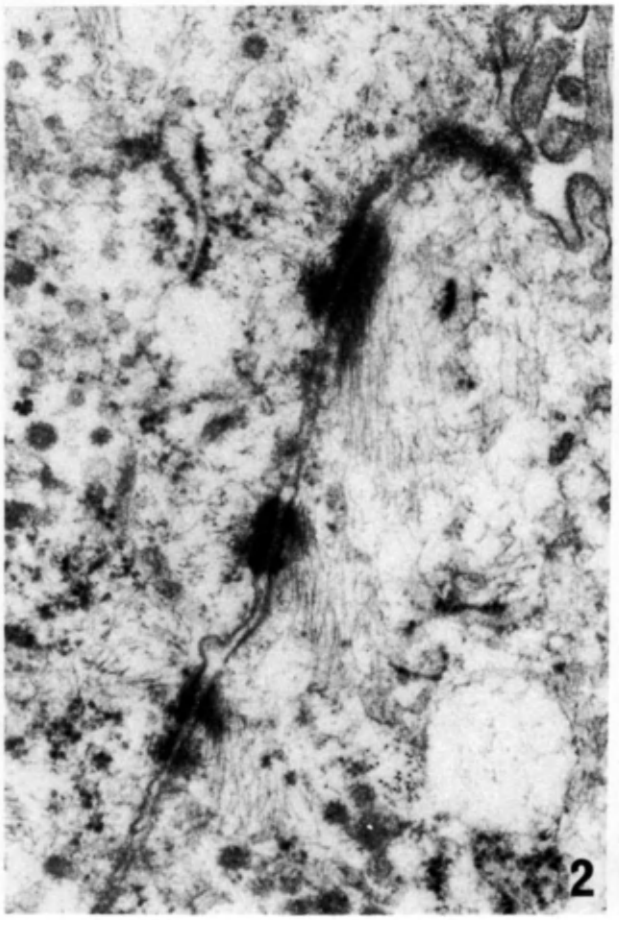
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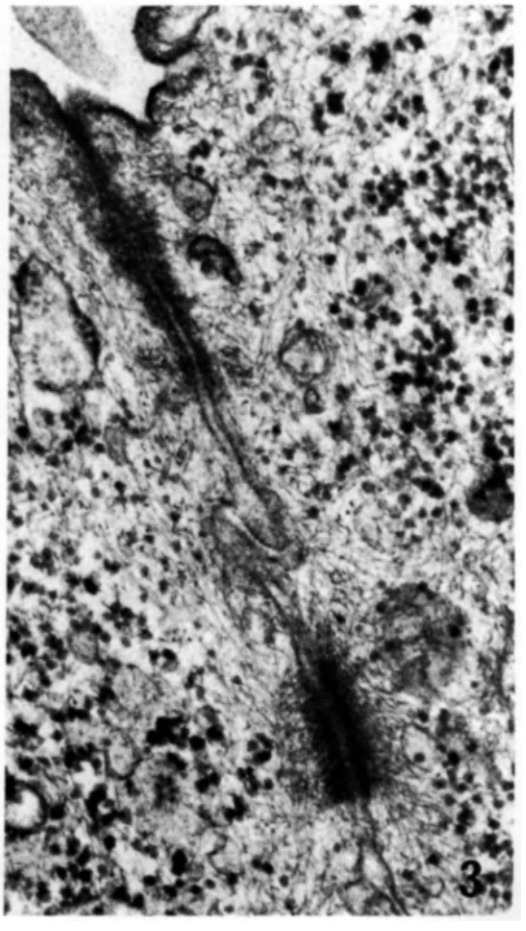

3

Cell junctions in connective tissues and haemopoietic tissues

An obvious minimum requirement for cell junctions to develop would be a close apposition of neighbouring cell surfaces. In keeping with this is the observation that in fibrous tissue, synovial tissue, or cartilage where the cells are usually separated by a fair amount of matrix, junctions or junction-like structures are, as a rule, not seen. However, when cellular proliferation (hyperplastic or neoplastic) brings such cells close together junctions or junction-like structures do develop. There are several old reports where structures variously described as 'close junctions', 'close association regions', 'attachments resembling intermediate junctions' have been seen between embryonic connective tissue cells and proliferating fibroblasts in culture (Ross and Greenlee, 1966; Devis and James, 1964; Trelstad *et al.*, 1966, 1967).

In retrospect it seems that what these authors are talking about are largely structures which we now call 'subplasmalemmal densities' and 'desmosome-like structures' (*see* pages 1114–1118). True desmosomes are not seen between the fibroblasts of fibrous tissue, fibroma or fibrosarcoma, but desmosome-like structures have been seen in a variety of human sarcomas including fibrosarcoma and also fibroadenoma (*Plate 471, Fig. 2*). Clarke (1970) has found desmosome-like structures in carcinogen-induced subcutaneous sarcomas in mice.

The chondrocytes of articular cartilage usually lie apart, separated from one another by abundant matrix. Even between chondrocyte pairs, there is a fair amount of matrix. However, occasionally when such cells come to lie very close together (e.g. in osteoarthrosis), desmosome-like structures may develop between them (Palfrey and Davis, 1966; Ghadially, 1983).

Synovial intimal cells are set apart in a matrix, so as a rule no junctions or junction-like structures are seen. However, in some species and in certain situations where the synovial cells are closely packed, junctions or junction-like structures may develop. Desmosome-like junctions are relatively frequent in the synovial membrane of rat (Roy and Ghadially, 1967) (*Plate 473, Fig. 1*) and cat (Groth, 1975) and they may also occasionally be found in the rabbit and calf (Langer and Huth, 1960; Groth, 1975). Rare gap junctions are said to occur in the cat, rabbit and chicken (Groth, 1975).

Neither desmosomes nor desmosome-like structures have been found in the normal human synovial membrane, but desmosome-like structures are often seen in pathological states (*Plate 473, Figs. 2 and 3*) where a hyperplasia of the synovial intimal cell has occurred; e.g. in traumatic arthritis, rheumatoid arthritis and villonodular synovitis (Grimley and Sokoloff, 1966; Ghadially and Roy, 1967, 1969; Ghadially *et al.*, 1979; Ghadially, 1983). It is thought that some of these desmosome-like structures develop as a result of the interaction of fibrin, trapped between the cell membranes of synovial cells (Ghadially *et al.*, 1978). The trapped fibrin may mimic the intermediate line of a true desmosome, but the absence of dense plaques and converging intermediate filaments clearly shows that they are not true desmosomes.

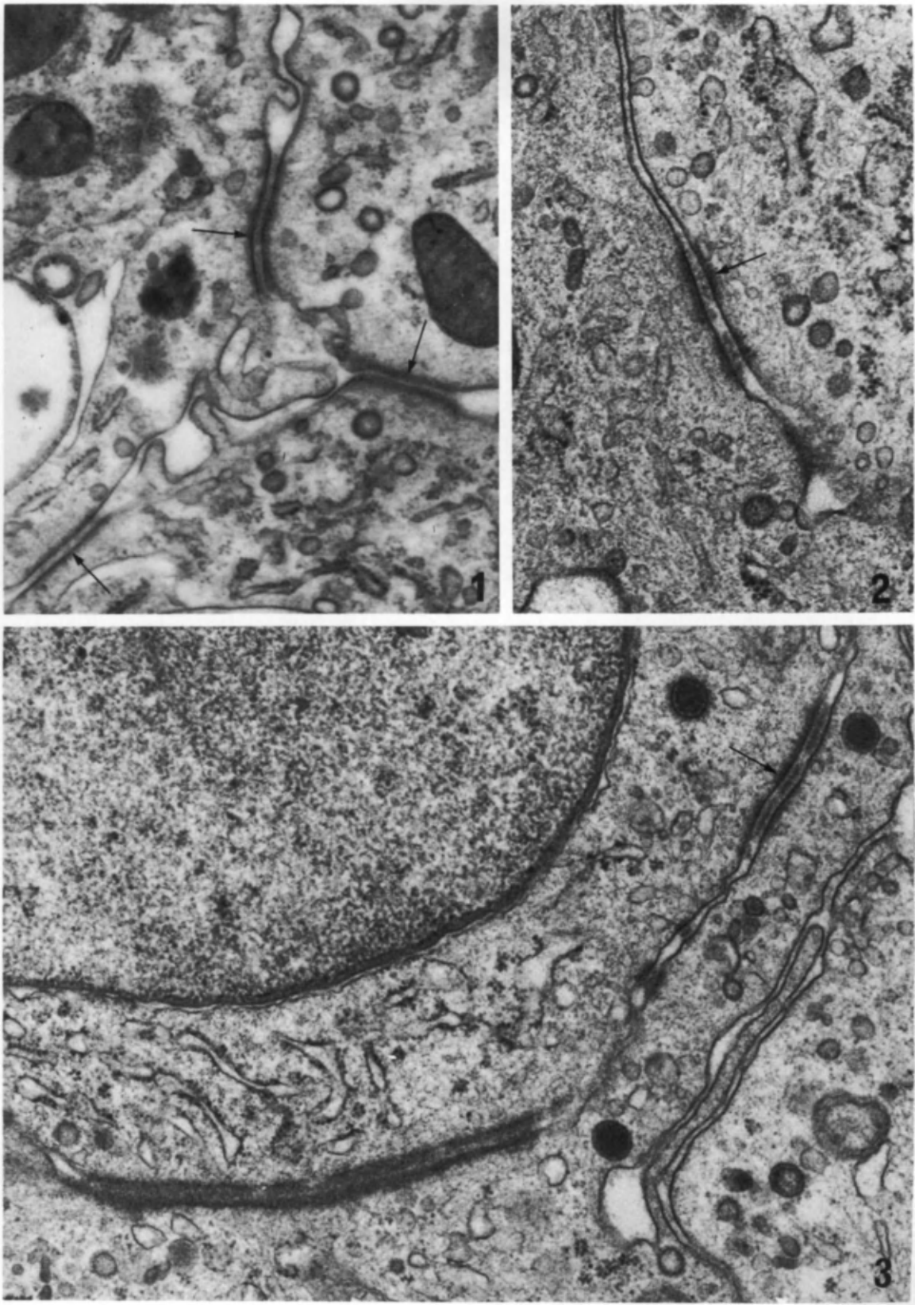
Plate 473

Desmosome-like structures (arrows) found in synovial tissue are depicted here.

Fig. 1. Normal rat synovial intima, showing desmosome-like structures between closely apposed synovial cells. ×36 000 (*From Roy and Ghadially, 1967*)

Fig. 2. Desmosome-like structures found in the synovial intima from a case of rheumatoid arthritis. ×48 000 (*From Ghadially, 1983*)

Fig. 3. Desmosome-like structures found in the synovial tissue from a case of villonodular synovitis. ×35 000 (*From Ghadially, Lalonde and Dick, 1978*)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3

Whether junctions occur in haemopoietic tissues (including lymphoreticular tissues and their neoplasms) is a matter of much dispute and uncertainty. It is difficult to understand why some workers can find junctions in these tissues but others do not. For example, junctions were not found in: (1) guinea-pig and rat bone marrow by Pease (1956); (2) human and rat spleen by Weiss (1957); (3) bone marrow of guinea-pigs by Zamboni and Pease (1961); and (4) bone marrow of albino rats by Weiss (1961). On the other hand, junctions were found in the bone marrow of: (1) rabbits, rats and guinea-pigs by Watanabe (1966); (2) rats by Weiss (1965), Ito (1965) and Ferguson *et al.* (1972); (3) guinea-pigs by deBruyn *et al.* (1971). It is difficult to be too precise about the type of junction or cell type between which junctions are alleged to occur, because of differences in interpretation and nomenclature. However, it would appear that the controversy revolves mainly around tight junctions between reticulum cells or reticuloendothelial cells*. The fact that some authors can find 'tight junctions', while others cannot, leads one to suspect that these junctions may be a preparative artefact—that is to say, they are probably labile appositions rather than tight junctions.

Desmosomes or desmosome-like structures have on rare occasions been demonstrated between dendritic reticulum cells in the germinal centres of the lymph nodes. For example, Kojima and Imai (1973) present a convincing illustration of desmosome-like structures between reticulum cells in the lymph node, while Lennert and Niedorf (1969) show a desmosome or desmosome-like structure between reticulum cells in the lymph node from a case of follicular lymphoma. The same desmosome-like structure is illustrated in Mori and Lennert (1969) and in Lennert (1973), attesting perhaps to the rarity of occurrence of desmosomes or desmosome-like structures in this site. A desmosome-like structure found in a histiocytic lymphoma with sclerosis (arising from a nodular lymphoma) is illustrated by Katayama *et al.* (1977), but this was between the stromal cells producing the collagenous stroma.

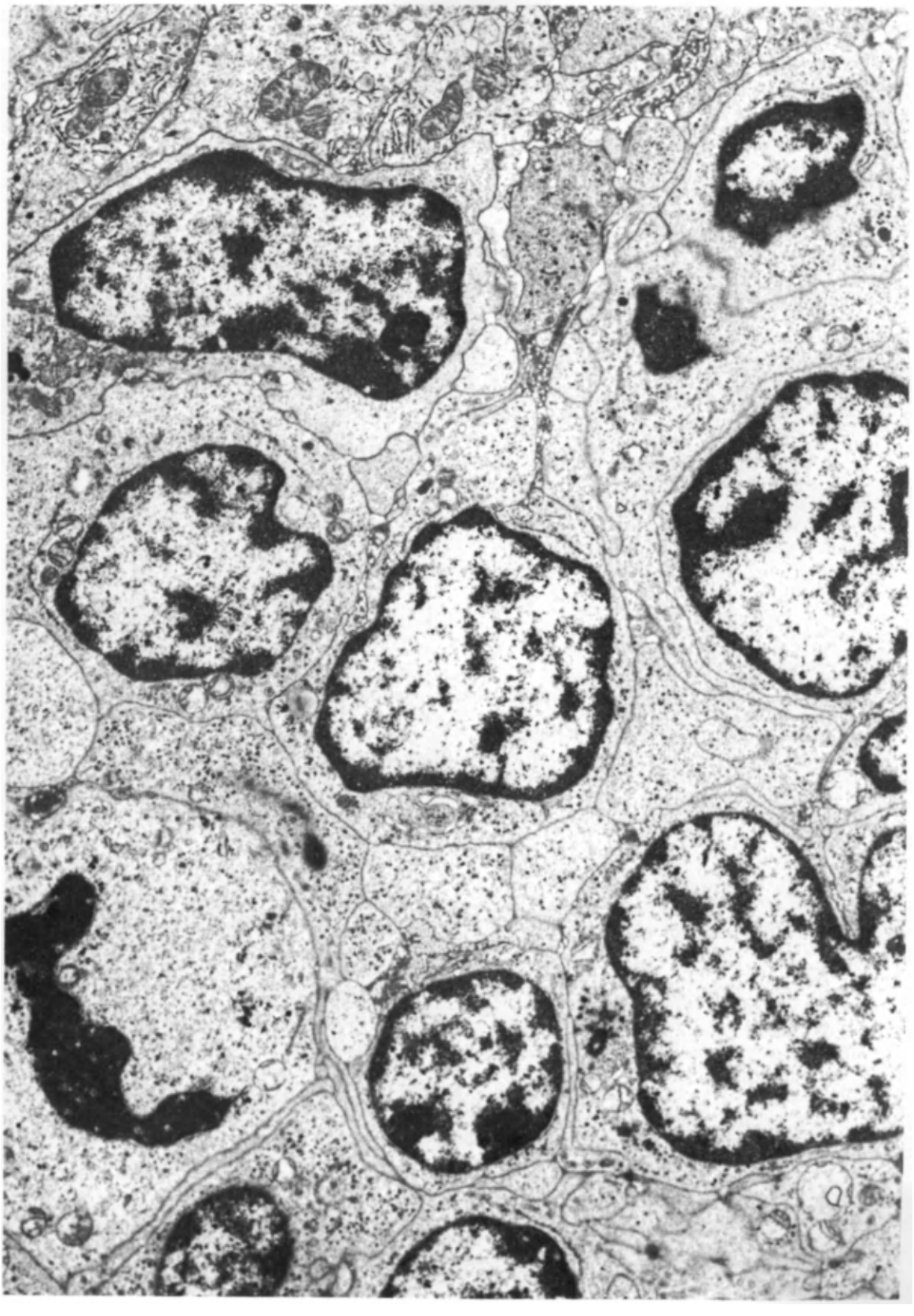
In an excellent paper on nodular lymphoma Levine and Dorfman (1975) show indubitable desmosomes with dense plaques, filaments and an intermediate line in the normal human tonsil. However, in a tissue such as this the possibility of squamous epithelial cells trapped in lymphoid tissue has to be kept in mind. Carr *et al.* (1977) present an electron micrograph (their *Fig. 1.6*) with a legend which reads: 'Electron micrograph of macrophage from sarcoid granuloma showing numerous lysosomes, interdigitating cytoplasmic processes and desmosomes'. No desmosome is seen in this illustration but some subplasmalemmal densities are present along the cell membrane of this macrophage. This is evident despite the poor quality of tissue preservation and/or preparation.

One may conclude by pointing out that in a majority of reports there is no mention of desmosomes or desmosome-like structures in haemopoietic (including lymphoreticular) tissues and their neoplasms (*Plate 474*) nor have I seen such structures in the cases that I have studied. However, I think one has to concede that on rare occasions desmosome-like structures do occur between reticulum cells but perhaps not between various other cells in haemopoietic tissues, except of course, the reticular epithelial cells of the thymus. Here, as one would expect (because of their epithelial nature), indubitable desmosomes perfect in every detail are found and the same is also true for epithelial thymomas, where classic desmosomes with dense plaques, filaments and intermediate line are seen (Ghadially and Illman, 1965; Levine, 1973; Bloodworth *et al.*, 1975; Llombart-Bosch, 1975).

*An exception to this is the report by Veerman and van Ewijk (1975) who illustrate structures that could be tight junctions between what they call an 'interdigitating cell' and a lymphocyte.

Plate 474

Bone marrow from a case of lymphoblastic leukaemia. Despite the close packing of tumour cells, nothing even remotely resembling a cell junction is present. ×10500



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16

Endocytotic structures and cell processes

Introduction

Extensions of the cell cytoplasm covered by cell membrane are collectively referred to as cell processes. Since some of these processes engage in endocytotic activity (e.g. pseudopodia in phagocytosis), it is convenient to consider together various cell processes and structures involved in endocytosis, and endocytotic phenomena such as: (1) micropinocytosis, whereby vesicles (called caveolae) springing from the cell membrane transport material into the cell; (2) micropinocytosis vermiformis, whereby material is transported via tubular channels; and (3) Langerhans' cell granule, a structure of unique morphology which is also thought to be involved in the transport of material into the cell.

A variety of cell processes are known to occur on the cell surface and attempts have been made to classify them in various ways. Thus one may try to divide them into motile and non-motile processes, and into stable and ephemeral or transient processes. Such attempts are not too successful, for within a single morphological category different patterns of function and behaviour may be found (e.g. both motile and non-motile cilia are known to occur) and there is controversy regarding the nature and function of others.

However, the morphological distinctions between most cell processes are reasonably clear-cut. Cilia and flagella are distinguished by the presence of a basal body and an axial microtubule complex (page 1176). In the striated or brush border of absorptive cells occur numerous uniform, slender processes called microvilli. A few microvilli occur also in a variety of other cells.

Pseudopodia are an example of blunt transient processes employed by cells such as amoeba or the neutrophil leucocyte for locomotion and the uptake of food particles and/or other extraneous matter. In ultrathin sections of tissues, profiles of long slender processes are at times encountered in some cells. Such an appearance often represents sections through folds* or ruffles of the cell membrane. Cells in culture demonstrate that fusion of the margins of such folds leads to the formation of a vacuole in which impounded fluid is transported by a process called pinocytosis. No convenient acceptable term has been coined to describe these long slender 'cell processes' seen in sectioned material. Such processes abound in synovial intimal

*Thin well-developed folds of this nature are at times referred to as lamellipodia but they can only confidently be identified by phase contrast microscopy or scanning electron microscopy. Hence this term is of limited value in describing cell processes in sectioned material.

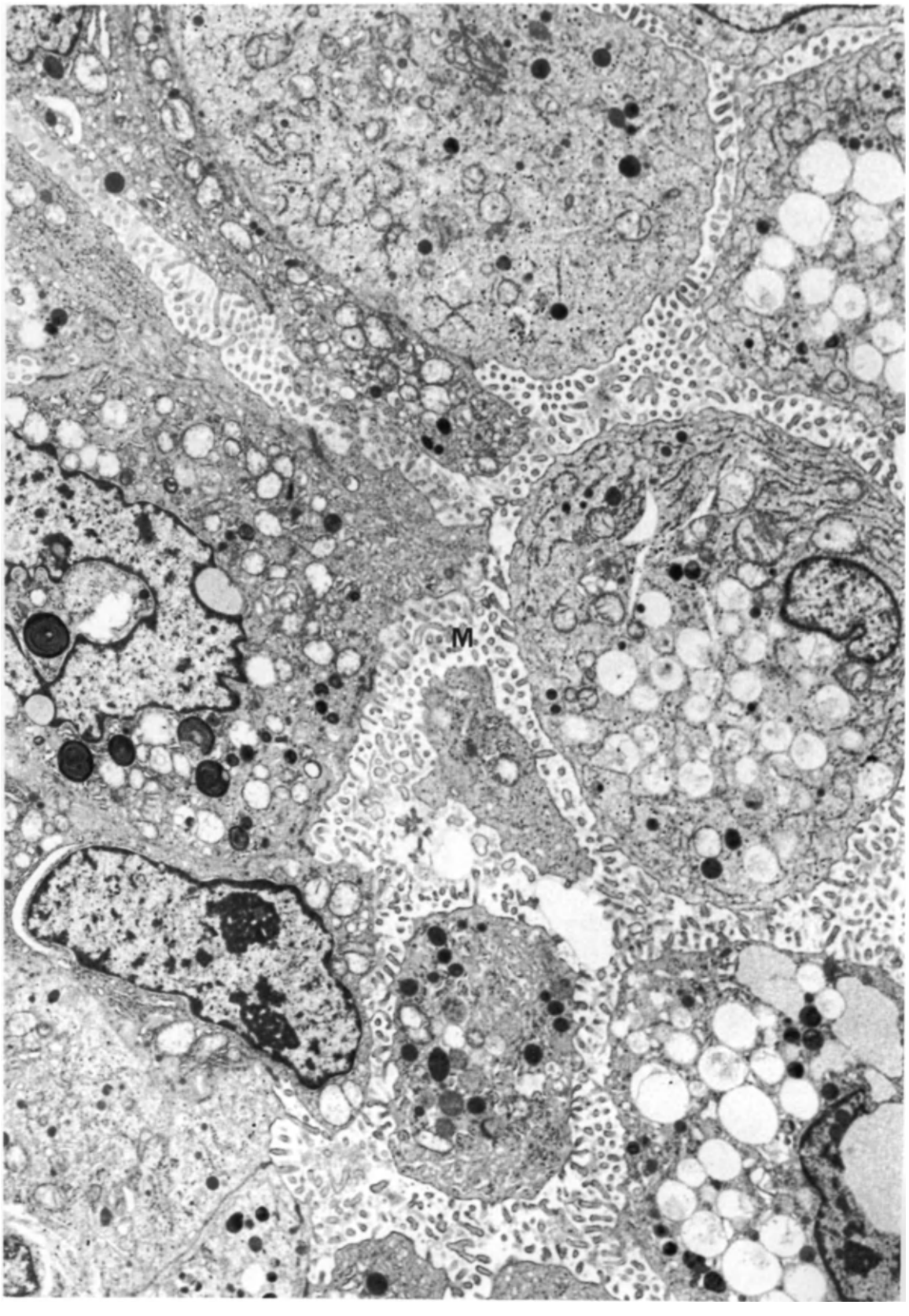
cells and students of this tissue have adopted the term 'filopodia' (L. *filium*, thread) to describe them, yet such filopodia may or may not all be thread-like (three-dimensionally) and they certainly are not little feet or locomotor organs*. However, the lack of a better term, and ignorance of the three-dimensional morphology forces us to use the term 'filopodia' and even to extend its usage to other sites and situations where slender cell processes are seen in sectioned material.

In pathological and experimental situations processes may develop on cells that do not normally bear such processes or have only a few processes. Such processes are frequently referred to as microvilli, even though some of them may be sections through folds and ruffles (i.e. circular profiles acceptable as transverse sections through microvilli are absent) and others bear only a vague resemblance to microvilli in that although they may be more or less 'finger-like' they are neither uniform in size (i.e. variable length and diameter) nor regularly arranged. Examples of such processes may be seen in cells in ascitic effusions and also in cells in oedematous tissues and in cells in culture. Atypical cell processes may be found in tumour cells. The most florid example of this is seen in a condition called 'hairy cell leukaemia' (page 1166). A few microvilli are seen in a variety of adenocarcinomas but some such as the alveolar cell carcinoma of the lung are noted for the abundance of microvilli on the surface of the tumour cells (*Plate 475*). However, much longer and quite numerous cell processes occur in many mesotheliomas. In conjunction with other findings these long cell processes aid in distinguishing mesothelioma from adenocarcinoma (for more details *see* Chapter 7 in Ghadially, 1985).

*Examples of true filopodia are found in some protozoa and in cultured cells spreading on a substrate. They may have a substrate-exploring function (Albrecht-Buehler, 1976).

Plate 475

Alveolar cell carcinoma showing innumerable microvilli (M) sprouting from the tumour cells. ×6800


M

Endocytotic vesicles and vacuoles

There are many ways by which substances can move in and out of the cell across the selective, semipermeable barrier imposed by the cell membrane. These include: (1) active transport, whereby substances are driven against a concentration gradient with energy originating from cell metabolism (e.g. from ATP; *see* page 1048); (2) passive transport, whereby substances are driven across the cell membrane with energy supplied from external sources (e.g. osmotic forces or the application of an electrical potential); and (3) vesicular transport, whereby material is transported within single-membrane-bound vesicles or vacuoles derived from the cell membrane. Vesicular and vacuolar transport into the cell is now described by the term 'endocytosis', whereby the engulfed material ultimately comes to lie in a cytoplasmic vesicle or vacuole derived from the cell membrane. Similarly, vesicular transport out of the cell is spoken of as exocytosis, whereby a membrane-bound structure such as a secretory granule, a telolysosome or a vesicle derived by endocytosis fuses with the cell membrane and discharges its contents into the external environment.

In some instances the material leaving the cell takes with it a coat derived from the cell membrane. Examples of this are seen in the case of viruses, and particles of milk-secretion, which are liberated by a process of evagination and pinching off of the cell membrane (for references supporting the statements made above, *see* Bennett 1969a,b; Schoffeniels 1969a,b).

The first two methods of transport (active and passive) which are sometimes collectively referred to as transmembrane transport, or permeation, do not concern us here, for the ions and small molecules moved in this fashion are not directly demonstrable in electron micrographs.

Some 23 or more names have been coined by various workers to describe the process of endocytosis as seen in different sites and by different techniques. Fortunately, only three terms—namely, phagocytosis, pinocytosis and micropinocytosis—are in current use and seem adequate to describe most observed phenomena (*Plates 476–478*).

The term 'phagocytosis' and 'pinocytosis' derive from the Greek roots for 'eating' and 'drinking' respectively*. Both phenomena are observable by light microscopy and have been recognized by students of the cell for a long time. The term 'phagocytosis' was coined by Metschnikoff (1883) to describe the process by which cells ingest food, and later he extended this to his well-known concept of microphages (neutrophils) and macrophages and their role in defence mechanisms. Based on morphological differences of vacuole content, size of particles ingested and other small differences, a host of names were coined by other workers (e.g. colloidoplexy, chromopexy, atherocytosis, phagotrophy and granulocytosis) to describe this

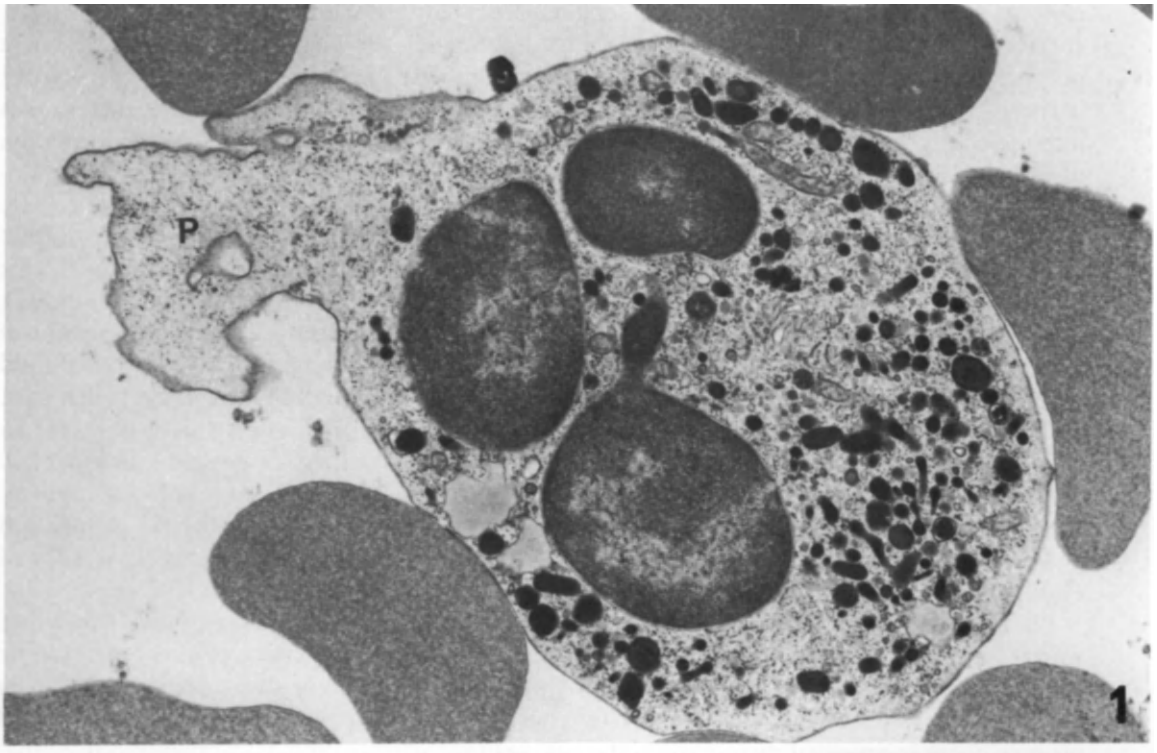
*This distinction between particulate uptake (eating) and fluid uptake (drinking) relate more to the resolving power of the microscope (light and electron) rather than the true state of affairs, for in the final analysis all matter is particulate. However, there is some value in retaining such distinctions from the morphological point of view.

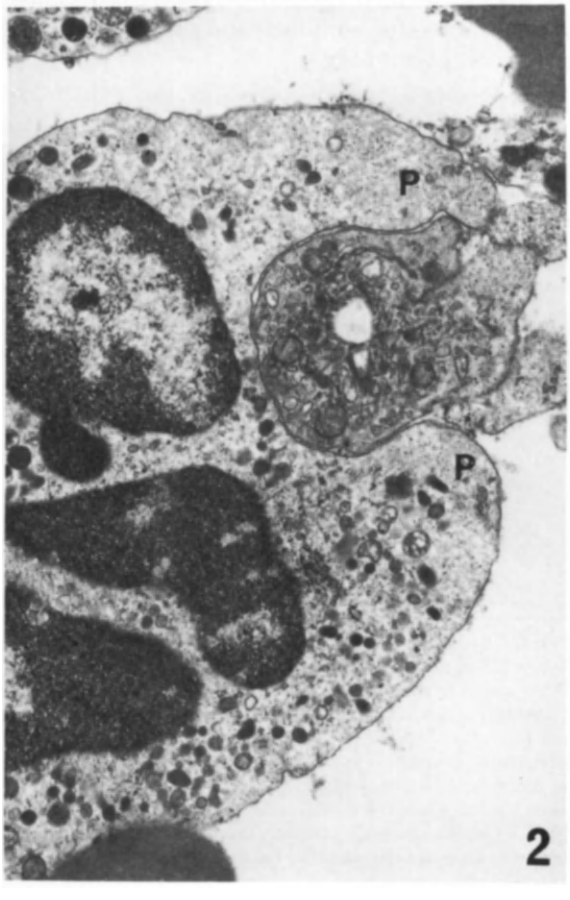
Plate 476

Fig. 1. A human neutrophil leucocyte from peripheral blood, showing a pseudopod (P), which consists mainly of ectoplasm. The various organelles are confined to the endoplasm. The amoeboid movement of these leucocytes is accomplished by such pseudopodia. ×13 000

Fig. 2. The pseudopodia (P) on this leucocyte found in human bone marrow appear to be about to phagocytose a cell fragment. ×14 000

Fig. 3. Neutrophils phagocytosing staphylococci (S). From an *in vitro* preparation consisting of buffy coat of blood mixed with staphylococci (S). The cells were fixed one minute after the addition of the organisms. ×20000 (*Skinnider and Ghadially, unpublished electron micrograph*)


P
1


P
P
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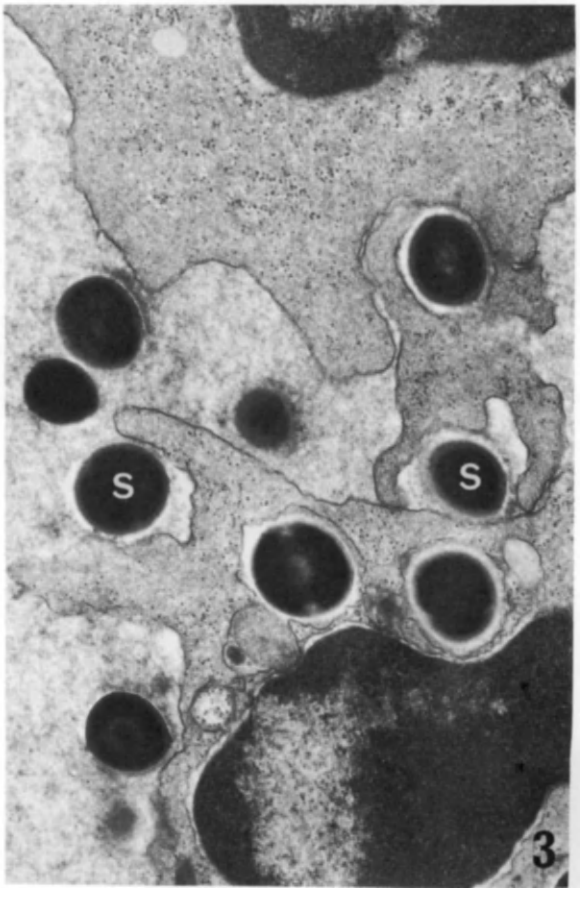
S
S
3

phenomenon but these names are no longer used. The term 'phagocytosis' has, however, survived. Here it is envisaged that blunt pseudopodia arising from a cell such as a polymorphonuclear leucocyte (*Plate 476*) or an amoeba encircle some 'solid' particle or particles, trapping the exogenous material in a membrane-bound vacuole which then moves into the cell. Usually, only the ectoplasmic zone of the cytoplasm is involved in the formation of pseudopodia. The major cytoplasmic organelles and inclusions as a rule do not flow into pseudopodia.

The term 'pinocytosis' was coined by Lewis (1931) to designate the vacuolar uptake of fluid by macrophages and sarcoma cells observed with phase-contrast microscopy. This phenomenon is commonly seen in tissue culture, where constantly moving and altering transient thin folds or ruffles are seen on the cell surface. Every now and again the margins of such folds fuse, or the margin of a fold fuses with the cell membrane to trap a droplet of fluid which then moves into the cytoplasm as a clear vacuole. It has long been debated whether such a phenomenon occurs *in vivo*. Certainly electron-microscopic images consistent with such a concept are seen, but, as is only to be expected, such folds and ruffles present as slender cell processes (filopodia) in sectioned material. Fawcett (1966) illustrates this in cells lining capillary endothelia, and the same is seen in the case of synovial cells which, with the aid of such cell processes, can trap not only fluid but also fibrin and a variety of other materials (*Plate 477* and *Plate 303*) (Ghadially and Roy, 1969).

Electron microscopy reveals that, besides fluid, pinocytotic vacuoles may contain particulate matter, and in some instances quite large particulate material (e.g. entire erythrocytes) can be impounded by the so-called 'filopodia' of synovial cells. Thus the distinction between pinocytosis and phagocytosis is singularly tenuous. Except for the size and shape of the cell process, there is little that distinguishes the two in electron micrographs. Certainly cell 'eating' and cell 'drinking' are not such separate and distinct events, for both fluid and particulate matter are often impounded in a single gulp (*see* footnote on page 1134).

Morphologically quite distinct from the above-mentioned processes is the process of micropinocytosis. Here small invaginations of the plasma membrane occur and later these detach by pinching off to form little vesicles which move into the cell. Both fluid and small particulate matter (e.g. ferritin, carbon or Thorotrast) is transported in this manner.

Micropinocytotic vesicles present in electron micrographs as alveolate or flask-shaped structures of uniform size (40–80 nm in diameter) attached to the cell membrane. Such an appearance could represent either a vesicle about to form and move into the cell (endocytosis) or a vesicle which has fused with the cell membrane and is discharging its contents into the cell environment (exocytosis). Since such distinctions cannot be made in a routine electron

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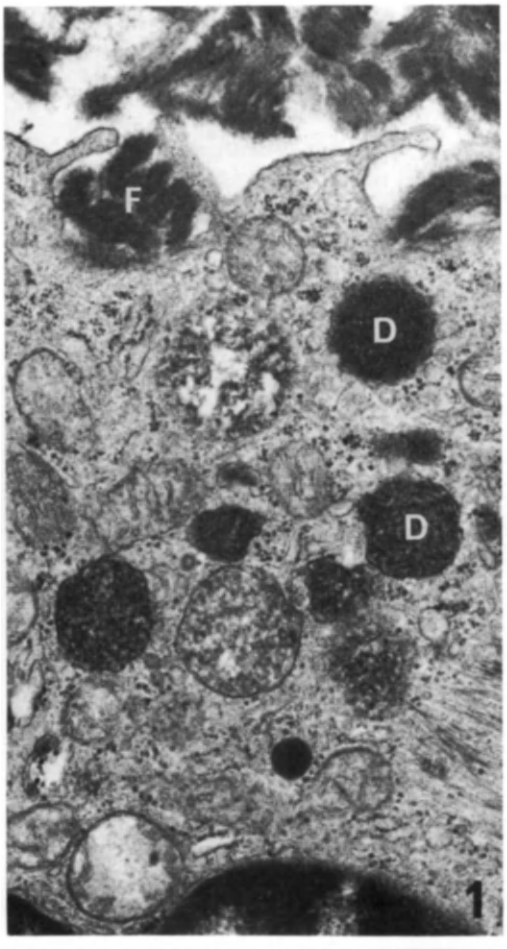
Plate 477

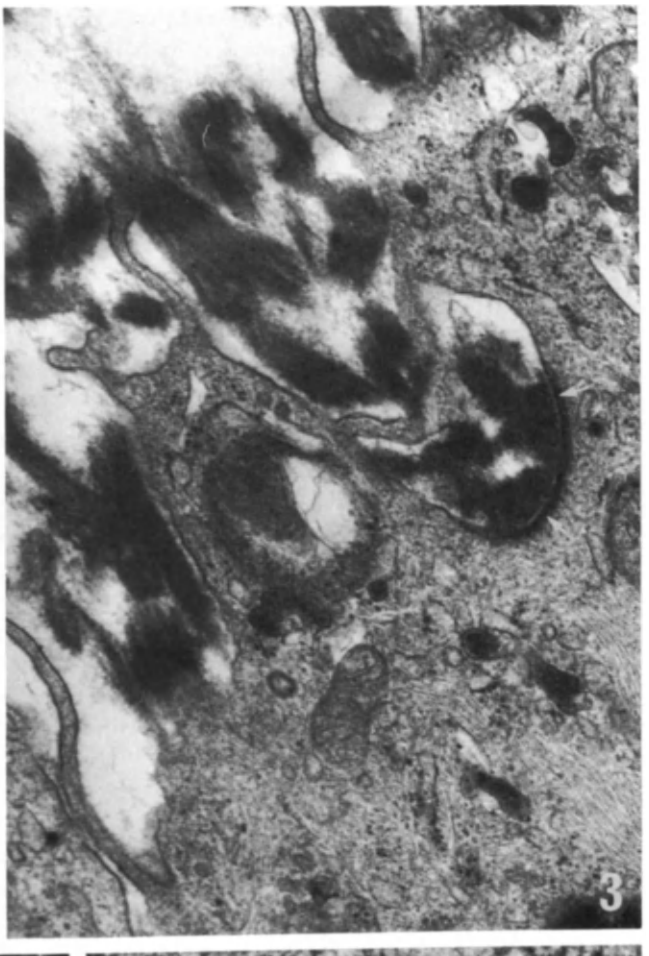
Synovial cells from an arthritic knee joint. (*Figs. 1 and 4 from Ghadially, Oryschak and Mitchell, 1974*)

Figs. 1 and 2. 'Filopodia' wrapping around fibrin (F). Also seen are dense bodies (D) in the cell cytoplasm which represent phagosomes and phagolysosomes (i.e. heterolysomes) derived from the endocytosed fibrin. For a more detailed account of this phenomenon, *see* the legend to *Plate 303*. ×26 000; ×25 000

Fig. 3. Here again, 'filopodia' are seen trapping fibrin. An unusual feature here is that part of the wall of an endocytotic vacuole is markedly thickened and electron dense (arrow). ×30 000

Fig. 4. At higher magnification, another forming or formed vacuole from the same specimen as *Fig. 3* shows that the thickening is due to the deposition of fuzzy electron-dense material on the cytoplasmic side of the cell membrane. Electron-dense material also coats the inner surface of the vacuole, but it is separated from the cell membrane by a less dense layer. ×65 000


F
D
D
1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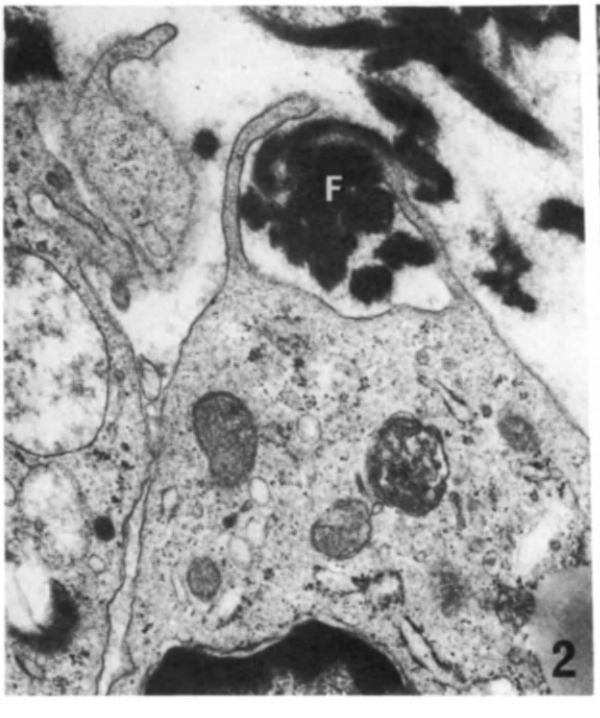

F
2


4

micrograph, it has been suggested that a morphologically descriptive but non-committal term such as 'caveolae' (first proposed by Yamada in 1955) would be more appropriate to describe these structures than 'micropinocytotic vesicles', which has functional connotations. Although this is a perfectly logical suggestion, the term 'caveolae' is used only occasionally.

A few micropinocytotic vesicles may be found in most cell types, but they are particularly common in capillary endothelia and in smooth and striated muscle (*Plate 478* and *Plate 365*). Such vesicles are also at times quite numerous in synovial cells and chondrocytes. An abundance of micropinocytotic vesicles is an indication of heightened endocytotic activity, and it is said that this can be provoked by presenting the cell with material which is ingested by this mechanism (Stockem and Wohlfarth-Bottermann, 1969).

Various terms such as 'fuzzy vesicles', 'coated vesicles', 'alveolate vesicles', 'acanthosomes' and 'decorated vesicles' have been used to describe micropinocytic vesicles bearing filamentous and spiny adornments on their limiting membrane. Coated micropinocytotic vesicles* form when protein is being transported into the cell. An example of this is the fuzzy or spiny vesicle which is believed to transport ferritin destined for haemoglobin synthesis into the erythroblast (*Plate 478*). Coated vesicles have been seen in many other situations, but the most conspicuous examples are found (Fawcett 1966) in the oocytes of insects at a stage when rapid uptake of protein is occurring.

We (Ghadially *et al.*, 1974) have noted that much larger endocytotic vacuoles may also develop a fuzzy or coated appearance when fibrin is being endocytosed by synovial cells. This was seen in a patient who had long-standing multiple sclerosis, psoriasis and arthritis. Here however the vacuoles were only partially coated, in the sense that only a portion of the circumference of the vacuole was coated and bore filamentous decorations on its cytoplasmic surface (*Plate 477, Figs. 3 and 4*).

Phagocytosis, pinocytosis and micropinocytosis all lead to the formation of a single-membrane-bound structure containing engulfed material. We have seen that distinction between these processes is at times subtle and difficult. Endocytosis may be followed by exocytosis so that the material is transported across the cell from one border to another (e.g. in capillary endothelium), but often the endocytotic vacuole is a phagosome which subsequently fuses with a primary lysosome or lysosomes to form a heterolysosome. The development and fate of these structures has been dealt with in Chapter 7.

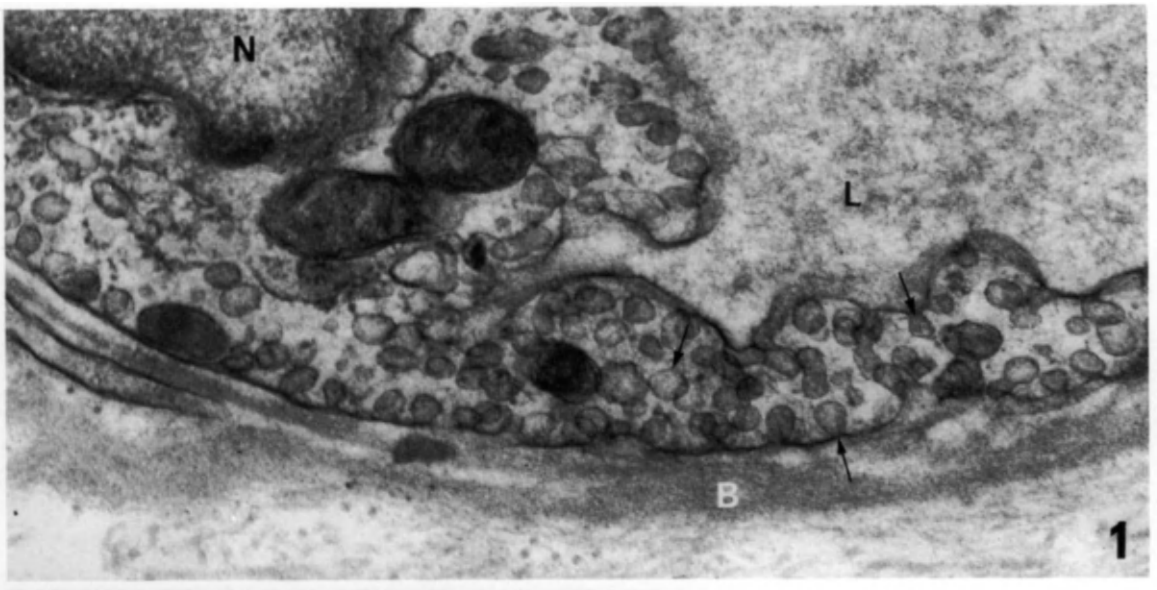
*The coated micropinocytotic vesicle is but one variety of coated vesicle. For example, vesicles with or without a coat can also be derived from the Golgi complex. Here they transport enzymes (e.g. hydrolases in primary lysosomes) and secretory products. It would be absurd to call these micropinocytotic vesicles because they are not involved in 'cell drinking'. One must not confuse such vesicles with quite different origins and functions as do Carr and Wright (1979) when they state that in the mature macrophage 'the primary lysosome is a micropinocytic vesicle'. Incidentally, the correct term is 'micropinocytotic' not 'micropinocytic'.

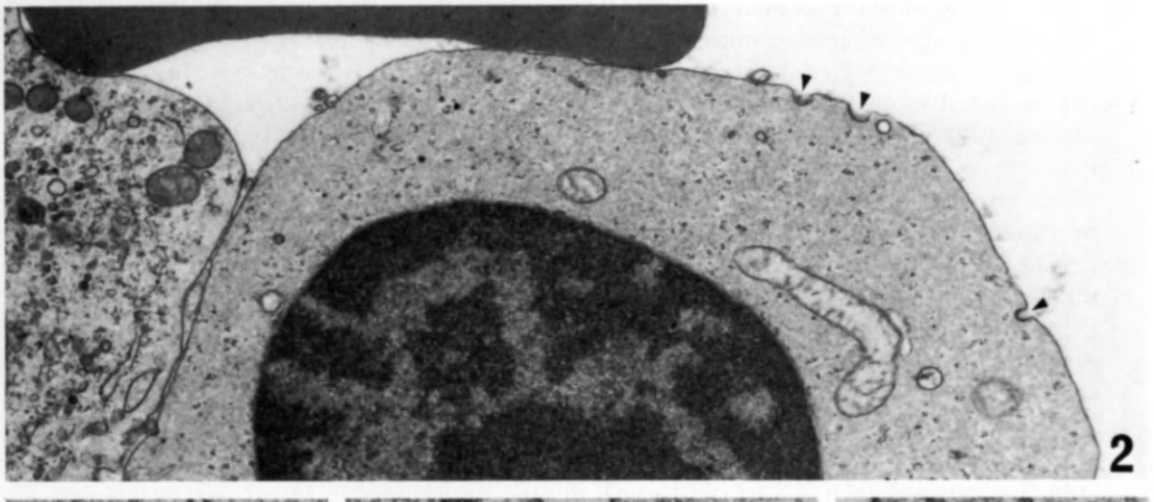
Plate 478

Fig. 1. A vascular capillary from normal rabbit synovial membrane, showing unusually numerous caveolae (arrows). The nucleus of an endothelial cell (N), the lumen of a vessel (L) and the basal lamina (B) are easily identified. ×56 000 (*Ghadially and Roy, unpublished electron micrograph*)

Fig. 2. Erythroblast (normoblast) from human bone marrow, showing three coated vesicles (arrowheads) in the process of formation from the cell membrane. ×17 500

Figs. 3, 4 and 5. The manner in which coated vesicles transporting ferritin develop is depicted here. *Fig. 3* shows the cell membranes of two erythroblasts which were lying side by side. One of these shows a saucer-shaped depression where the cell membrane has acquired a fuzzy coat and some electron-dense particles (arrowhead) believed to be ferritin. *Fig. 4* shows two vesicles (arrowheads) which depict further stages of development, while *Fig. 5* shows a vesicle which appears detached from the cell membrane but about this one cannot be absolutely certain because the membrane limiting the vesicle is poorly defined, suggesting that this is an off-centre section. ×90 000; ×90 000; ×90 000


N
L
B
1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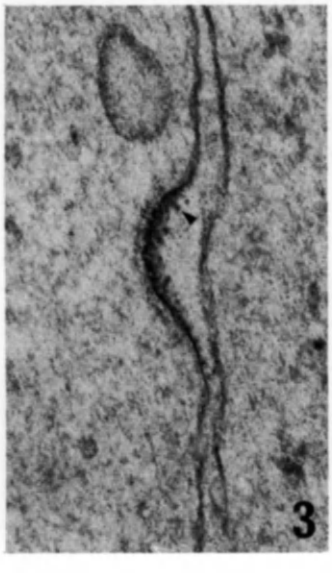
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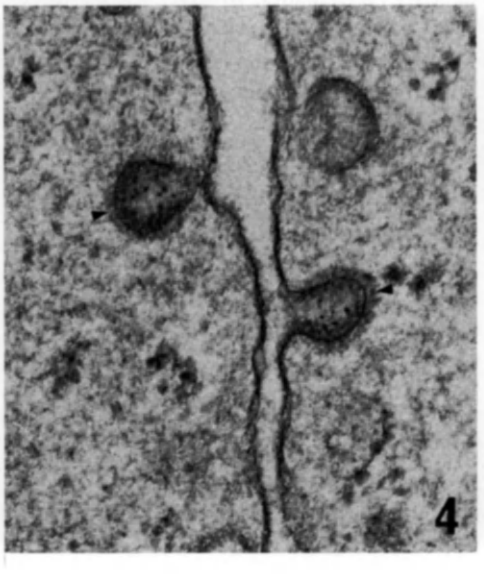
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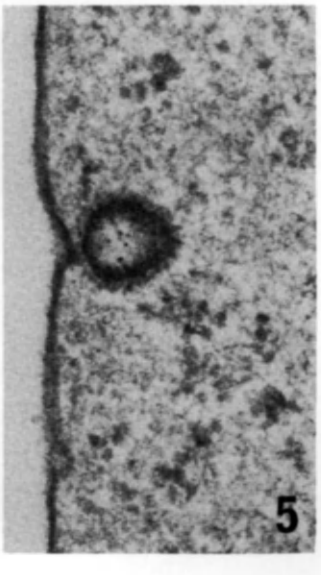
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Micropinocytosis vermiformis

Micropinocytosis vermiformis, a term coined by Matter *et al.* (1968), is used to describe a unique type of endocytotic activity executed via certain 'worm-like structures' first described by Toro *et al.* (1962) in the Kupffer cells of the rat liver. These formations are in fact lamellar (not tubular or worm-like) invaginations of the cell membrane of a fairly constant width (80–125 nm) which meander into the cell.

Also seen is a dotted or shaggy median 'line' running along their length, produced by apposition of the free margins of the doubled-up cell coat as it extends into these invaginations. Coated vesicles, at times continuous with these lamellae, are also seen. This may represent fusion of pre-existing vesicles with the lamellae or the genesis of coated vesicles from the wall of this structure.

Micropinocytosis vermiformis is not routinely seen in Kupffer cells; when present, it is thought to represent an exaggerated or intensified form of endocytosis. Since the discovery of this structure by Toro *et al.* (1962), various other workers have seen micropinocytosis vermiformis in Kupffer cells of the rat and rabbit under normal and experimental situations (Rohlich and Toro, 1964; Melis and Orci, 1967; Orci *et al.*, 1967; Matter *et al.*, 1968; Horn *et al.*, 1969; Wisse and Daems, 1970a, b; Pfeifer, 1970; Fahimi, 1970; Emeis and Wisse, 1971).

These studies have shown that micropinocytosis vermiformis (in Kupffer cells and occasionally also in splenic macrophages) can be induced by the injection of a variety of substances or by partial hepatectomy. Micropinocytosis vermiformis has also been demonstrated in macrophages harvested from the peritoneal cavity of the rat (Brederoo and Daems, 1972). These authors report that the omission of washing and centrifugation prior to fixation was important in preserving these structures and they point out that in previous studies where micropinocytosis vermiformis has been demonstrated in the Kupffer cells, *in situ* fixation by vascular perfusion has been employed.

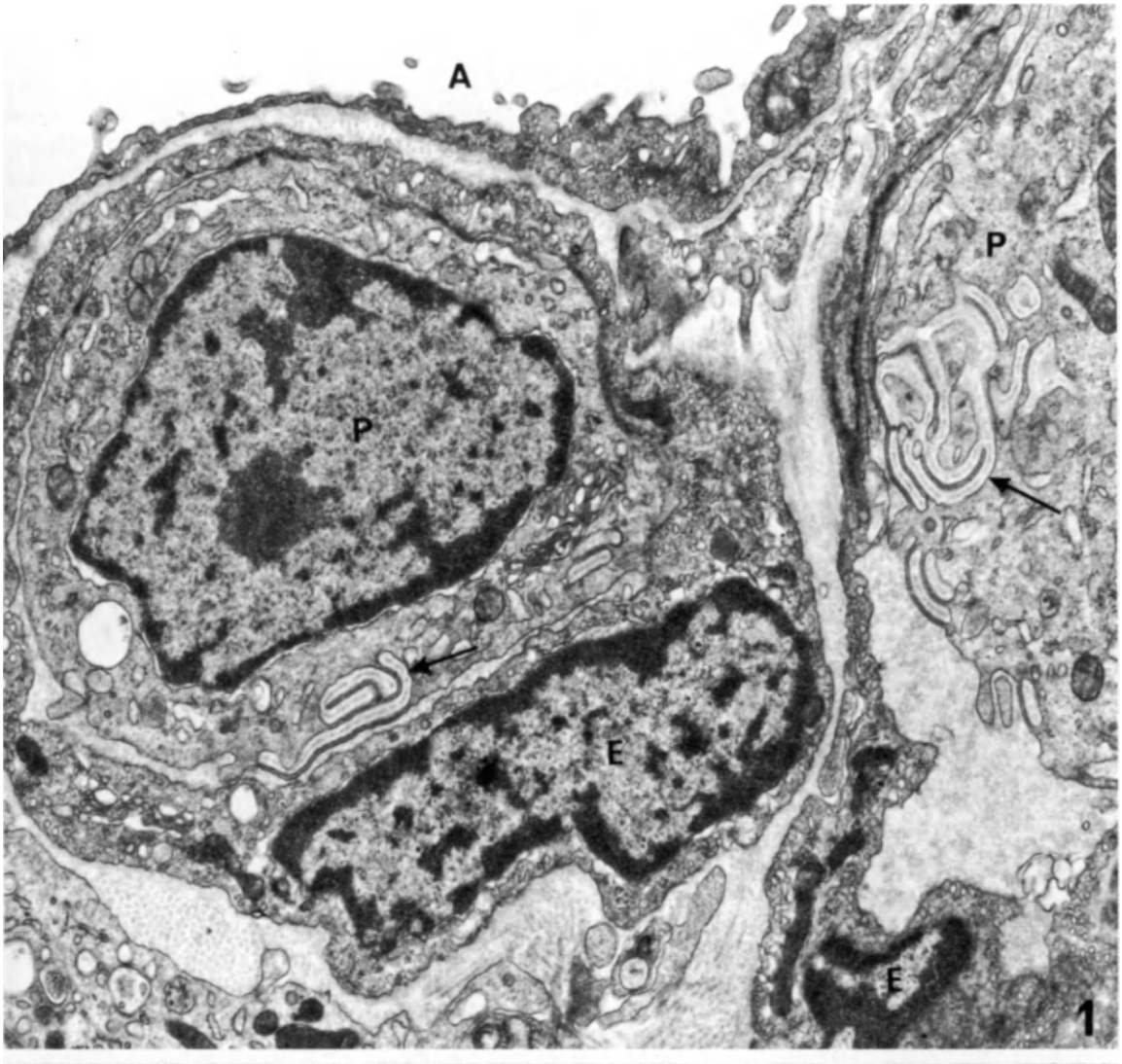
It has long been suspected from observations on animals injected with colloidal substances that Kupffer cells can migrate to the lung (Simpson, 1922; Irwin, 1932; Nicol and Bilbey, 1958; Patek and Bernick, 1960; Collet and Policard, 1962; Frankel *et al.*, 1962), and it has been suggested that alveolar macrophages may derive from such cells trapped in pulmonary capillaries (Florey, 1958).

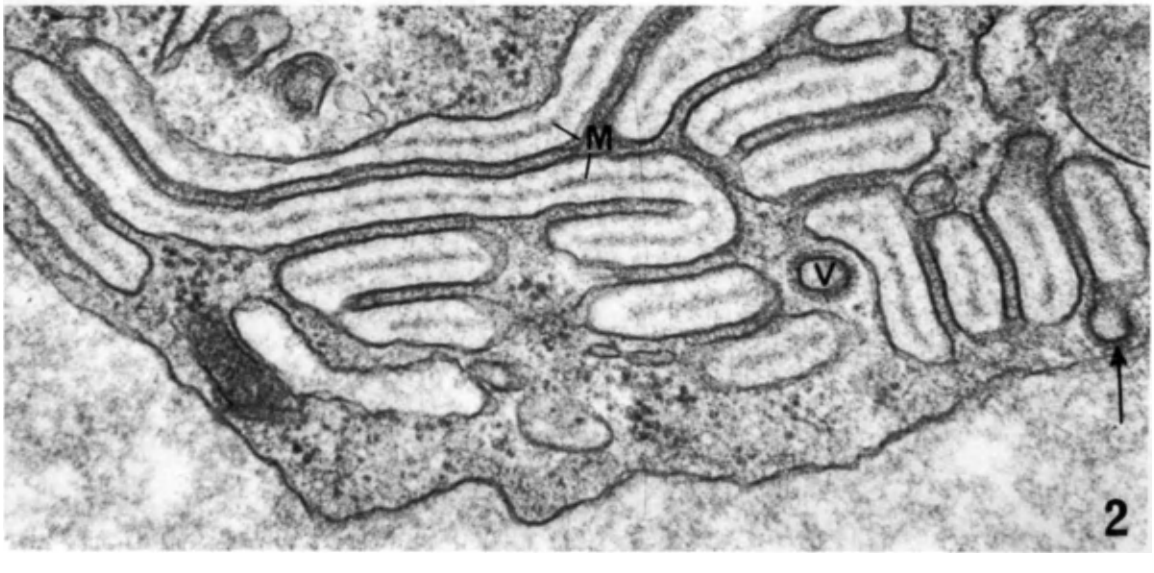
These ideas gain fresh support from the elegant experiments of Schneeberger-Keeley and Burger (1970) (*Plate 479*). In these experiments Kupffer cells of cats were first labelled with a critical dose of colloidal carbon. When such animals were subjected a week later to one hour of open-chest ventilation the lungs came to contain numerous cells similar to Kupffer cells, showing both micropinocytosis vermiformis and vacuoles containing the carbon label. Hardly any cells showing micropinocytosis vermiformis were seen in the lungs of control animals. These authors conclude that 'the manipulations and drugs used in open-chest ventilation are powerful enough stimuli in the cat to induce migration of hepatic macrophages to the lung and cause micropinocytosis vermiformis in them'.

Plate 479

Fig. 1. An air space (A) and two pulmonary capillaries lined by endothelial cells (E) are seen here. Each capillary contains a phagocytic cell (P) showing micropinocytosis vermiformis (arrows). From the lung of a cat subjected to open-chest ventilation. (Kupffer cells were not labelled with carbon in this experiment.) ×15 000 (*From Schneeberger-Keeley and Burger, 1970*)

Fig. 2. Periphery of a phagocytic cell, showing micropinocytosis vermiformis. Numerous lamellae with a shaggy median line (M) are seen. Also present are coated vesicles (V), one of which (arrow) is continuous with a lamella. ×56 000 (*From Schneeberger-Keeley and Burger, 1970*)


A
P
P
E
E
1


M
V
2

Langerhans' cell granules (Birbeck's granules)

The intra-epidermal gold-chloride-positive dendritic cells described by Langerhans (1868) were at one time regarded as intra-epidermal neural elements (*see* Niebauer, 1968) or as effete melanocytes (Masson, 1948, 1951; Billingham and Medawar, 1953; Billingham and Silvers, 1960). Ultrastructurally, this cell (*Plate 480*) is characterized by the presence of cytoplasmic granules* which usually present rod-shaped or tennis-racket-shaped profiles in sectioned material. The rod-shaped profile or the handle of the racket shows a median striated line. Three-dimensional reconstruction of these granules by Wolff (1967) and Sagebiel and Reed (1968) show that they are basically discoid bodies† (straight, curved or cup-shaped) with a focal vesicular expansion at the margin of the disc. The stippled line represents a transverse section through a paracrystalline net or lattice with a periodicity of 9 nm. The crystalline lattice is best appreciated in tangential cuts through this flat discoid granule.

Several other profiles are at times seen. Thus for example, a transverse section through a cup-shaped granule will produce a circular profile, while a vertical section will produce a U-shaped profile. Various other profiles indicate that more than one vesicle is at times present on a disc, or that a single vesicle is present in the centre rather than the periphery of the disc.

Since Langerhans' cell granules are at times seen in continuity with the cell membrane (*Plates 481* and *482*), it was argued that they are either produced here and represent a unique variety of endocytotic structure or that they are 'secretory' granules arising from the Golgi complex and discharging their contents on to the cell surface. It is the former hypothesis which is now generally accepted (Hashimoto, 1970), and it is clear that the Langerhans' cell granule is formed by a lamellar infolding of the cell membrane and its coat. The paracrystalline structure in the granule is thought to represent modified cell coat. There is controversy about the nature of the vesicle which gives the granule a racket-shaped profile, because the size and frequency of occurrence of the vesicle apparently depends on the osmolarity of the fixative. Falck *et al.* (1985) believe that the vesicle is largely a swelling artefact stemming from the hypotonic fixatives (compared with blood) which electron microscopists routinely employ.

Langerhans' cells‡ characterized by the presence of these specific granules have now been found in various squamous epithelia such as cutaneous, ungual, gingival, buccal, glossal, nasal and cervical of humans (normal and pathological) and experimental animals (Birbeck *et al.*, 1961; Breathnach, 1964, 1965; Schroeder and Theilade, 1966; Hackemann *et al.*, 1968;

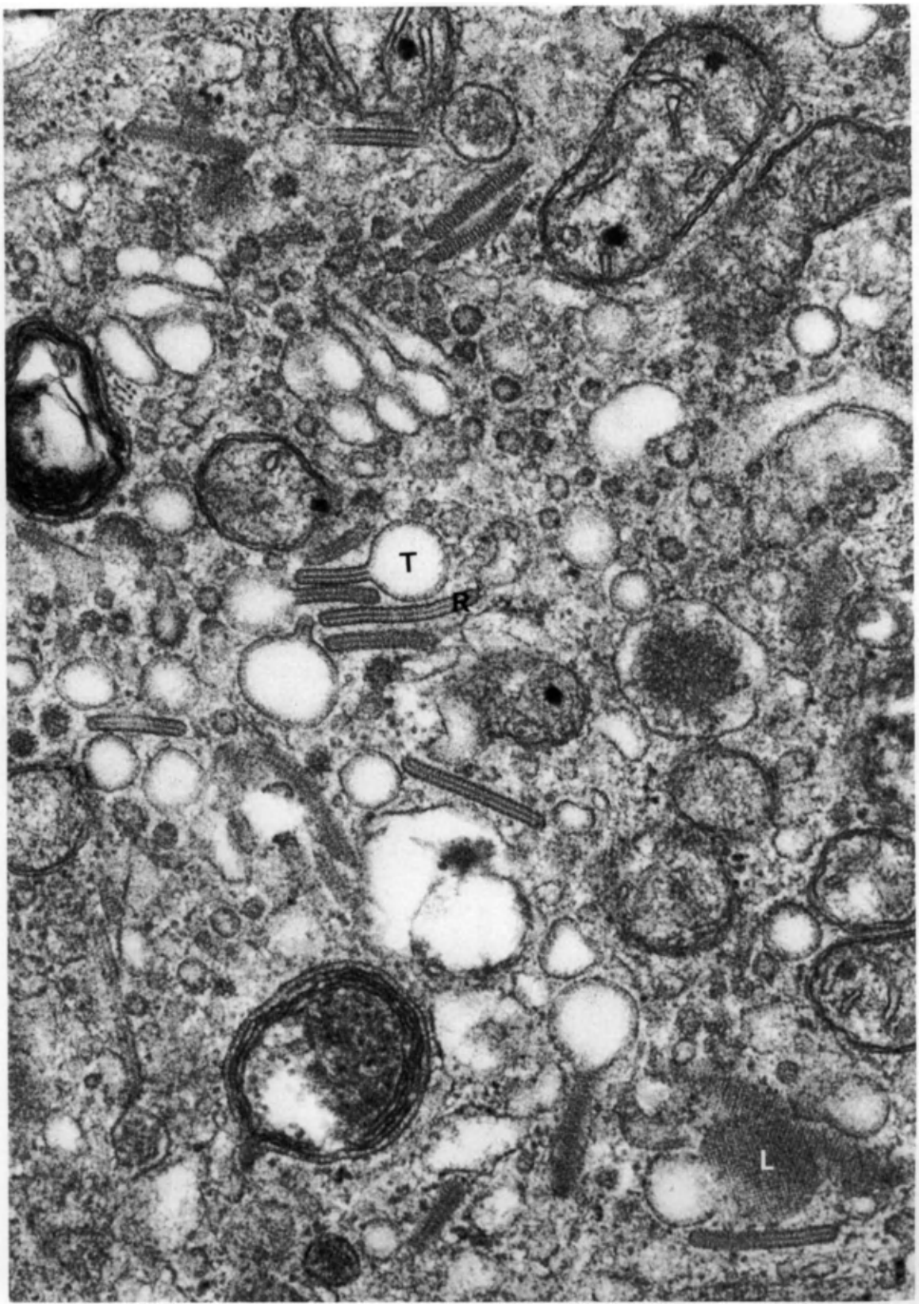
*These granules were discovered by Birbeck *et al.* (1961); hence some workers refer to them as 'Birbeck's granules', others refer to them as 'Langerhans' cell granules', perhaps because it is not customary to give a granule or body a different eponym from that used for the cell itself.

†It is at times erroneously imagined that the Langerhans' cell granule is a tubular structure. For example, Carr (1973) states that these are 'curious cytoplasmic inclusions, elongated and probably tubular with an electron dense core'. This assumption cannot possibly explain all the complex profiles seen, or explain why circular profiles of a diameter and internal structure acceptable as transverse sections through these alleged 'tubules' are not seen. One may object also to the use of the term 'inclusion' for a structure more deserving to be regarded as an 'organelle' performing the function of endocytosis.

‡At this point some comments are needed about the so-called 'indeterminate cells' which some believe are a discrete population of dendritic cells different from melanocytes and Langerhans' cells. An indeterminate cell is defined as 'an epidermal non-keratinocyte where neither melanosomes nor Langerhans' cell granules are present, but the cell is otherwise similar to a melanocyte or Langerhans' cell'. Using the presence of Ia antigen as a marker for Langerhans' cell, Rowden *et al.* (1979) classified 85 per cent of indeterminate cells as Langerhans' cell precursors and the remaining 15 per cent as immature melanocytes. However, the studies by Andersson *et al.* (1981) and Karás *et al.* (1984) show that there is no such thing as an indeterminate cell, for examination of serial sections with a goniometer stage always reveals melanosomes or Langerhans' cell granules in these so-called 'indeterminate cells'. One must remember that when a marker organelle is not seen in a section it does not necessarily mean that it is not present in the cell.

Plate 480

Langerhans cell from human epidermis, showing granules presenting rod-shaped (R) and tennis-racket-shaped (T) profiles. The discoid shape of the granule and the paracrystalline lattice (L) is better appreciated in tangential cuts through these structures. ×58 000


T
R
L

Hashimoto, 1970, 1971; Hutchens *et al.*, 1971; Jahnke, 1974; Wilborn *et al.*, 1978; Basset *et al.*, 1986; Sauget *et al.*, 1986). Occasionally, melanin granules have been demonstrated in Langerhans' cells (Breathnach and Wyllie, 1965), but they take the form of compound melanosomes as seen in keratinocytes and melanophages, so there is no reason to believe that melanosome formation and melanin synthesis occur here as in melanocytes. In achromatic skin such as that found in vitiligo, pinta, the halo of halo naevus, the forelock area of human skin in piebaldism and the white areas of skin from recessively spotted black and white guinea-pigs, there is said to be an increase in the number of Langerhans' cells and a diminution or absence of melanocytes (for references, *see* Rodriguez *et al.*, 1971).

This suggests a close relationship between melanocytes and Langerhans' cells, but it is now difficult to accept that Langerhans' cells are derived from melanocytes or that these two share a common lineage because cells containing these granules have been seen not only in the epidermis but also in: (1) the dermis (Zelickson, 1967; Kustala and Mustakallio, 1968); (2) the dermis and lymphatic vessels during experimentally produced contact-allergic dermatitis in humans and guinea-pigs (Silberberg *et al.*, 1974, 1976; Silberberg-Sinakin *et al.*, 1977); (3) dermis in actinic reticuloid (Schnitzler *et al.*, 1975); (4) pustules produced by vaccinia virus inoculation (Nagao *et al.*, 1976); (5) human lymph nodes of dermatopathic lymphadenopathy (Jimbow *et al.*, 1969); (6) normal lymph nodes of rabbit (Kondo, 1969); (7) human hyperplastic lymph nodes (Shamoto *et al.*, 1971); (8) peripheral lymph (from leg) of normal humans (Sokolowski *et al.*, 1978); (9) thymus of rat (Olah *et al.*, 1968; van Haelst, 1969); (10) human thymus (Hoshino *et al.*, 1970); (11) liver in viral hepatitis (Scotto, 1977); (12) Hodgkin's and non-Hodgkin's lymphomas (Burns *et al.*, 1983); (13) reticulum cell sarcoma and pityriasis rosea (Hashimoto and Tarnowski, 1968); (14) acute monocytic and monomyelocytic leukaemia* (Sanel and Serpick, 1970); (15) histiocytosis X (Hand–Schuller–Christian disease, Letterer–Siwe disease and eosinophilic granuloma) (*Plates 481* and *482*) (Basset *et al.*, 1965; Turiaf and Basset, 1965; Basset and Nezelof, 1966; Hashimoto and Tarnowski, 1967; Cancilla *et al.*, 1967; Gianotti and Caputo, 1969; de Man, 1968; Morales *et al.*, 1969; Shamoto, 1970; Imamura *et al.*, 1971; Carrington and Winklemann, 1972; Cutler and Krutchkoff, 1977; Basset *et al.*, 1986); (16) basal cell carcinoma (20 out of 32 tumours examined contained Langerhans' cells) (Macadam, 1978); (17) various pulmonary tumours, particularly bronchiolo-alveolar carcinoma (Hammar *et al.*, 1980; Nakajima *et al.*, 1985); and (18) renal cell carcinoma (clear cell variety) (Navas Palacios *et al.*, 1984).

*Zipper-like junctions and structures resembling Langerhans' cell granules developed in these monocytoid cells during *in vitro* experiments to test the phagocytic competence of these cells.

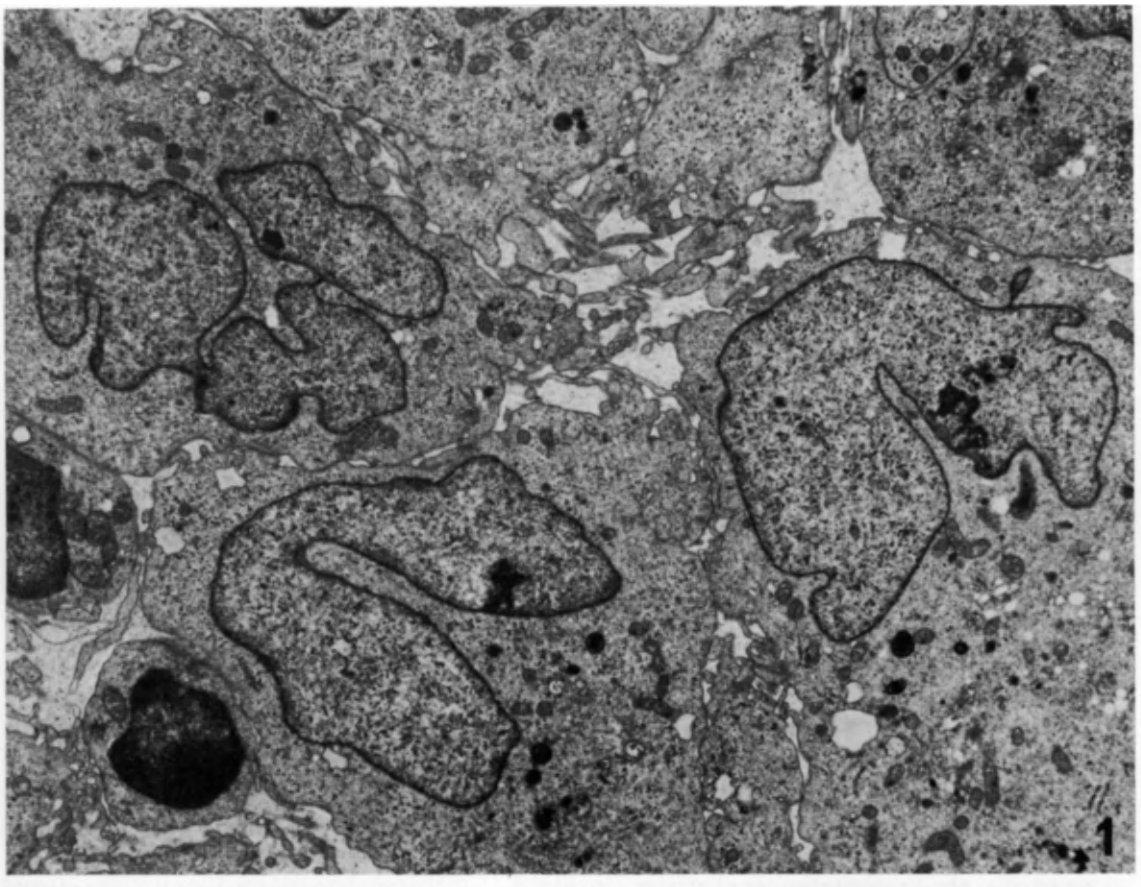
Plate 481

Histiocytosis X. A lymph node from a child with Hand-Schuller-Christian disease. (*From a block of tissue supplied by Dr Imamura*)

Fig. 1. Histiocytic cells with contorted nuclei. At higher magnifications (*Fig. 2*) most of these cells were found to contain the characteristic granules. ×5700

Fig. 2. High-power view showing the characteristic racket-shaped profiles of granules. ×80 000

Fig. 3. Appearances seen here (arrow) are consistent with the idea that a Langerhans' cell granule is developing by invagination of the cell membrane. ×59000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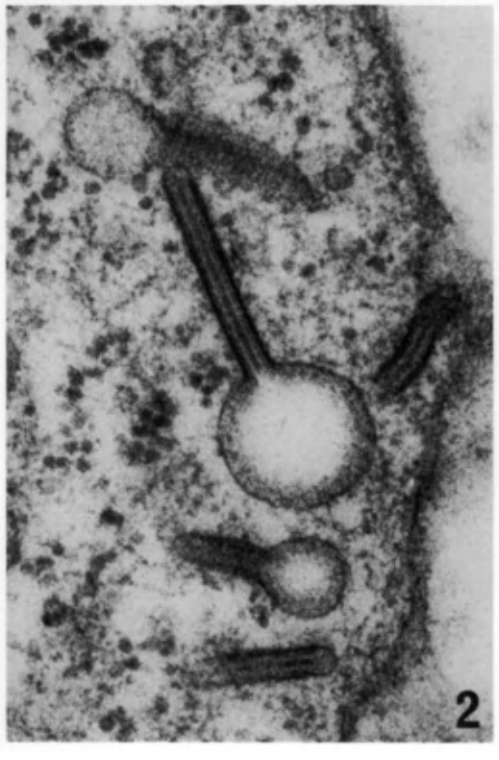
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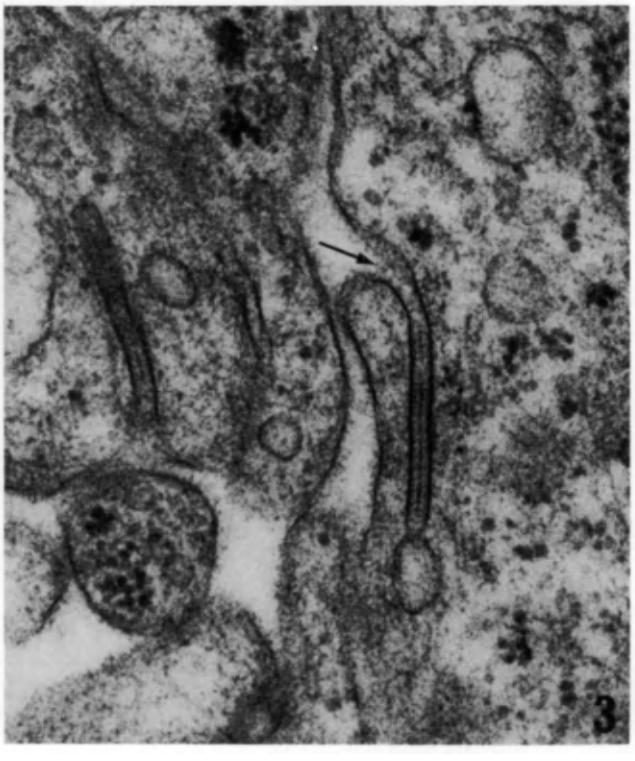

3

Since Langerhans' cells have now been seen crossing the basement membrane by more than one observer, and since such cells have also been observed dividing, Hashimoto and Tarnowski (1968) proposed that they 'may constitute a self-perpetuating "intraepidermal phagocytic system" to which histiocytes from the dermis are added from time to time'. Further, like macrophages or histiocytes, Langerhans' cells have: (1) Fc and C3 surface receptors (Stingl *et al.*, 1977; Nezelof *et al.*, 1973); (2) Ia antigen (Rowden *et al.*, 1977); (3) non-specific esterase, acid phosphatase and ATPase (Berman and France, 1979); and (4) an origin in the bone marrow (Katz *et al.*, 1979).

However, unlike macrophages, Langerhans' cells: (1) do not as a rule endocytose large particulate matter (except for melanosomes as mentioned above); (2) do not contain lysozyme or α_1-antitrypsin (Beckstead *et al.*, 1984); (3) contain S-100 protein (for references *see* Rowden, 1985); and (4) express T_6 antigen on their surface (*see* Rowden, 1985). This suggests that Langerhans' cells may be related to T lymphocytes, but Rowden (1985) presents an excellent critique on expression of T_6 antigen by Langerhans' cells and concludes that 'It is difficult at this stage to interpret the findings on T_6 antigen as evidence for a direct relation to T lymphocytes'.

However, the fact that these cells have now been found in lymph nodes and thymus and seem to be involved in allergic contact hypersensitivity indicates that Langerhans' cells are a cell population of immunological importance. Further the fact that they have now been seen in lymphatics suggests that this might be a circulating population of cells. Macadam (1978) states that 'the Langerhans' cell can accept antigenic material and convey it to the regional lymph node where blast cell transformation takes place with the subsequent production of immunologically competent small lymphocytes'.

The fact that Langerhans' cells contain a characteristic granule which permits their identification has proven to be of value in the electron microscopic diagnosis of the group of conditions collectively referred to as 'histiocytosis X' (*Plates 481* and *482*). For example, Morales *et al.* (1969) report a case of a tumour composed of large compact cell aggregates admixed with eosinophils, lymphocytes and a few plasma cells. On the basis of light microscopy of paraffin sections, the diagnostic possibilities included, amelanotic melanoma, undifferentiated carcinoma and eosinophilic granuloma. Electron microscopic examination ruled out melanoma because of the absence of melanosomes and carcinoma because of the absence of desmosomes and tonofilaments. However, numerous characteristic granules similar to those found in Langerhans' cells were found in the histiocytic cells and so the diagnosis of eosinophilic granuloma was readily established.

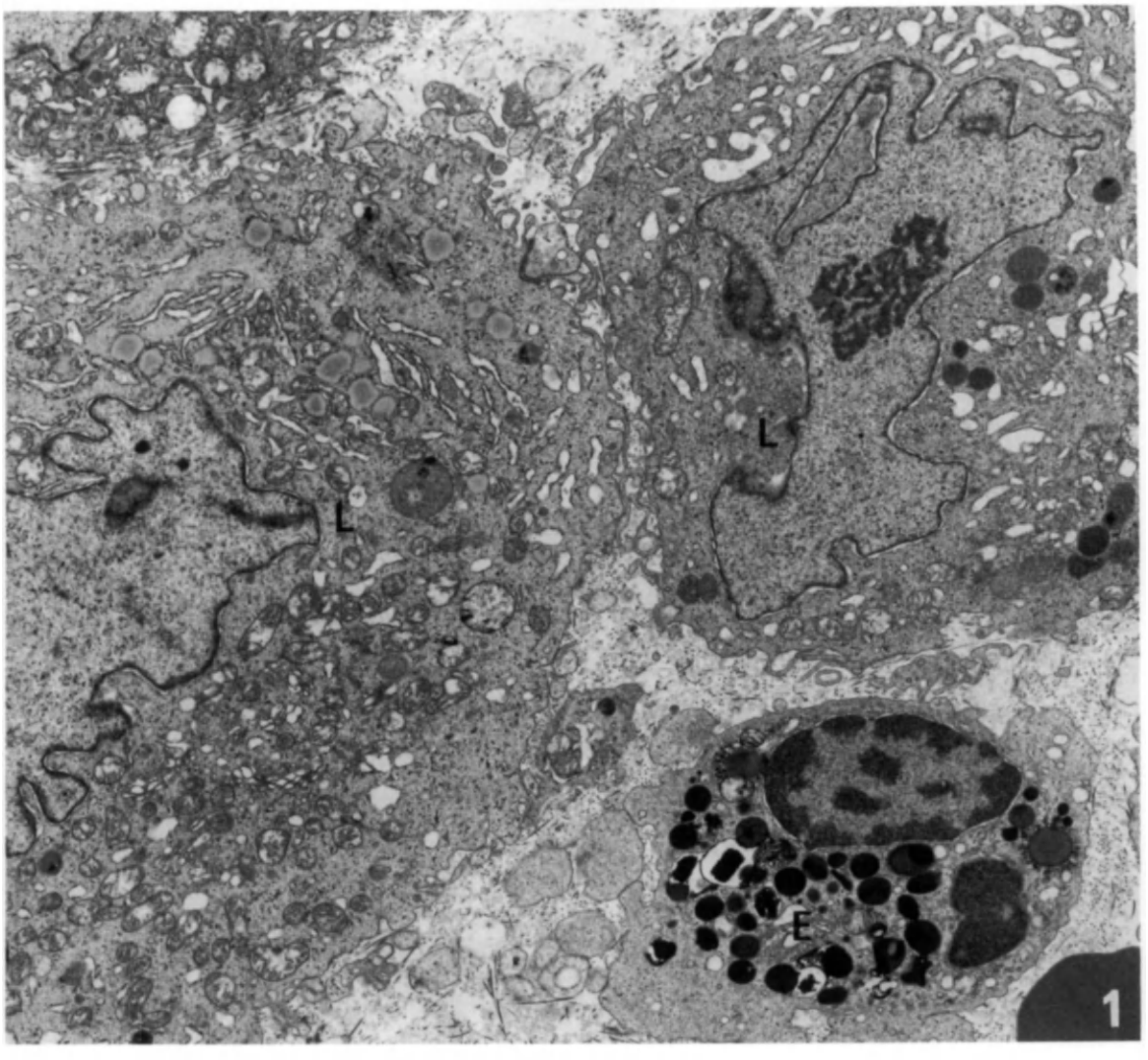
However, some caution is needed in arriving at the diagnosis of histiocytosis X because Langerhans' cells have now been seen in many lesions including the stroma of some carcinomas. Thus, the mere finding of cells with Langerhans' cell granules is not diagnostic of histiocytosis X, but when taken in conjunction with clinico-pathological findings, the finding of Langerhans' cells with their characteristic granules does help to establish the diagnosis of histiocytosis X.

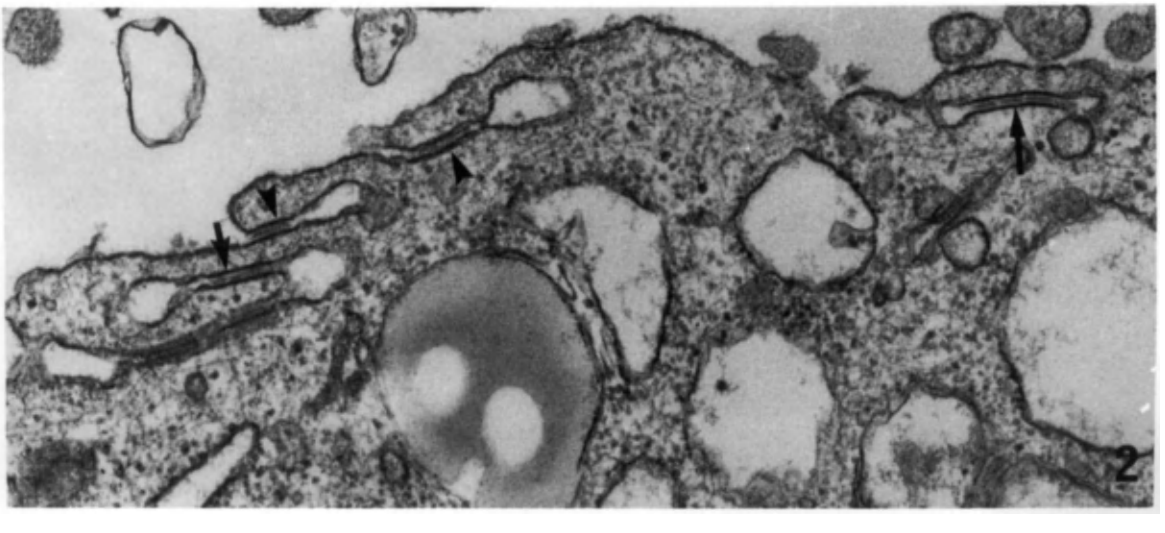
Plate 482

Histiocytosis X. Eosinophilic granuloma of bone (fifth lumbar vertebra)

Fig. 1. Langerhans' cell (L) with contorted nuclei and an eosinophil (E) with characteristic granules. ×6900

Fig. 2. Langerhans' cell granules are seen in the peripheral part of a Langerhans' cell. One of the granules (short arrow) has a complex profile, two granules are continuous with the cell membrane (arrowheads) and the profile of another granule (long arrow) shows two vesicles. ×45 000


L
L
E
1


2

Emperipolesis

The term emperipolesis was coined by Humble *et al.* (1956) to describe what they called 'inside round about wandering' of lymphocytes within malignant cells in tissue culture. However, the phenomenon itself had previously been described by Fischer and Dolschansky (1929) who saw (*in vitro* studies) lymphocytes wandering in and out of stromal cells of chicken spleen.

Since then other cells besides lymphocytes have been seen wandering in cells. Therefore, emperipolesis (Gk. *en* + *peripolesis*, a going about) may now be defined as a phenomenon whereby a cell (which I will call 'visiting cell') enters another cell (which I will call 'host cell') wanders within it and may* then depart.

Emperipolesis may be regarded as a variety of endocytosis in that a cell is seen lying within another cell in a single-membrane-bound vacuole. However, there are some important differences between the common variety of endocytosis namely phagocytosis and emperipolesis.

In the former, the endocytosed cell is picked up (presumably a passive process as far as the endocytosed cell is concerned) by a phagocytic cell and comes to lie in a single-membrane-bound vacuole (heterophagosome). Fusion of primary lysosomes with the vacuole (heterolysosome) leads to the digestion and destruction of the endocytosed cell. In contrast to this is emperipolesis where a visiting cell enters (presumably an active process on the part of the visiting cell) a host cell and comes to lie or wander about in a single-membrane-bound vacuole in the host cell. Neither the visitor nor the host show any sign of damage or morphological alteration†.

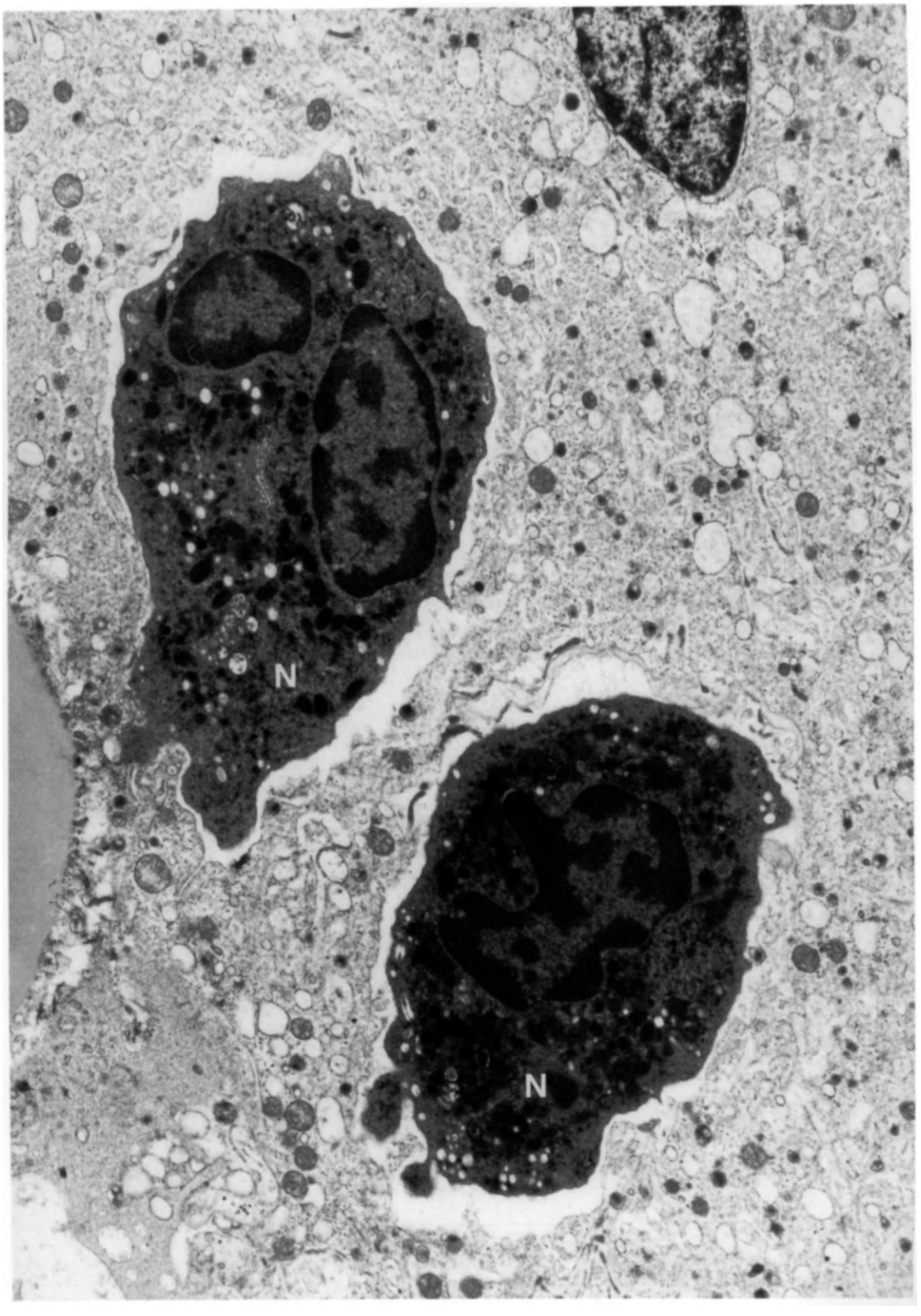
Granulocyte or at times granulocyte and lymphocyte emperipolesis (*Plates 483* and *484*) has been seen within: (1) megakaryocytes in bone marrow from a 59-year-old uraemic, diabetic woman with anaemia and thrombocytopenia (Larsen, 1970); (2) megakaryocytes in bone marrow of cases of acute and chronic myeloid leukaemia (Shamoto, 1981); (3) megakaryocytes in bone marrow of patients with several different types of chronic myeloproliferative disorders (Thiele *et al.*, 1984); and (4) large platelets and megakaryocytes in presumably normal bone marrow (Breton-Gorius and Reyes, 1976).

*From some studies one gets the impression that the visiting cell takes up 'permanent residence' in the host cell, while in others the visiting cell departs after a period of time.

†There are, however, rare reports where the host (item 15) or the visitor (item 7) is said to be destroyed. One wonders whether these can be accepted as examples of emperipolesis. Be that as it may, the term 'cytolytic emperipolesis' is used by Khavkin *et al.* (1986) when the visiting lymphocyte destroys the host macrophage.

Plate 483

Neutrophil leucocyte emperipolesis in a megakaryocyte. Two dark neutrophil leucocytes (N) are lying within single-membrane-bound vacuoles in a megakaryocyte, found in the bone marrow from a patient with uraemia, diabetes mellitus, anaemia and thrombocytopenia. All neutrophils (within or outside megakaryocytes) in this specimen showed the dark cell phenomenon, but other cells were not affected in this manner. ×9900 (*From a block of tissue supplied by Dr T.E. Larsen*)


N
N

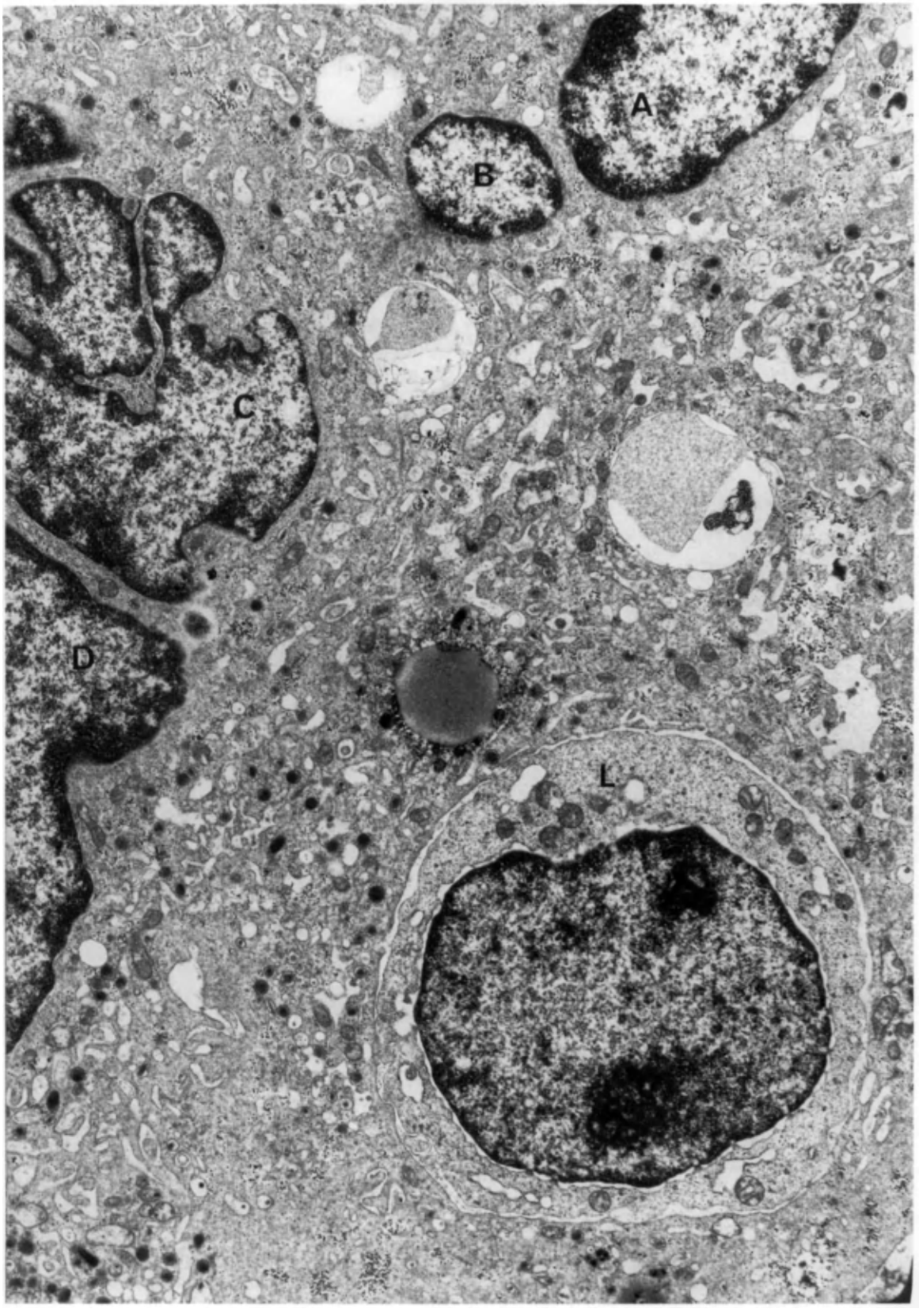
Lymphocyte (non-neoplastic) emperipolesis has been seen within: (5) stromal cells of cultured chicken spleen (Fischer and Dolschansky, 1929); (6) hepatocytes of guinea pig (Biermann-Dörr, 1944); (7) developing ova in amphibian ovary (Liebman, 1945); (8) intestinal epithelial cells in fish, amphibia, mouse, rat, hamster, cat, dog and humans (for references *see* Trowell, 1958); (9) fibroblasts in mixed cultures of rat thymus and chicken fibroblasts (Trowell, 1949); (10) epithelial cells of thyroid and submandibular gland homografts in rabbits (Darcy, 1952); (11) cultured thyroid epithelial cells (normal and Hashimoto's disease (Pulvertaft *et al.*, 1959; Ling *et al.*, 1965)); (12) cultured rat sarcoma cells (Koller and Waymouth, 1953); (13) cultured human malignant tumour cells (Humble *et al.*, 1956); (14) cultured mesenchymal cells (Ioachim, 1965); (15) IgG sensitized Chang liver cells (Sandilands *et al.*, 1978); and (16) cultured monocytes exposed to mitotic lectins (Khavkin *et al.*, 1986).

It is impossible to guarantee that in every one of the above-mentioned instances lymphocytes were actually seen within host cells and one may also question as to whether the phenomenon of emperipolesis or phagocytosis was witnessed. However, most observers noted a narrow space separating the lymphocyte from the host cell and concluded that the lymphocyte really lay in a cytoplasmic vacuole (presumably closed or communicating with the exterior). It would appear that in most recorded cases the phenomenon of emperipolesis (in contrast to phagocytosis) was witnessed and one has to conclude that lymphocytes can and do invade a variety of cells, *in vivo* and *in vitro*.

Neoplastic lymphocyte emperipolesis has been seen within: (17) macrophages in a lymph node biopsy from a patient with a diffuse B-cell lymphoma (Takeya, personal communication which included numerous illustrations and blocks) (*Plate 485*); (18) macrophages from a case of acute lymphatic leukaemia (Pulvertaft and Humble, 1962); (19) macrophages in mouse ascites lymphoma (Shelton and Rice, 1958; Shelton and Dalton, 1959); (20) 'large elongated cells' in bone marrow culture from a case of chronic myeloid leukaemia (Sinkovics, 1962); and (21) fibroblast-like cells in cultures of lymph nodes from cases of malignant lymphoma (Dreyer *et al.*, 1964).

Plate 484

Lymphocyte emperipolesis in a megakaryocyte. A lymphocyte (L) is seen lying in a single-membrane-bound vacuole in a megakaryocyte. Note the multiple nuclear profiles (A–D) one finds in megakaryocytes. ×10 500 (*From a block of tissue supplied by Dr T.E. Larsen*)


A
B
C
D
L

Tumour cell emperipolesis* has been seen within; (1) Ehrlich's ascites tumour cells (Burns, 1967); (2) JB-1 ascites tumour (hypotetraploid plasmacytoma) cells (Chemnitz and Bichel, 1973); (3) cultured JB-1-E tumour cells after addition of Bt_2cAMP (Chemnitz and Skaaring, 1978).

From the static picture seen by light or electron microscopy of a cell lying within a vacuole† in another cell one cannot be absolutely certain as to whether one is witnessing an act of phagocytosis or emperipolesis. When the cell lying in a vacuole appears to be non-viable, degenerate, necrotic or fragmented one may conclude that what one is witnessing is phagocytosis followed by lysosomal degradation. However, if an unaltered viable cell is seen it could either be emperipolesis or an early stage in the sequence of events leading to heterolysosome formation. This difficulty is more apparent than real for in specimens where emperipolesis is in progress, many viable cells are seen in vacuoles and disintegrating cells are hard to find, the converse being correct for phagocytosis and heterolysosome formation.

However, proof of emperipolesis is more convincingly obtained by the dynamic technique of time-lapse phase-contrast cinephotography of living cells in culture. Using this technique Larsen (1970) found (item 1) a granulocyte which moved around in a megakaryocyte for 30 minutes before leaving the cell. In this elegant study Larsen also shows (in marrow crushes and light microscopy and electron microscopy of tissue sections) that up to ten visiting granulocytes‡ may be found in a single host megakaryocyte and up to five granulocytes may be found in a single vacuole. He states: 'serial sections of megakaryocytes clearly revealed the intracytoplasmic location of granulocytes'.

There has been debate in the literature as to whether the visiting cell is ever totally sequestrated in a vacuole. Clearly, there is bound to be an early phase when sequestration is not total or absolute.

Tracer studies (Breton-Gorius and Reyes, 1976) using horseradish peroxidase have shown that in the case of emperipolesis in megakaryocytes, the vacuole containing the visiting cell communicates with the extracellular milieu, but tracer studies (Chemnitz and Skaaring, 1978) using ruthenium red showed that in tumour cell–tumour cell emperipolesis the vacuole is totally isolated from the extracellular milieu. Hence, Chemnitz and Skaaring state that: 'Coexistence of cells—one within another—is possible'.

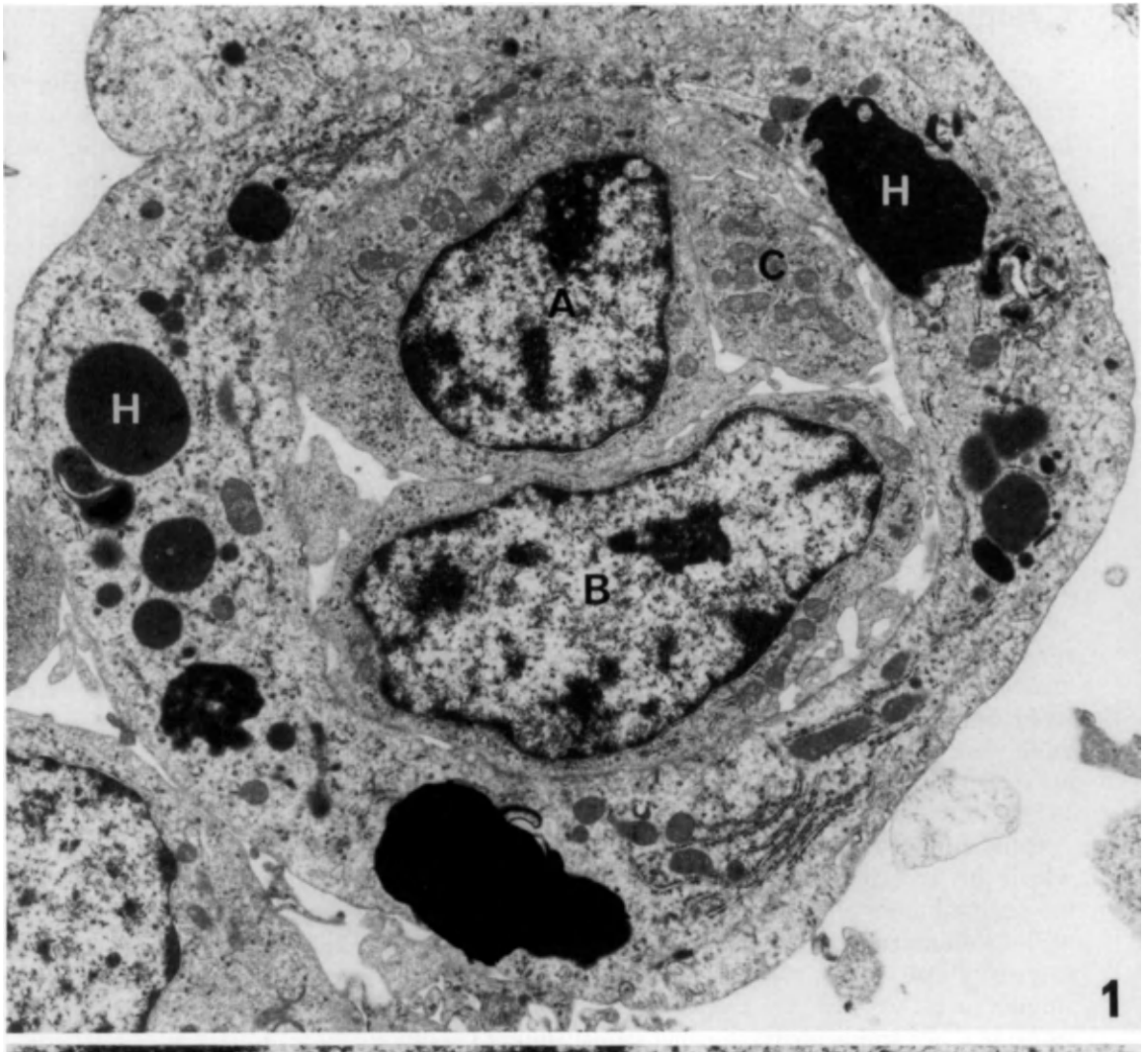
*This is described in the literature as 'tumour cell–tumour cell emperipolesis'
†There is also the problem of deciding whether the cell is truly sequestrated in a vacuole or whether it is lying in a deep bay or recess in a cell which looks like a vacuole because of the plane of sectioning.
‡Mainly neutrophils, occasionally also eosinophils and metamyelocytes.

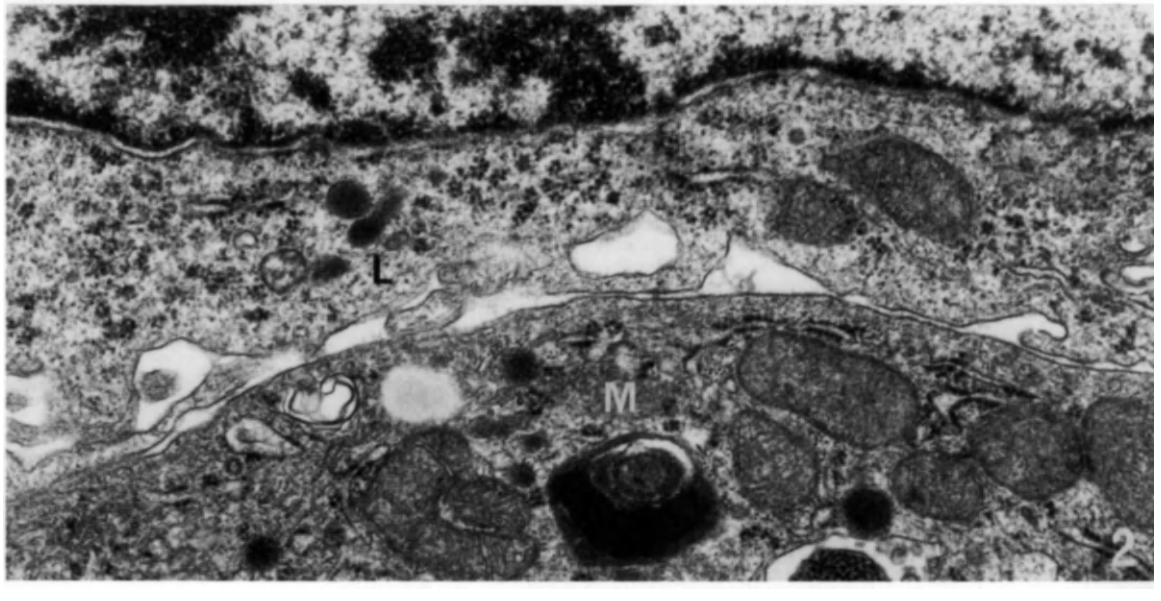
Plate 485

From a lymph node biopsy from a case of diffuse B-cell lymphoma showing marked emperipolesis (*From a block of tissue supplied by Dr M. Takeya*)

Fig. 1. Profiles of neoplastic lymphocytes (A,B,C) are seen lying within a large vacuole in a macrophage containing several heterolysosomes (H), presumably derived from endocytosis of erythrocytes and fibrin. ×7200

Fig. 2. Higher-power view of the wall of a vacuole. Note that there is no cytoplasmic continuity between the visiting lymphocyte (L) and host macrophage (M). ×28 000


H
A
C
H
B
1


L
M
2

Cytoplasmic bubbling, blebs and blisters

Tissues culturists have long been aware of a phenomenon called 'cytoplasmic bubbling' or 'cellular bubbling' (for references *see* Price, 1967). With the phase contrast microscope, bubbling is seen as a relatively rapid projection and retraction of spherical structures (i.e. bubbles) on the cell surface. However, at times some of these 'bubbles' detach and float away from the cell.

Costero and Pomerat (1951) saw this phenomenon on the surface of cultured human neurons and, since the appearance was reminiscent of a boiling viscous fluid, they coined the term 'zeiosis' (Gk. *zeiein*, to boil) to describe this phenomenon. Several studies (*see* Price, 1967) have now established that these 'bubbles' or 'zeiotic blebs' occur: (1) *in vitro* and *in vivo*: (2) in normal and malignant cells; and (3) more frequently or prominently in mitotic than interphase cells. Further, zeiotic blebbing can also be induced by several chemicals, drugs, high hydrostatic pressure and virus infection.

Two transmission electron microscopic* studies (Landau and McAlear, 1961; Price, 1967) carried out with the specific purpose of determining the fine structure of zeiotic blebs show that these are single-membrane-bound projections on the cell surface containing cytoplasmic matrix and ribosomes. Larger organelles are as a rule excluded from the blebs, but Landau and McAlear (1961) reported that occasionally mitochondria are seen in blebs induced by hydrostatic pressure. We (Skinnider and Ghadially, 1977) have seen structures acceptable as zeiotic blebs in circulating neoplastic histiocytes from two cases of malignant histiocytosis and a case of leukaemic reticulum cell sarcoma. As a rule, the zeiotic blebs were attached to the cell (*Plate 486, Fig. 1*) but at times appearances were seen which supported the idea that some of them were probably detached and cast off from the cell (*Plate 486, Fig. 2*). Scanning electron microscopy also supports the idea that at times blebs are cast off from the cell (Porter *et al.*, 1973).

Quite distinct and different from zeiosis is the blistering of cells described by Zollinger (1948), which he thought was engendered by a phenomenon called 'potocytosis'† whereby membrane-bound blisters containing clear fluid develop on the surface of cells. This elegant well-documented study executed with the phase-contrast light microscope shows that suspensions of various types of normal cells (kidney, liver, adrenal and others) from various animals (e.g. frog, rabbit and mouse) and transplantable tumours show blistering when suspended in various media (normal saline, Ringer's solution and serum).

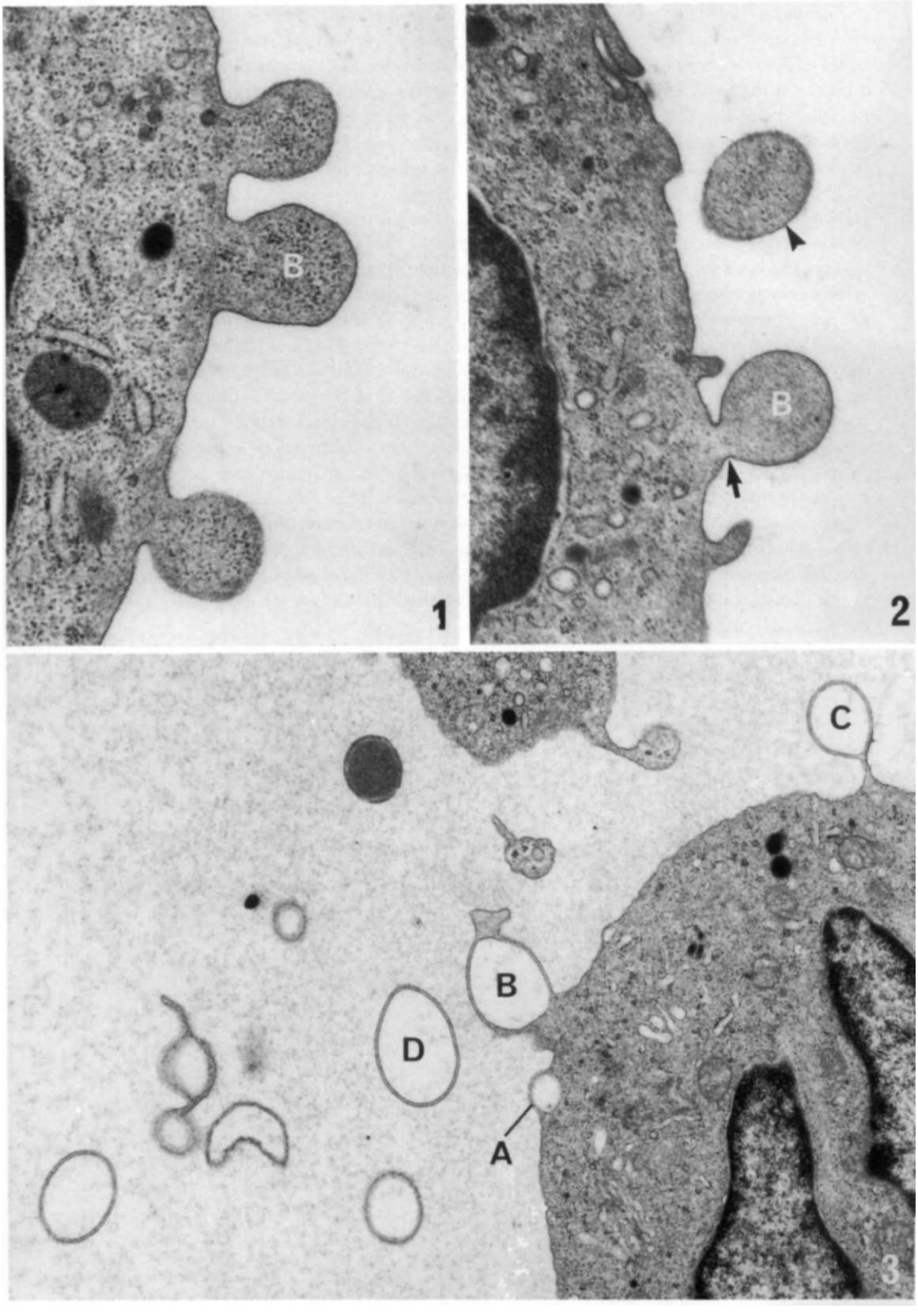
*Scanning electron microscopy of cultured cells often shows a few or many spherical excrescences (see for example Porter *et al.*, 1973; Porter and Fonte, 1973; Kessel and Shih, 1974) which are thought to be zeiotic blebs. However, one cannot be absolutely certain about this because by just looking at the surface it is not possible to say whether a structure is a zeiotic bleb filled with cytoplasm or a blister filled with watery fluid.

†Meltzer (1904) introduced the term 'potocytosis' for drinking, sipping or imbibing of minute quantities of fluid by cells. The modern equivalent of this would probably be pinocytosis or micropinocytosis. The term 'potocytosis' is rarely if ever used now.

Plate 486

Figs. 1 and 2. Circulating leukaemic cells from a case of malignant histiocytosis. Seen here are classic zeiotic blebs (B) containing cytoplasmic matrix and ribosomes. Appearances seen in *Fig. 2* suggest that blebs are pinched off (arrow) and cast away (arrowhead) from the cell but serial sections would be needed to prove this. Note that the blebs are bounded by a single-membrane. ×31 000; ×31 000 (*From Skinnider and Ghadially, 1977*)

Fig. 3. Circulating leukaemic cell from a case of acute myelomonocytic leukaemia. Appearances seen here suggest the sequential stages (A–D) of development and discharge of cytoplasmic blisters. (It is of course possible that some of the apparently free-floating blisters are attached to a cell above or below the plane of section.) Note that the blisters are bounded by two membranes. ×15 000


B
1
B
2
C
B
D
A
3

Zollinger (1948) states: 'The first signs of blister formation appear as early as 3 or 4 minutes after the suspension is made: small "cavities" arise in the protoplasm. They are usually adjacent to the cellular membrane, and later, as they grow in size, the cellular membrane bulges out until a blister is formed'. Unfortunately there is no transmission electron microscopic study which has elucidated the ultrastructural morphology of these blisters, but during the course of my studies I have seen structures which can be regarded as blisters, in that they largely contain clear or flocculent fluid and not cytoplasm as do the quite distinct and different zeiotic blebs.

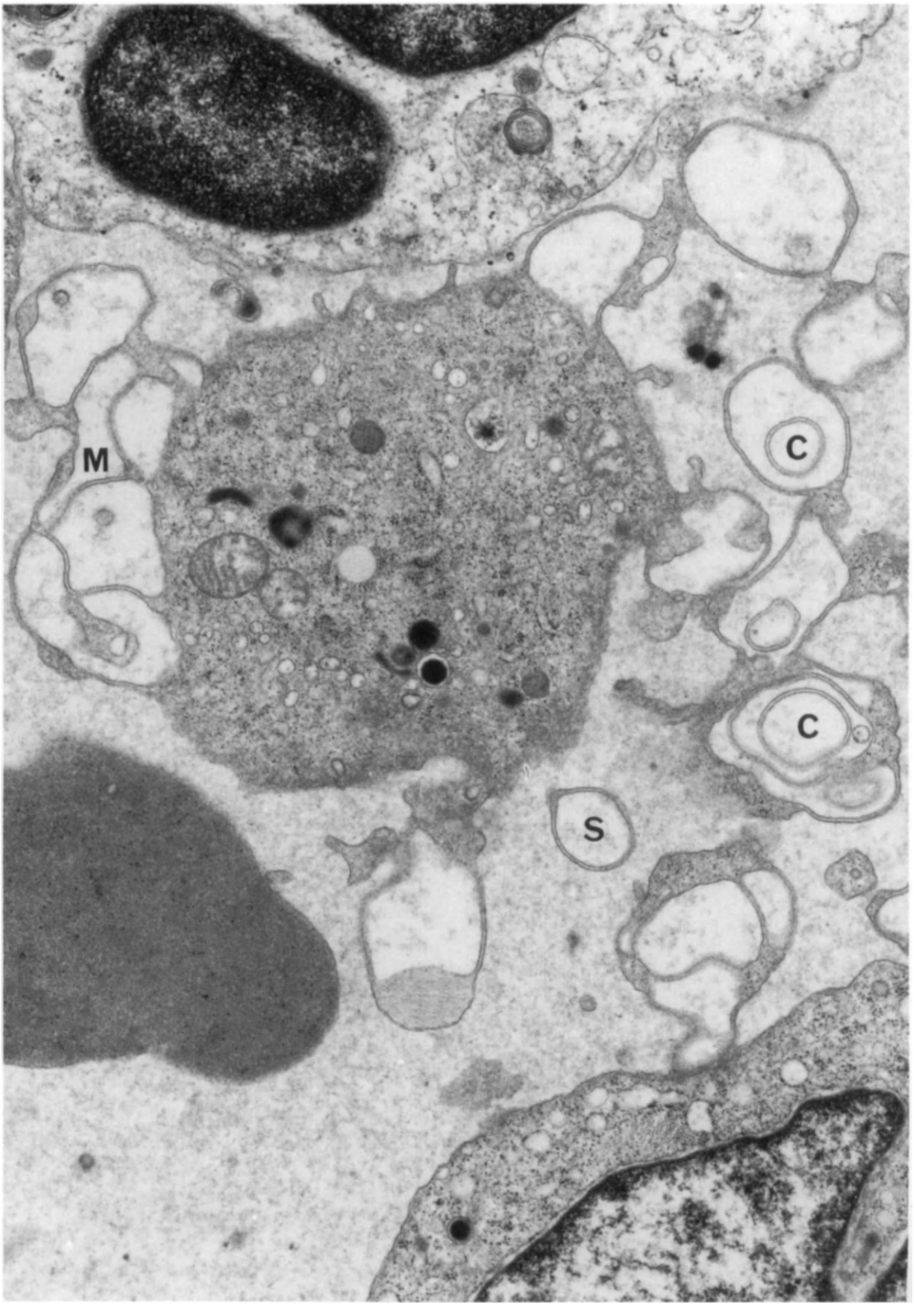
These blisters (*Plate 486, Fig. 1, Plates 487* and *488*) can be divided into two main categories: (1) single-membrane-bound blisters; and (2) double-membrane-bound blisters. The latter can be sub-divided into: (1) solitary blisters; (2) multiloculated blisters which presumably form by juxtaposition of solitary blisters; and (3) concentric blisters where a blister (or two) is seen lying within another blister. All the above-mentioned blisters usually develop from the cell surface proper, but sometimes they seem to develop in association with or atop a zeiotic bleb.

Zollinger (1948) gives a remarkably accurate description of how blisters form (see above) but the 'cavities' which he describes turn out to be membrane-bound vacuoles with the electron microscope. My observations lead me to believe that the double-membrane-bound blister commences as a single-membrane-bound vacuole which migrates to the surface of the cell. As it enlarges and rises above the cell surface it acquires an additional or second membranous coat from the cell membrane. This double-membrane-bound structure may become pedunculated and be discharged from the cell. From static pictures (*Plate 486, Fig. 2*) one may surmise that blisters have been cast off from the cell but this phenomenon is more convincingly seen in living cells. About this Zollinger (1948) writes 'Just before detachment, the blister becomes drop-shaped and the stalk appears more and more elongated until finally it is torn off. As soon as the blister is detached, it becomes spherical and behaves like a rubber ball, its surface being momentarily indented if it bounces against an obstacle...'.

Transmission electron microscopic studies commenting on these blisters are remarkably few. Unaware of well established light microscopic nomenclature, electron microscopists have referred to them as 'cytoplasmic blebs', 'cytoplasmic projections', 'handles', and 'characteristic cytoplasmic surface activity', but it is best to avoid these inferior terms and continue to call them 'blisters'.

Plate 487

Peripheral blood from a case of acute myelomonocytic leukaemia. Same case as *Plate 486, Fig. 3*. Massive blistering is evident here. One can identify solitary (S), concentric (C) and multiloculated (M) blisters. ×21 000


M
C
C
S

In common usage the terms 'blebs' and 'blisters' denote (amongst other things) any fluid-filled or gas-filled excrescence or projection on a surface such as the surface of skin or metals. However, in the case of cytoplasmic 'blebs' and 'blisters' it would be wise to restrict the term 'blebs' for projections containing cytoplasmic material and 'blisters' for those containing largely clear or flocculent fluid*, for this is how they were first described before electron microscopists messed up the nomenclature.

Double-membrane-bound blisters (transmission electron microscopic studies) have been seen in: (1) malignant histiocytic or monocytic cells from two cases of malignant histocytosis and a case of leukaemic reticulum-cell sarcoma (Skinnider and Ghadially, 1977); (2) leukaemic cells in a case of lymphoplasmacytoid immunocytoma (Kaiserling and Muller-Hermelink, 1984); (3) normal lymphocytes which have been cooled to 4°C and abnormal lymphocytes in pathological conditions in which the tissue has not been cooled (Bockus *et al.*, 1985); and (4) leukaemic cells of acute lymphoblastic leukaemia of childhood and non-neoplastic polymorphs in bone marrow biopsies (de Chadarévian and Corriveau, 1985).

The observations of Bockus *et al.* (1985) suggest that blisters can be produced by cooling blood to 4°C. However, de Chadarévian (1986) comments that the 'cooling to 4°C mentioned by Bockus *et al.* in relation to their first case is probably coincidental'. My view is that, since several chemical and physical agents (see above) can produce blistering, it is quite possible that cooling may have the same effect; but this needs investigating. Zollinger (1948) notes that blistering is not seen in fresh preparations and that it starts 3–4 minutes later. Thus, delays in specimen preparation rather than storage at 4°C may be involved in blister production.

de Chadarévian (1986) found that blistering is seen in 50 per cent of cases of acute lymphoblastic leukaemia and that it is four to five times more common in such neoplastic than non-neoplastic bone marrow. Needless to say, blistering is not specific for leukaemic cells, indeed Zollinger (1948) has shown that a variety of cells can develop blisters. In conclusion one may surmise that altered milieu can produce blistering and that malignant cells are more susceptible to such changes than normal cells.

I have not found a study that deals specifically with single-membrane-bound blisters (*Plate 488, Figs. 1 and 2*). My view is that they are probably artefacts of tissue preparation which have attracted little attention.

*Sometimes blisters contain vesicles and rarely also some ribosomes (*Plate 488*). The important difference being that they do not contain a portion of cytoplasm identical to that seen in the cell.

Plate 488

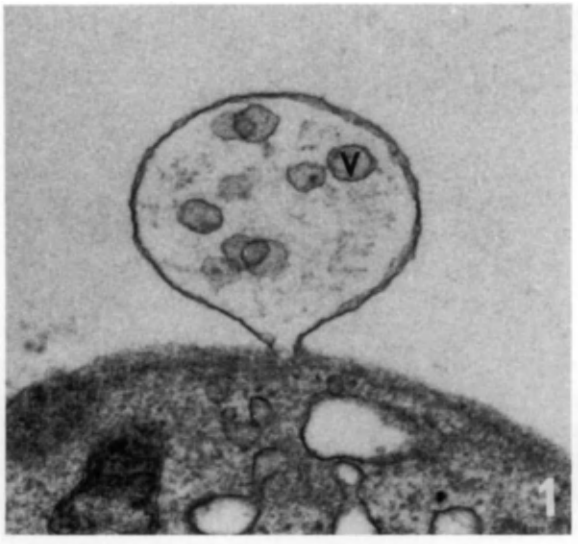
Fig. 1. Leukaemic cell from a case of malignant histiocytosis. Note the single-membrane-bound blister containing a few vesicles. ×48 000

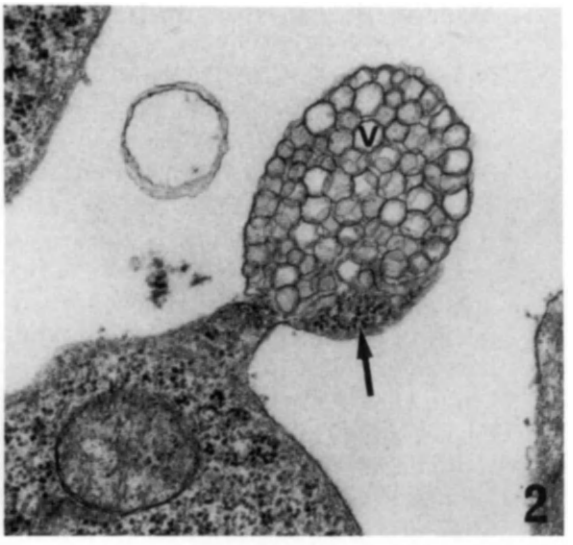
Fig. 2. Neoplastic cell in a lymph node biopsy from a case of diffuse lymphocytic leukaemia. Note the single-membrane-bound blister containing numerous vesicles (V) and some ribosomes (arrow). ×35 000

Fig. 3. Leukaemic cell from a case of malignant histiocytosis. Appearances seen here may be interpreted as an early stage of development of a concentric blister. It takes little imagination to see that if the two flattened blisters or vesicles (V) were to rise above the surface, we would have a vesicle lying in another vesicle. ×58 000

Fig. 4. Leukaemic cell from a case of malignant histiocytosis. Note the concentric blister composed of two double-membrane-bound blisters or vesicles lying in a larger blister. ×41 000 (*From Skinnider and Ghadially, 1977*).

Fig. 5. Leukaemic cell from a case of malignant histiocytosis. Seen here are double-membrane-bound blisters containing electron-lucent fluid (F) associated with blebs containing cytoplasm (C). ×38 000


V
1


V
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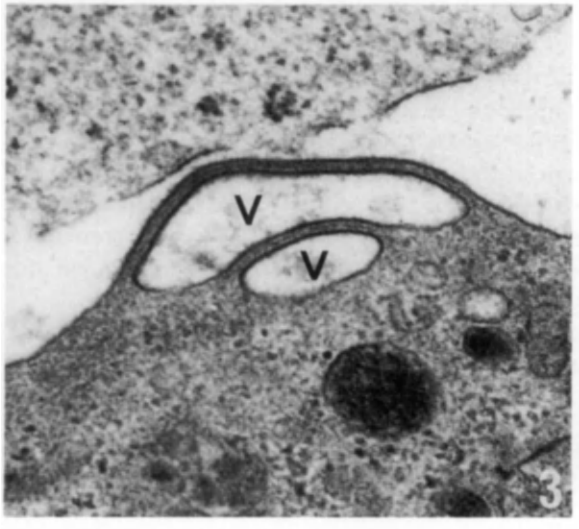
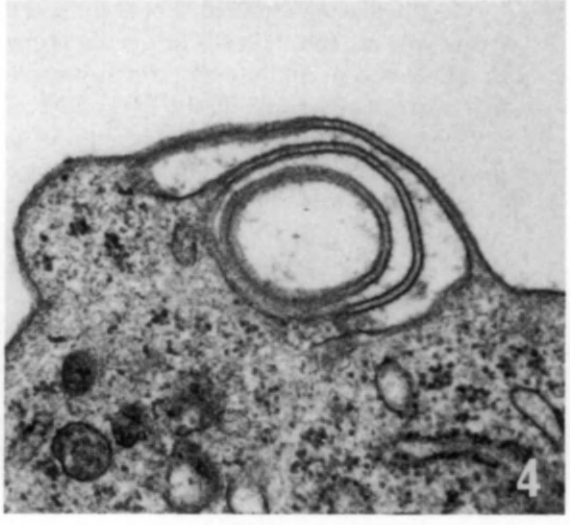
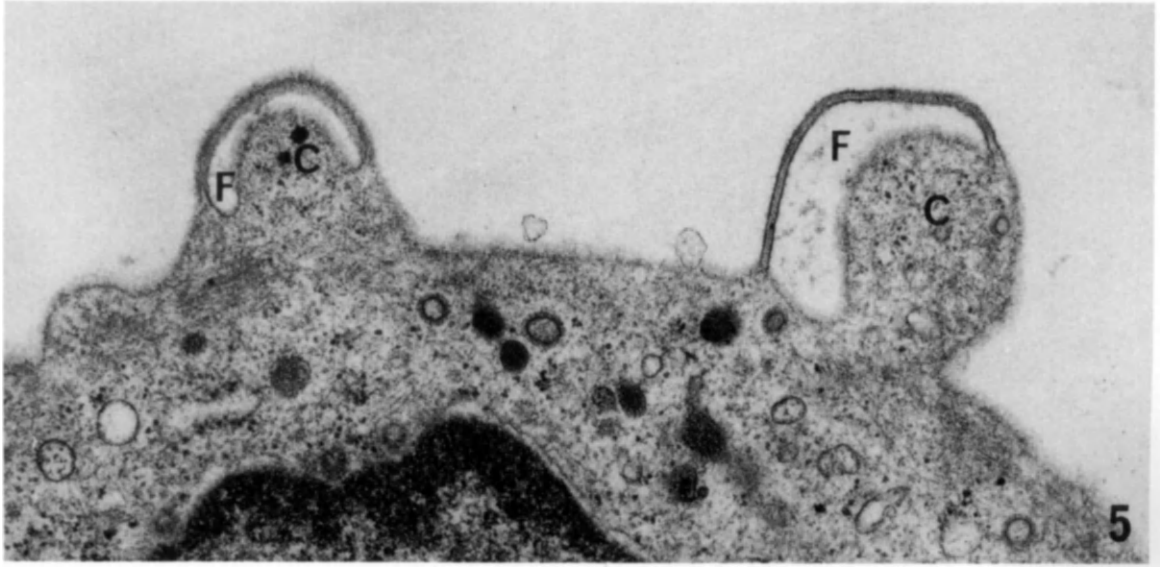

V
V
3


4


F
C
F
C
5

Microvilli and stereocilia

Numerous prominent, regularly arranged microvilli are usually found on the free surface of cells whose principal function is absorption. Fewer, less tidily arranged microvilli may, however, be seen on a variety of other cells. Microvilli are considered to be stable differentiations of the cell surface, in contrast to transient cell processes such as those formed during pinocytosis and phagocytosis. Microvilli are distinguished from cilia and flagella by the absence of a basal body and the characteristic 9+2 microtubule complex found in the latter two structures. It is thought that microvilli facilitate absorption by amplification of the cell surface.

Microvilli, remarkably uniform in size and shape, are found in the proximal convoluted tubule of the kidney and the intestinal mucosa (*Plates 439* and *440*), where they constitute the well-known striated or brush border seen at light microscopy. Straight fine filaments* run the length of such microvilli and extend downwards into the apical cell cytoplasm to mingle with the network of fine filaments in this region which forms the terminal web. Studies (Rostgaard and Thuneberg, 1972) on the isolated brush border of proximal tubule cells of mammalian kidneys have shown that the core of the microvillus contains filaments (5–7 nm in diameter) forming an axial bundle with 6+1 configuration (i.e. one central filament surrounded by a ring of six filaments). These filaments are said to resemble actin filaments and it is therefore thought that these microvilli have contractile properties which may play a part in absorptive processes.

Microvilli occur not only on absorptive surfaces but also at times on secretory and other surfaces. For example, in the liver, microvilli are found on both the absorptive (space of Disse) and secretory (bile canaliculus) poles of hepatocytes (*Plate 489, Fig. 1*). These microvilli are, however, not as regular in size or arrangement as those found in the gut.

Singularly long slender microvilli (three to four times longer than those seen in the brush border of the gut) are found on epididymal epithelium (*Plate 489, Fig. 2*). The term 'stereocilia' has long been used to describe these structures, but it is now evident that they are not cilia for they lack the characteristic basal body and axial microtubule complex (*Plate 497*).

Fine filaments are, however, readily demonstrated in these microvilli (*Plate 489, Figs 3 and 4*). Another variation of microvillus form is the clavate microvillus found in the choroid plexus. These club-shaped microvilli show much irregularity of size, shape and arrangement.

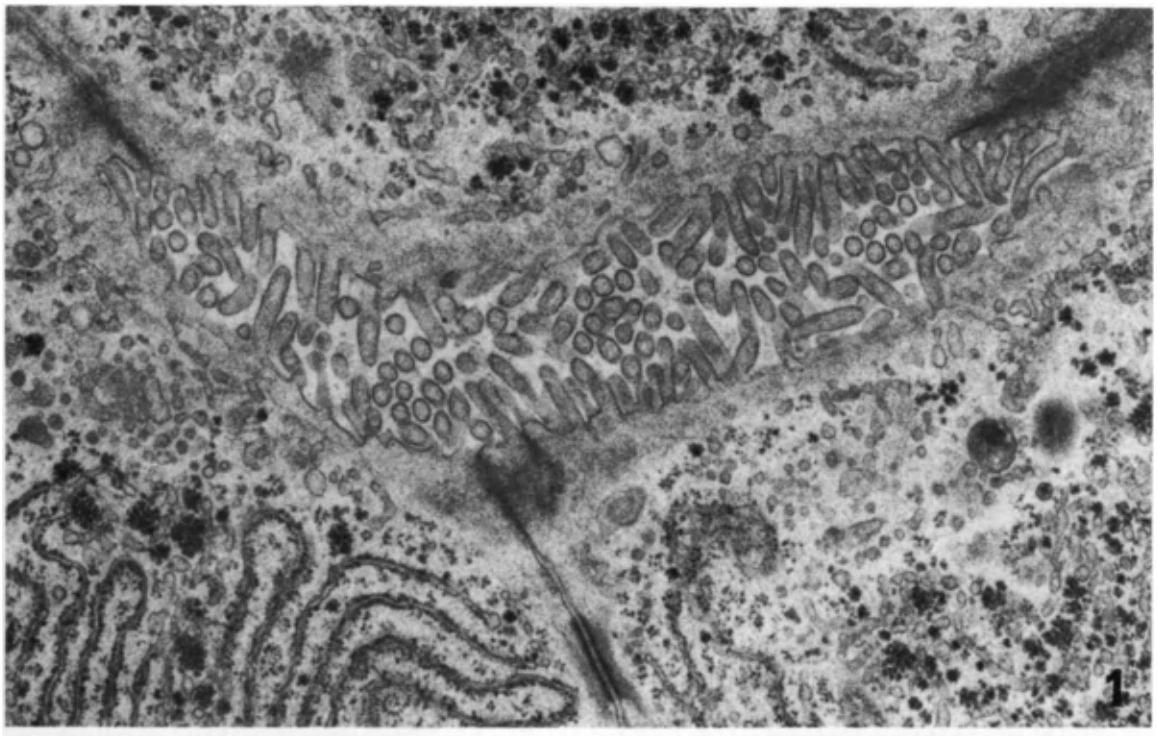
*The collection of filaments extending from the core of the microvillus to the apical cytoplasm of the cell is referred to as the 'filamentous core rootlet' or 'core rootlet' of the microvillus. It can be quite a prominent structure (*Plate 453*) in microvilli from certain sites, but in many other instances the filaments are not particularly prominent and sometimes they can be sparse and difficult to discern.

Plate 489

Fig. 1. A bile canaliculus from cow liver, showing numerous microvilli. Note also the junction complexes between the adjacent hepatocyte cell membranes forming the canaliculus. ×27 000

Fig. 2. Stereocilia. From mouse epididymis. ×7000

Figs. 3 and 4. Longitudinal and transverse sections through stereocilia, showing filaments (arrows) in their interior. From the same specimen as *Fig. 2.* ×68 000; ×98 000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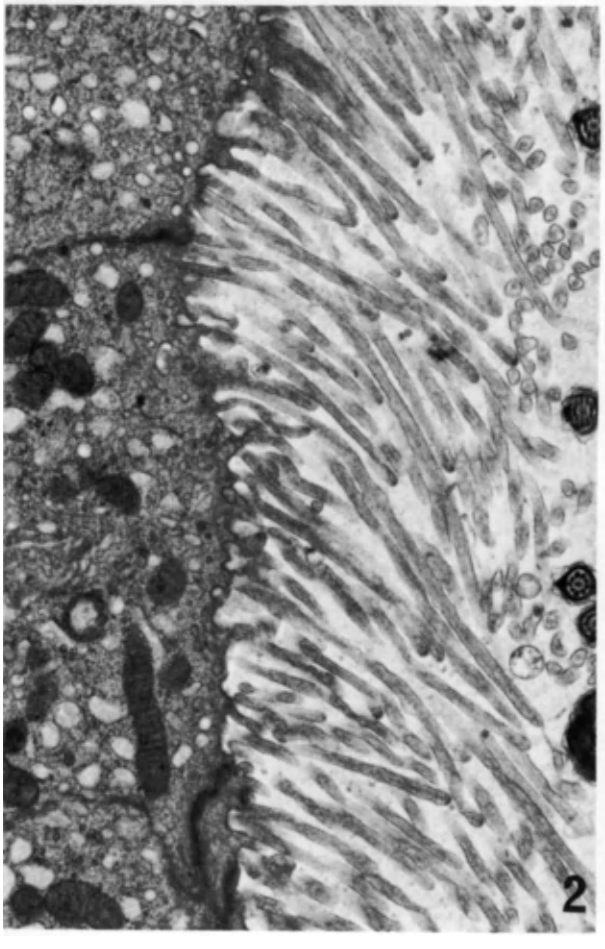
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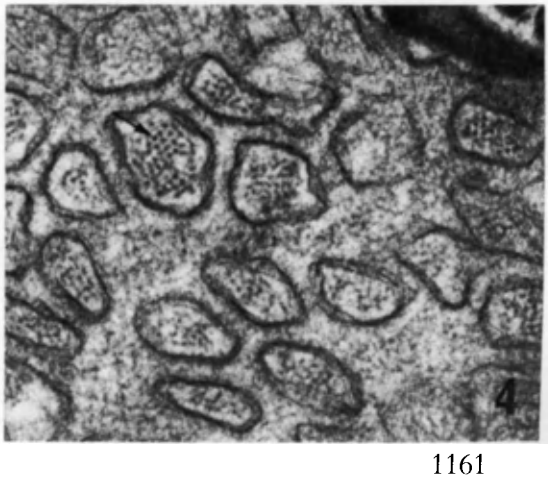
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Morphological alterations in microvilli

Numerous alterations in microvilli have been noted in experimental situations and pathological states. Such changes include an increase or decrease in size and number of microvilli, ballooning of microvilli and fusion of microvilli. Such changes are quite common and only a few examples relating to microvilli in the liver and gut are presented here.

A reduction or absence of microvilli in bile canaliculi is seen after partial hepatectomy in the rat. This is part of the 'simplification' or dedifferentiation which occurs in hepatocytes preparing for mitosis. In hepatomas also, one of the ways in which the dedifferentiation of neoplasia may be expressed is by a loss of microvilli (Ghadially and Parry, 1966). We have seen a reduction of bile canalicular microvilli in the liver of rats bearing transplanted and carcinogen-induced tumours in their flanks (*Plate 490*). An increase in the number of microvilli in the space of Disse has been noted in: (1) rats after talcum-injury (Baum and Nishimura, 1964); (2) rabbits after injection of antigen-antibody complexes (Steiner, 1961); and (3) mice infected with hepatitis agent (Svoboda *et al.*, 1962). A ballooning of microvilli in the space of Disse 30 minutes after the administration of carbon tetrachloride was described by Reynolds (1963).

Alterations in the structure of intestinal villi and microvilli have received much attention. In coeliac sprue, electron microscopy of absorptive cells reveals an irregularity of arrangement, shortening, widening and fusion of microvilli (for references, *see* Yardley *et al.*, 1962; Trier and Rubin, 1965). Such changes are thought to reduce markedly the absorptive surface and impair the functional capacity of the gut. It is interesting to note, however, that in the majority of patients with clinically detectable malabsorption such morphological alterations are not evident. Such examples include postgastrectomy malabsorption, diabetic steatorrhoea, pancreatic insufficiency and many others (Trier, 1967). Fusion of microvilli seems to be a common response to acute and chronic stressful states, for such changes have been seen in the rat jejunum after: (1) ischaemia (15 minutes); (2) hypothermia (20 minutes); (3) lactose-induced diarrhoea (three days); (4) mecamylamine-induced malabsorption (three days); (5) protein malnutrition (three weeks); and (6) protein-calorie malnutrition (three weeks) (Wehman *et al.*, 1972).

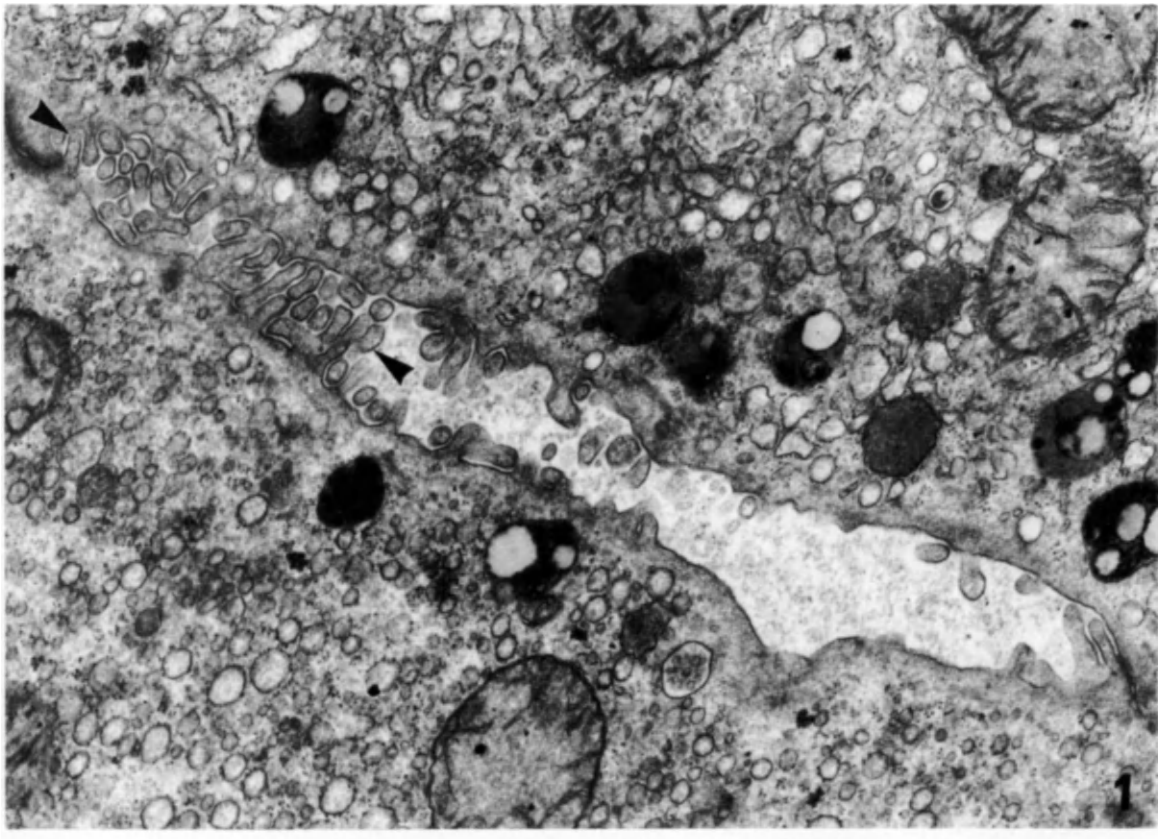
Dilatation and vesiculation of the microvilli have been observed in human jejunal biopsies after a single dose of intravenous methotrexate (Trier and Rubin, 1965) and within one hour in mouse small intestine after a high dose of x-rays (Quastler and Hampton, 1962; Hugon *et al.*, 1963). At a later stage of the experiment the intestinal villi are populated by cuboidal cells with large nuclei and sparse short microvilli. Similar changes have also been described in the human small intestine after therapeutic field irradiation with 2000–3000 rad (Trier and Browning, 1965).

In cholera a voluminous outpouring of fluid occurs from the surface of the intestinal mucosa and it is now well known that this loss occurs through a fairly intact mucosa and not through a necrotic mucosa denuded of cells. Ultrastructural studies (Chen *et al.*, 1971) have shown that fluid released from the vessels into the lamina propria is effectively prevented from reaching the lumen via an intercellular route by the tight junctions of the terminal bar. Instead, the fluid finds its way into the cells via the lateral cell borders and appears in vesicles and vacuoles formed from the endoplasmic reticulum. The final route of escape seems to be via the microvilli, for many bullous microvilli are seen containing either oedematous cytoplasmic material or numerous fluid-containing membrane-bound vacuoles.

Plate 490

Fig. 1. Bile canaliculus from the liver of a rat bearing a subcutaneous sarcoma. A portion of the canaliculus (between arrowheads) is well populated by microvilli and gives some idea as to what a normal canaliculus looks like (*see also Plate 489, Fig. 1*). The remainder of the canaliculus shows a paucity of microvilli. ×26 000 (*Ghadially and Parry, unpublished electron micrograph*)

Fig. 2. Bile canaliculus from another rat bearing a subcutaneous sarcoma, showing a more severe loss of microvilli. ×52 000 (*Ghadially and Parry, unpublished electron micrograph*)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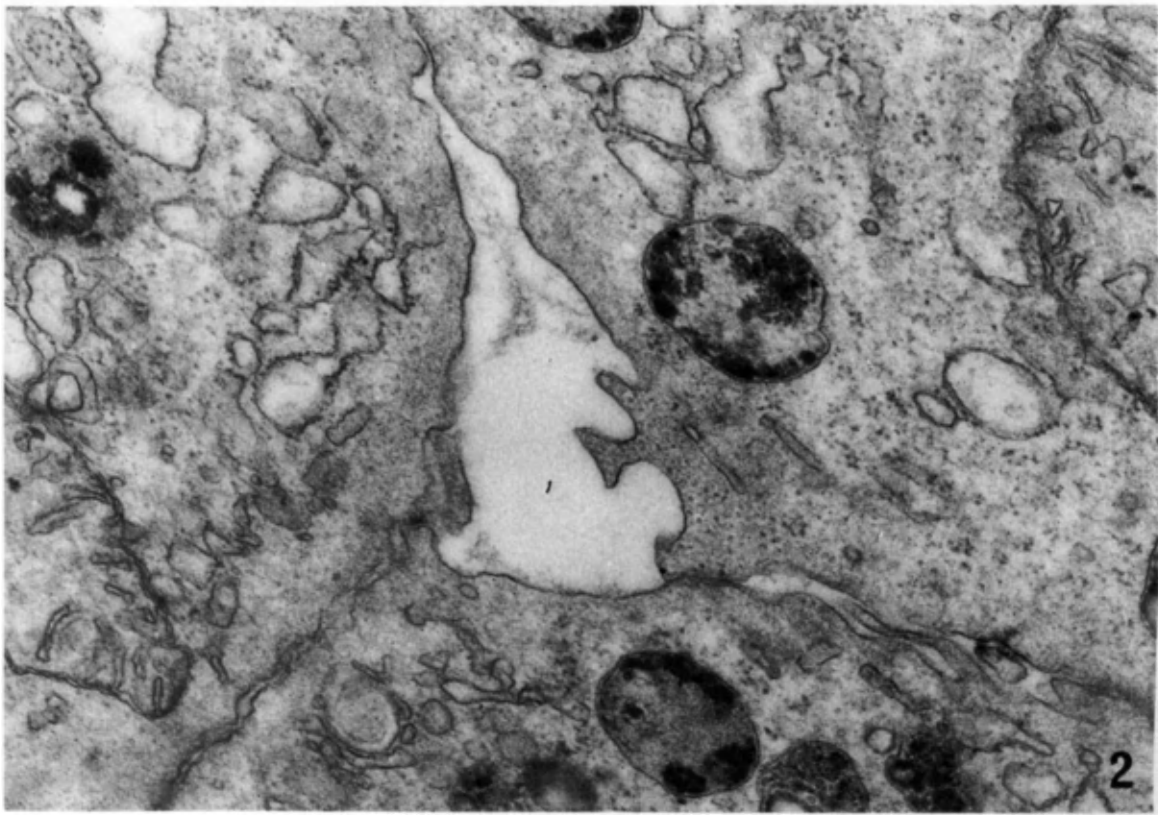
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Vermipodia

The term 'vermipodia' was coined to describe long cylindrical, wormlike processes (Ghadially and Skinnider, 1976; Skinnider and Ghadially, 1977). These cell processes (*Plate 491*) have to date been seen only in circulating neoplastic histiocytic or monocytic cells but not in hundreds of other haematological disorders (including various leukaemias) which we have studied. Thus at the moment it seems that this point may be of some diagnostic value.

The idea that vermipodia are a preparative artefact may be ruled out, because we have seen them in: (1) wet preparations of peripheral blood; (2) blood films stained by Wright-Giemsa stain; (3) transmission electron microscopic preparations from the buffy coat; and (4) scanning electron microscopic preparations of leucocyte-rich fraction of freshly collected blood.

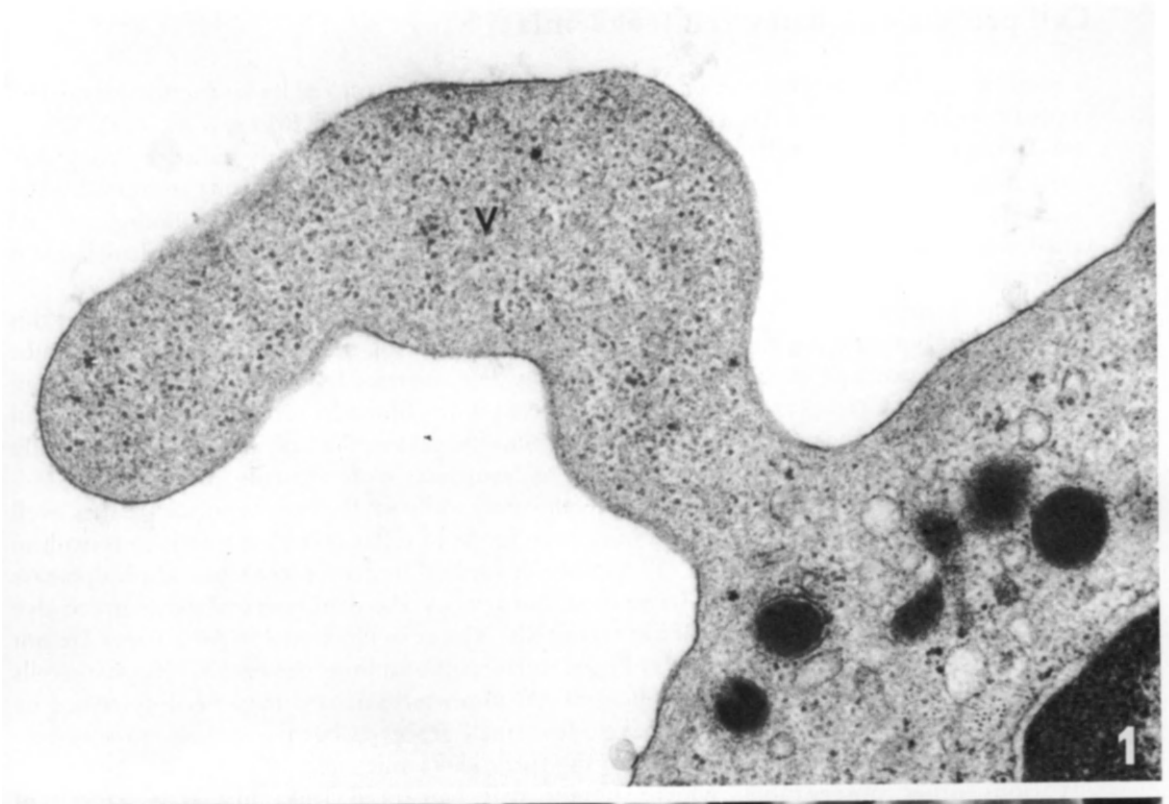
Vermipodia are clearly quite different from uropodia (*Plate 495*) which are solitary broad-based, tapering processes which terminate in microspikes. Further only one uropod occurs on a lymphocyte, while multiple vermipodia occur on neoplastic monocytes. Vermipodia also bear no resemblance to pseudopodia (*Plate 476*), which are blunt projections (as seen in sectioned material) of the cell surface, used by cells such as neutrophils and amoeba for phagocytosis and locomotion; nor are they akin to cilia (*Plate 497*), or microvilli (*Plate 430*).

The exact nature and significance of vermipodia is not known. The fact that they have been seen on malignant cells suggests that they might be an atypical or pathological alteration of the cell surface with little functional significance. The idea that they are atypical locomotor organs is conceivable but does not appear very likely. It has been suggested that some projections such as uropodia may serve to explore contact with other cells (Bessis, 1973). Similarly, one may argue that vermipodia may also serve such a function, for histiocytes and lymphocytes are known to establish connections (Schoenberg *et al.*, 1964; McFarland and Heilman, 1965; McFarland *et al.*, 1966). But once again direct proof that vermipodia subserve such a function is lacking.

Plate 491

Fig. 1. Malignant histiocytosis. A leukaemic cell from peripheral blood showing a vermipodium (V). Note absence of larger cell organelles in the vermipodium. ×44 000 (*From Skinnider and Ghadially, 1977*)

Fig. 2. Malignant histiocytosis. Different case from that shown in *Fig. 1.* In this scanning electron micrograph, five vermipodia (1–5) can be detected on this leukaemic cell from peripheral blood. ×8500 (*From Ghadially and Skinnider, 1976*)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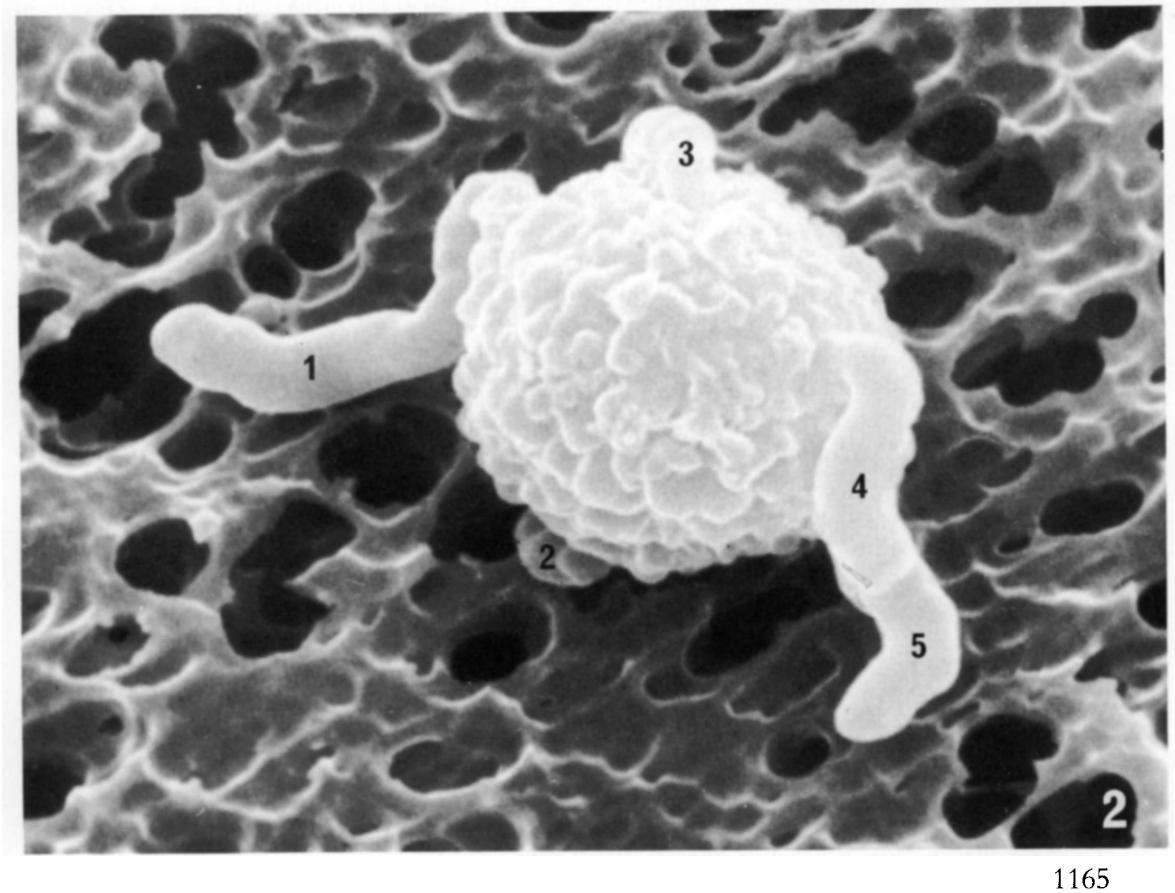
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Cell processes in hairy cell leukaemia

Several cases of this unusual type of leukaemia, which is characterized by leukaemic cells with a ragged appearance and numerous cell processes, have been described (Mitus *et al.*, 1961; Schrek and Donnelly, 1966; Plenderleith, 1970; Mitus, 1971). It is now aptly called a 'hairy cell' leukaemia. It would appear that similar cases have also been reported by some authors (Ewald, 1923; Bouroncle *et al.*, 1958; Yam *et al.*, 1968, 1971) as leukaemic reticuloendotheliosis and reticulum cell leukaemia. The nature of the hairy cell and the classification of this leukaemia is uncertain.

At light microscopy the dense nucleus and scant cytoplasm lend support to the idea that this is a variety of lymphocyte, the only disconcerting feature being the cell processes which can be seen at light microscopy but are much more clearly demonstrated by electron microscopy (*Plate 492*). These cell processes are somewhat reminiscent of the filopodia seen on synovial cells, but they do not seem to be engaged in endocytotic activity, and in the case we studied (Ghadially and Skinnider, 1972), dense bodies acceptable as lysosomes were virtually absent.

Since reticulum cells and histiocytes are the only cells in the haemopoietic tissues well endowed with cell processes, attempts have been made to relate these hairy cells to reticulum cells or histiocytes. Katayama *et al.* (1972) did not succeed in demonstrating acid phosphatase activity in these cells with the Gomori method, but activity was demonstrated using an azo-dye method in some of the clear vacuoles in these cells. The cells illustrated in their paper are not particularly hairy, nor are the clinical and light microscopic findings presented. Neoplastic cells from three patients in the leukaemic phase of reticulum cell sarcoma have been described by Schnitzer and Kass (1973). Such cells have a few small processes but the authors quite rightly conclude that these cells are different from the hairy leukaemic cells.

Various other observations which suggest that hairy cell leukaemia is a variety of lymphocytic leukaemia and that these cells are not related to reticulum cells or monocytes are as follows: (1) reticulum cells and monocytic or histiocytic cells have a pale nucleus with a prominent nucleolus, but hairy cells have a dense nucleus and a small nucleolus; (2) the virtual absence of pinocytotic, micropinocytotic and lysosomal activity seen in our case (Ghadially and Skinnider, 1972), and the inability of hairy cells to phagocytose latex particles (Schrek and Donnelly, 1966), also argue against the idea that these cells are related to histiocytic cells; (3) it is now well documented that lymphocytes often cluster around and establish connections with macrophages, and we have shown that hairy cells behave in the same way (Ghadially and Skinnider, 1972); (4) hairy cells do not contain lysozyme or myeloperoxidase, the two characteristic enzymes of monocytes (Catovsky *et al.*, 1975); (5) the demonstration of surface immunoglobulins and functional studies argue strongly in favour of the idea that the hairy cell is a neoplastic B-lymphocyte (Preud'homme and Seligmann, 1972; Aisenberg *et al.*, 1973; Burns *et al.*, 1973; Catovsky *et al.*, 1974, 1975; Haak *et al.*, 1974; Schnitzer and Hammack, 1974; Deegan *et al.*, 1976; Arrenbrecht *et al.*, 1982; Merchant *et al.*, 1985); (6) a review of 100 cases of reticulum cell sarcoma has shown that in no instance did hairy cells appear in the blood, while cases of hairy cell leukaemia do not develop the characteristic lymph node masses and skin infiltrates of reticulum cell sarcoma (Plenderleith, 1970); and (7) the average survival time for reticulum cell sarcoma is about nine months, while for hairy cell leukaemia it is about 4–13 years (Plenderleith, 1970).

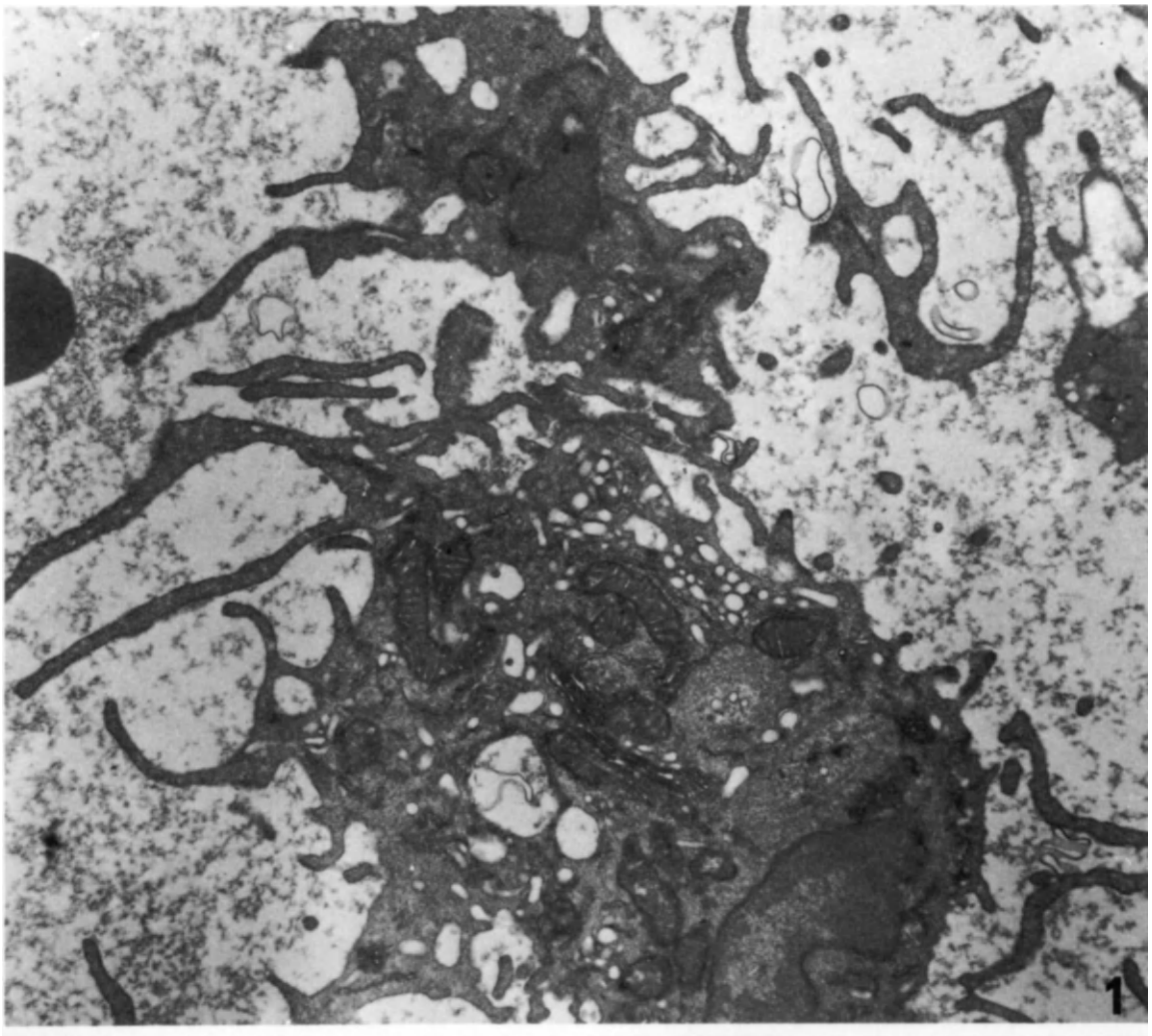
Plate 492

Leukaemic cells from a case of hairy cell leukaemia. Note the dense nucleus, and the absence of dense bodies acceptable as lysosomes.

Fig. 1. Shaggy leukaemic cells with long cell processes. ×14 500

Fig. 2. A ragged leukaemic cell. ×9000 (*From Ghadially and Skinnider, 1972*)

Fig. 3. A cell with relatively few short processes. ×12 000 (*From Ghadially and Skinnider, 1972*)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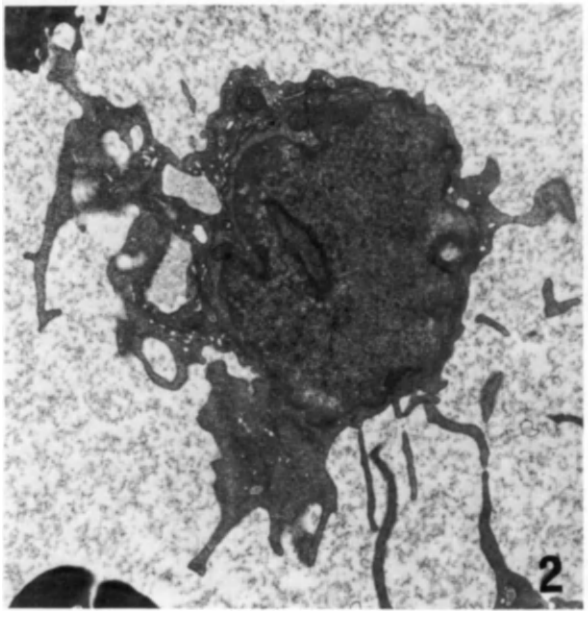
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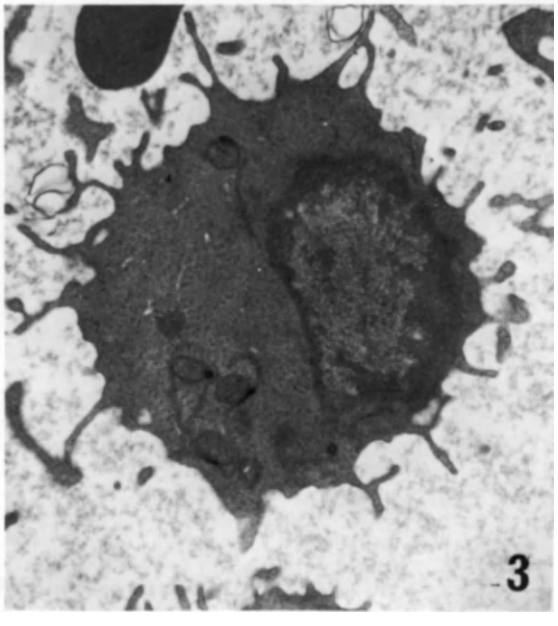
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Ropalocytes and ropalopodia

During the course of our studies (Ghadially and Skinnider, 1971, 1972) on a case of hairy cell leukaemia (page 1166) we observed a remarkable abnormality in the form of the erythrocytes and their precursors. A normocytic, normochromic anaemia is commonly associated with this condition, but light microscopy of blood films and bone marrow showed no overt alterations of erythrocytic morphology, apart from the occurrence of an occasional burr cell.

However, ultrathin sections of marrow and peripheral blood from our case revealed complex alterations in the morphology of normoblasts (*Plate 493, Figs. 1 and 2*), reticulocytes (*Plate 493, Figs. 3 and 4*), and erythrocytes (*Plate 494*). The alterations in form are often quite complex, but the basic feature here seems to be the occurrence of branched and unbranched cell processes which often present club-shaped profiles. It is more likely that a three-dimensional view would reveal that many of these processes are complex folds or deep invaginations of the cell membrane.

Since the basic defect presents in ultrathin sections as club-shaped processes and the Greek for club is *ropalon*, we coined the term 'ropalocytosis' to describe this abnormality of erythrocytic form (Ghadially and Skinnider, 1971), and in keeping with the nomenclature of other cell processes (e.g. uropodia, pseudopodia, lamellaepodia, filopodia, etc.) one may call the club-shaped process 'ropalopodia'.

It will be evident that ropalocytes are quite distinct and different from other well-known forms of abnormal erythrocytes such as schistocytes, echinocytes and target cells. The abnormality of form that characterizes these latter cells is best recognized by light microscopy of entire erythrocytes. In ultrathin sections they present little evidence of their characteristic form and are thus difficult or impossible to recognize with any degree of confidence. In contrast to this, ropalocytosis is a fine structural manifestation and, as already noted, light microscopy gives no clear indication of its presence. A further distinguishing feature is that ropalocytosis affects the entire series of erythroid cells and not just mature erythrocytes.

Ropalopodia have now been seen also on: (1) trypsin-treated mouse embryonic cells in culture (Friedmann and Hodges, 1975); (2) cytochalasin D-treated normal lymphocytes (Skinnider, 1977a); and (3) neoplastic lymphocytes in cases of acute leukaemia (Skinnider, 1977b) and the term ropalocyte had been used in this connection. Therefore in order to avoid confusion it would be better to speak about 'erythrocytic ropalocytes' when describing this change in the erythrocytic series of cells.

Erythrocytic ropalocytes have been seen in: (1) hairy cell leukaemia (Ghadially and Skinnider, 1971, 1972); (2) idiopathic thrombocytopaenic purpura, pernicious anaemia and Hodgkin's disease (Skinnider and Ghadially, 1973); (3) paroxysmal cold haemoglobinuria (Djaldetti *et al.*, 1975; Djaldetti, 1978); (4) acute lymphoblastic leukaemia (Djaldetti *et al.*, 1973); (5) 10–18 weeks old embryonic human blood (Djaldetti *et al.*, 1978); (6) a case of Ewing's tumour treated with desacetyl vinblastine amide sulphate (Jumean *et al.*, 1979); (7) refractory anaemia and myelomonocytic leukaemia (Maldonado and Mandon, 1976); and (8) 30 per cent of 130 cases with haematological diseases (e.g. myelomonocytic leukaemia, acute and chronic leukaemia), two cases with transplanted kidneys and several cases with brucella hepatitis (Nedelkovski *et al.*, 1984; Grozdev, 1986).

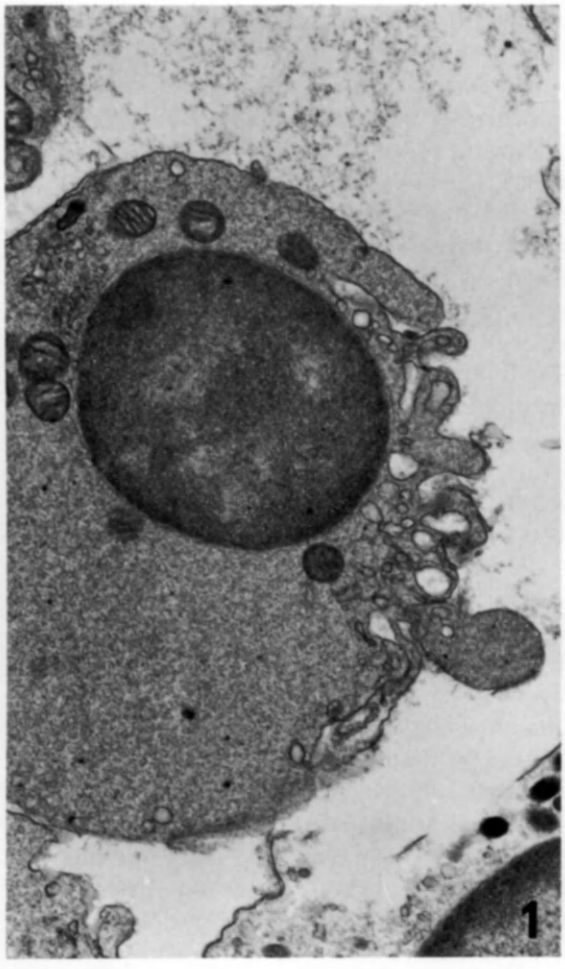
Plate 493

Fig. 1. A normoblast showing a mild degree of focal ropalocytosis. ×16 000 (*From Skinnider and Ghadially, 1973*)

Fig. 2. Normoblast showing marked ropalocytosis. The entire cell has a ragged appearance produced by the abundance of processes and vacuoles. Fine vesicles are seen in the main cytoplasmic mass and also in the cell processes. ×15 000 (*From Skinnider and Ghadially, 1973*)

Fig. 3. A reticulocyte showing short club-shaped, sessile and pedunculated processes. ×11 000 (*Ghadially and Skinnider, unpublished electron micrograph*)

Fig. 4. A reticulocyte from peripheral blood, showing a picturesque alteration of overall shape and cell processes. ×10 500 (*From Ghadially and Skinnider, 1971*)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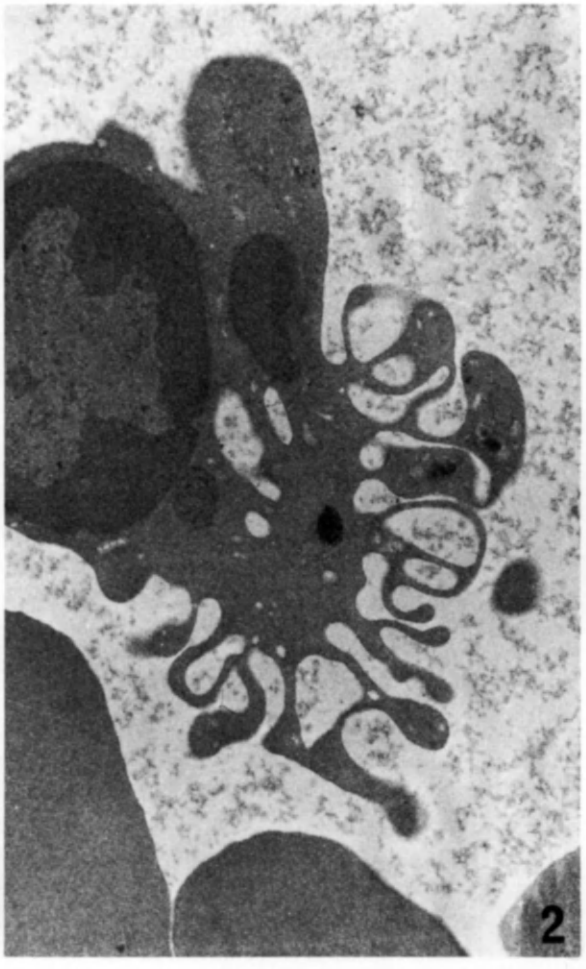
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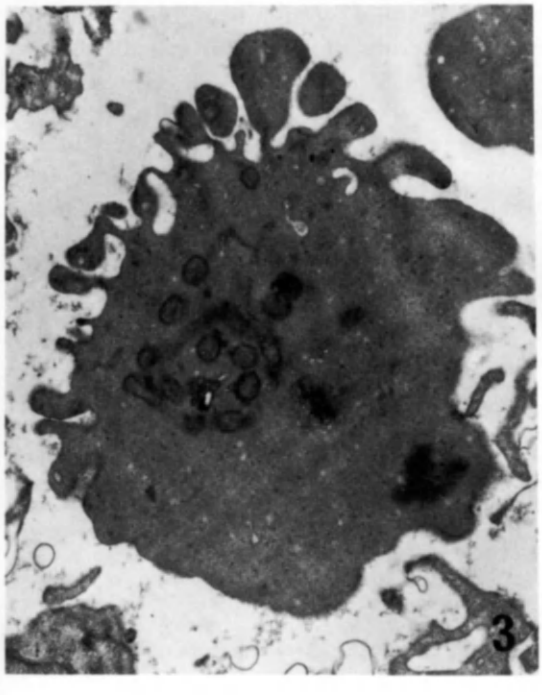
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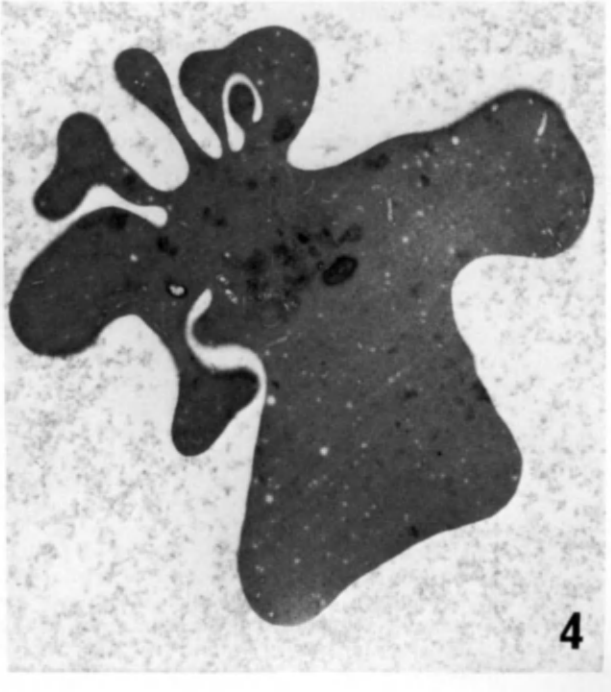
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As noted earlier this change is seen in erythrocytes (*Plate 494*), reticulocytes (*Plate 493*) and normoblasts (*Plate 494*), but it is the reticulocytes that are most frequently involved, the defect being less frequent in normoblasts and quite rare in erythrocytes. In some instances, the entire cell may be grossly deformed, with numerous processes springing from its entire surface, in other instances the defect is focal, with only a segment of the cell affected.

The significance of this change and the manner in which it is produced is obscure. In studies on the denucleation of normoblasts in dogs rendered anaemic by various means, Simpson and Kling (1967) noted the occurrence of numerous small vesicles on the 'underside' of the nucleus apposed to the cell membrane. They postulated that fusion of such vesicles leads to the detachment of the marginated nucleus and may leave behind a ragged area of cell wall. This in some instances bears a resemblance to the change we are describing (*see* their *Fig. 10*). However, our experience with denucleating normoblasts in human bone marrow is not in keeping with this and the fact that ropalocytosis is seen in normoblasts with intact nuclei argues against the idea that ropalocytosis is invariably or even usually produced in this fashion (Skinnider and Ghadially, 1973).

However, published illustrations lead me to conclude that a normoblast that has just lost its nucleus (i.e. an early reticulocyte) does in some instances show focal ropalocytosis. Somewhat confused and confusing is the report by Maldonado and Mandon (1976) who presumably found ropalocytosis in cases of refractory anaemia and myelomonocytic leukaemia and concluded that the cells they found were 'not necessarily pathologic, although they were observed in patients who had a very abnormal red blood cell line'. The logic behind this peculiar statement is difficult to fathom.

The significance of ropalocytosis and the manner in which this change is produced is obscure, but certain points are worth commenting on. As noted previously (pages 646–649) autolysosomes occur in erythrocytes and when their contents are discharged or when such bodies are removed by the spleen, clear tracts extending from within the cell to its surface are left behind. The possibility that ropalocytosis results from a heightened activity of this kind was not borne out by our investigations, for there was no positive correlation between the occurrence of autolysosomes and ropalocytosis in the cases we studied. The only positive correlation seemed to be between the occurrence of vesicles in these cells and ropalocytosis. It is likely that some but not all such vesicles represented sections through invaginations of the plasma membrane, for often such vesicles were too numerous, not near the cell surface and quite small.

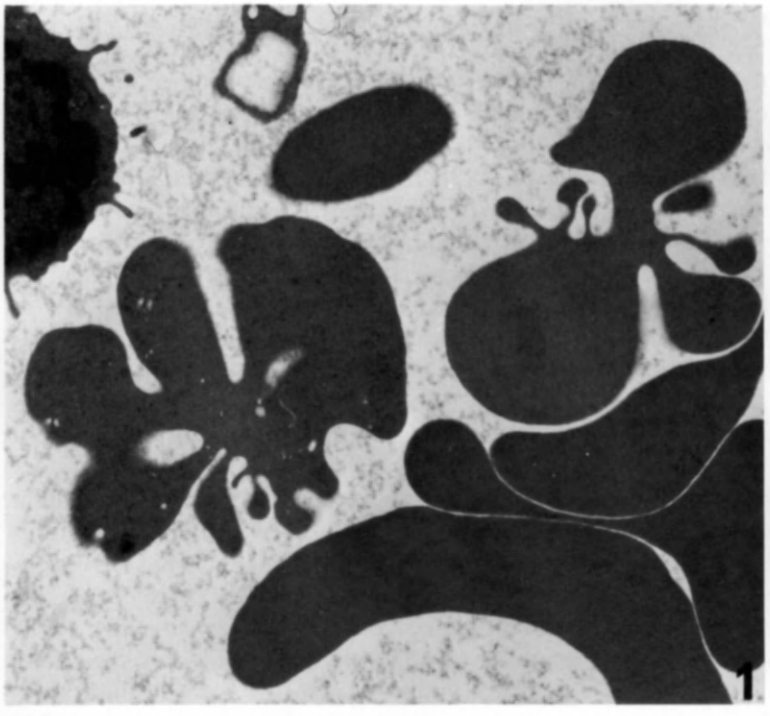
It seems to us that both pinocytotic and micropinocytotic activity are heightened in these cells (Skinnider and Ghadially, 1973) but the stimulus which provokes this change remains obscure. If this tentative supposition is correct, then one could speculate that it could be due to a deficiency of some factor needed by the cell, or to the presence of some stimulating agent in the cell environment.

Plate 494

Fig. 1. Two cells showing ropalocytosis are seen here. The one in the top right-hand corner may be regarded as an erythrocyte and the one in the bottom left-hand corner as a late reticulocyte, for it contains a few ribosomes (not clearly evident here but seen at higher magnification). Note also the numerous fine vesicles in its cytoplasm. Three normal-looking erythrocytes are seen in the bottom right-hand corner. ×10 000 (*From Skinnider and Ghadially, 1973*)

Fig. 2. An erythrocyte showing ropalocytosis. ×14 000 (*From Skinnider and Ghadially, 1973*)

Fig. 3. A singularly picturesque alteration of erythrocytic morphology is seen here. Islands and bands of erythroid substance are seen as a network or a maze from which spring many club-shaped processes. The general pattern here is continuous and the overall maximum dimension is about 6 μm: hence it is conceivable that this is a single altered erythrocyte rather than two or more erythrocytes lying close to one another, but one cannot be certain. ×22 000 (*From Ghadially and Skinnider, 1972*)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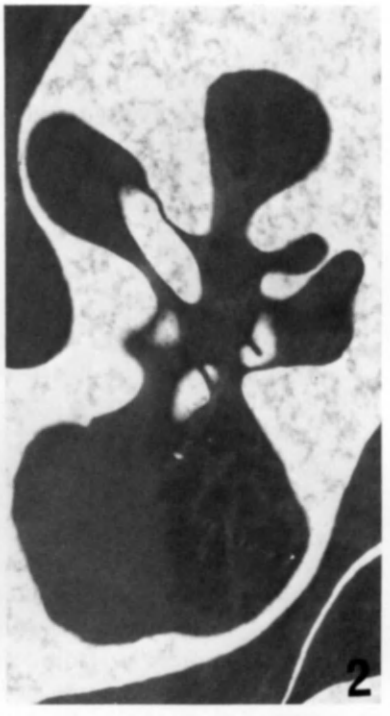
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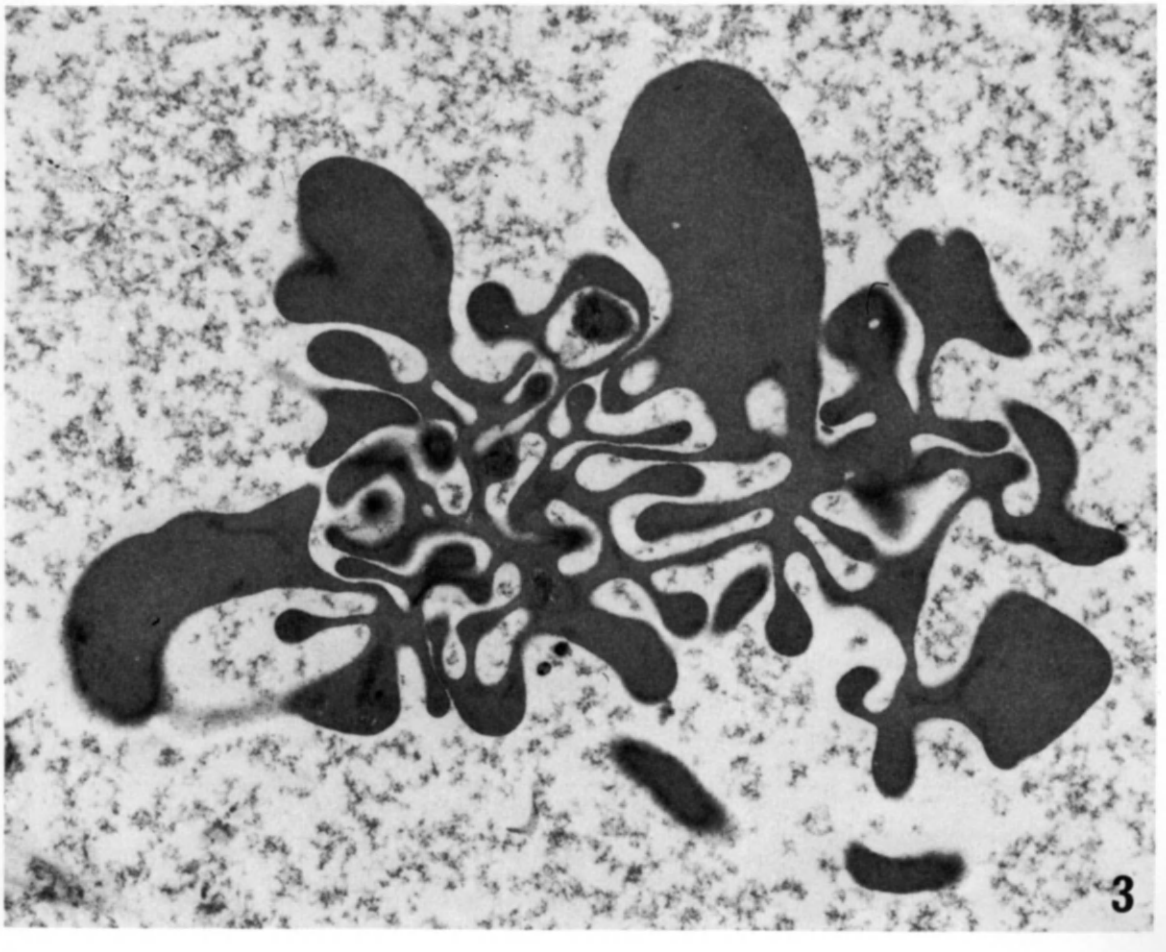

3

Uropod of the lymphocyte

It is generally thought that the leucocytes of peripheral blood are not actively motile during their passive transport in blood vessels. Their rounded form as seen in smears of peripheral blood supports this idea. In culture, however, cells in locomotion abandon their rounded form and assume an elongated or triangular form. Lewis (1931) noted that cultured rat lymphocytes develop an unusual morphological configuration with apposing polarization of nucleus and cytoplasm which (i.e. the cytoplasm) presents as an elongated cell process. Later, he and his colleagues (Rich *et al.*, 1939) coined the term 'hand mirror cell' to describe such cells. The term 'uropod' (Gk. *oura*, tail and *podos*, foot or stalk) was coined by McFarland *et al.* (1966) to describe the 'tail' (or 'handle' of the 'hand mirror') of the cell. The uropod of the lymphocyte is so prominent that it can be readily distinguished from other motile leucocytes which may irregularly and briefly display a small 'tail'. By means of phase contrast light microscopy and transmission and scanning electron microscopy, threadlike projections or microspikes are seen projecting from the uropod (*Plate 495*).

Uropods have been seen in: (1) cultures of rat lymphocytes (Lewis, 1931); (2) cultured rabbit lymph nodes and human leukaemic blood (Rich *et al.*, 1939); (3) mixed lymphocyte cultures (McFarland *et al.*, 1966; McFarland, 1969; McFarland and Schechter, 1969); (4) phytohaemagglutinin stimulated lymphocytes (Biberfeld, 1971; Gormley and Ross, 1972); (5) bone marrow from cases of myeloblastic leukaemia and a case of lymphosarcoma (Norberg *et al.*, 1974, 1977); (6) bone marrow from cases of acute lymphoblastic leukaemia (hand mirror cell variant) (Sjögren, 1976; Sjögren *et al.*, 1977; Stass *et al.*, 1978; Schumacher *et al.*, 1978, 1979; Sharp *et al.*, 1979); (7) American Burkitt's lymphoma (hand mirror cell variant) (Schumacher *et al.*, 1978); (8) virus-induced murine lymphoma (Schumacher *et al.*, 1979).

Various properties and functions have been ascribed to the uropod of the lymphocyte. These include: (1) locomotion (the uropod comprises the posterior end of the moving cell); (2) endocytotic activity (interiorization of immune complexes from the cell membrane); (3) attachment to other cells (particularly macrophages), glass (culture containers) and debris; (4) tactile exploration by continual thrusting out and retraction of microspikes ('probe and attach' function); (5) immunologic activation (interaction between lymphocytes and monocytes).

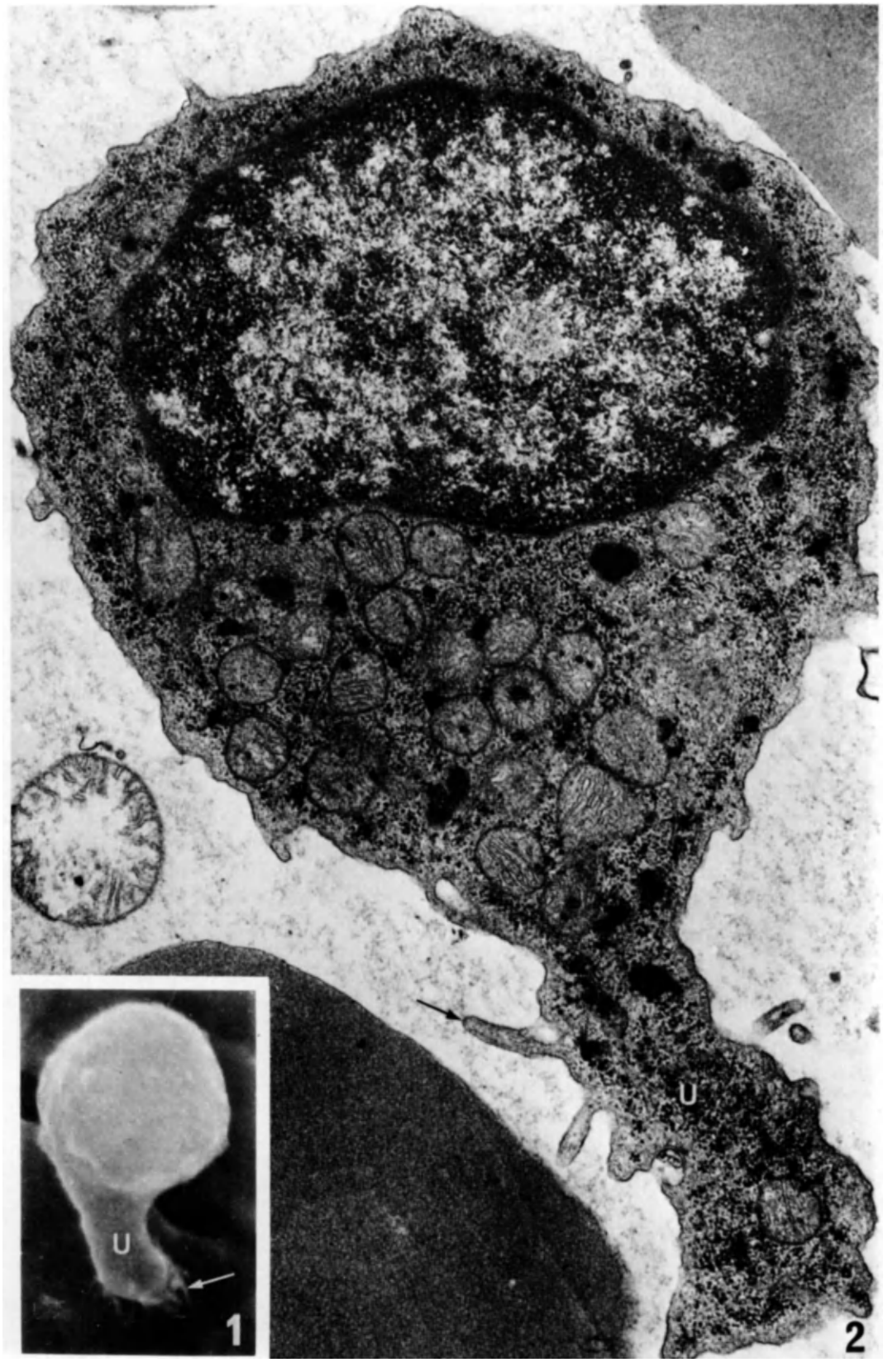
An increase in the number of hand mirror cells has been noted in several types of leukaemias, particularly certain cases of acute lymphoblastic leukaemia. Such cases are now designated as the hand mirror cell variant of acute lymphoblastic leukaemia. Various studies (Norberg *et al.*, 1974; Sjögren, 1976; Stass *et al.*, 1978) suggest that patients with greater than 40 per cent hand mirror cells in the bone marrow have a better prognosis than other types of acute lymphoblastic leukaemia. However, this suggestion does not appear to be supported by the studies of Schumacher *et al.* (1979).

Plate 495

Acute lymphoblastic leukaemia—hand mirror cell variant.

Fig. 1. Scanning electron micrograph of a hand mirror cell. Note uropod (U) bearing microspikes (arrow). ×6000 (*Stass, Perlin, Jaffe, Simon, Creegan, Robinson, Holloway and Schumacher, unpublished electron micrograph*)

Fig. 2. Transmission electron micrograph of a hand mirror cell showing uropod (U) bearing microspikes (arrow). ×27 000 (*From Stass, Perlin, Jaffe, Simon, Greegan, Robinson, Holloway and Schumacher, 1978*)


U
U
1
2

Foot processes of podocytes

The glomerular or visceral epithelium of the kidney is composed of cells with several radiating and branching processes (collectively referred to as 'primary processes') which embrace the glomerular capillaries. These major or primary processes give rise to many small secondary processes which are usually referred to as 'foot processes' or 'pedicles'. The foot processes of a cell (podocyte) may interdigitate with foot processes from the same cell (rare occurrence) or adjacent cells (usual situation), to create an elaborate system of intercellular clefts called 'slit pores', 'filtration slit pores' or 'filtration slits'.

In transmission electron micrographs (Bloom and Fawcett, 1969; Rhodin, 1974) the foot processes are seen aligned upon the outer surface of the glomerular basal lamina (*Plate 496, Fig. 1*). On the inner side of the lamina lie the fenestrated glomerular capillaries. The ends of the foot processes resting on the basal lamina are widened, hence they appear triangular in section. The slit pore or gap (about 20–50 nm wide) between the foot processes is bridged by an electron-dense 'line' (about 5 nm thick) which is commonly referred to as the 'filtration slit membrane'. However, strictly speaking this structure is a lamina for it does not show the trilaminar structure of a cytomembrane. It is in fact analogous to the tenuous diaphragm or lamina which bridges the pores of most fenestrated capillaries★.

The bodies of podocytes rarely contact the basal lamina, they seem to ride on top of cell processes of neighbouring podocytes. This arrangement makes available maximum surface area of the basal lamina to the foot processes and filtration slits.

The complex morphology of the podocyte and its cell processes is difficult to grasp from sectioned material. Although laborious reconstructions from serial sections had elucidated most of the morphological features of this region, it remained for scanning electron microscopy to convincingly demonstrate its detailed picturesque morphology (*Plate 496, Fig. 2*) (Arakawa, 1970; Arakawa and Tokunaga, 1972, 1974; Andrews, 1975; Fujita *et al.*, 1976).

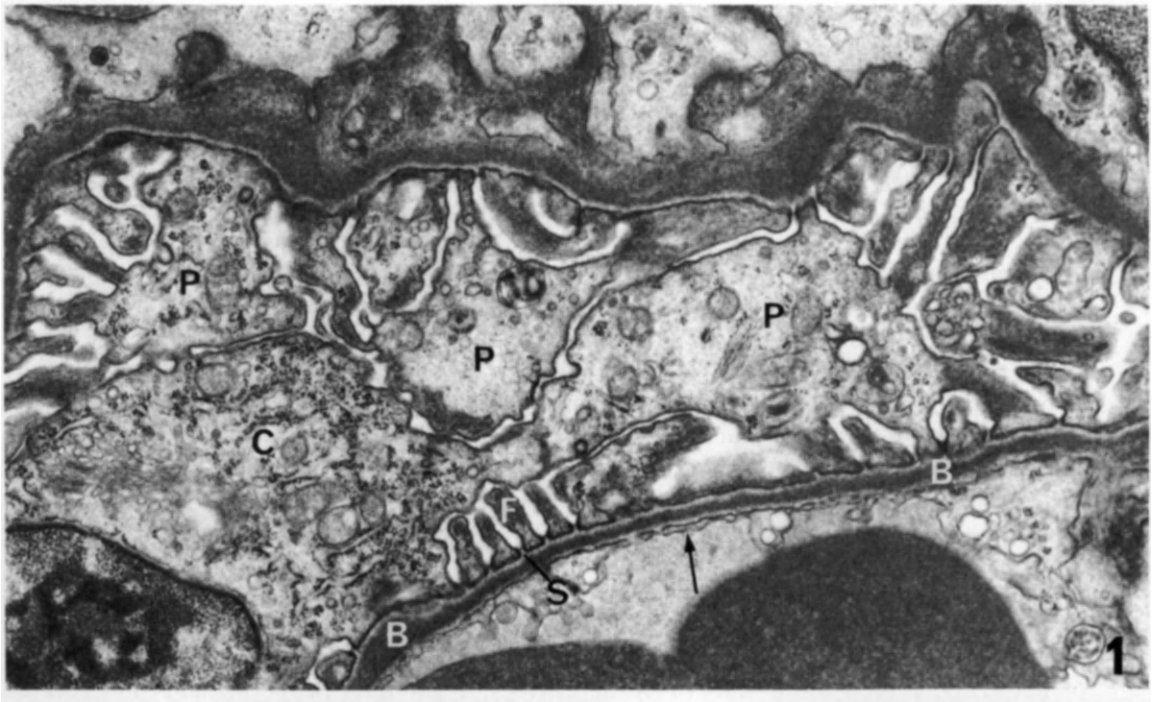
A change commonly referred to as 'fusion of foot processes' has been seen in a variety of nephropathies. It is characterized by a disappearance of the regularly spaced small foot processes and the appearance of much larger irregular cell processes or segments of podocyte cytoplasm resting on the basal lamina. It seems unlikely that this change results from the actual fusion of neighbouring foot processes of different podocytes, although images suggesting fusion of the cell membrane of two neighbouring foot processes have been seen on rare occasions (Sternberg, 1970). Scanning electron microscope studies show that the appearance called 'fusion of foot processes' is more likely due to a swelling and retraction of foot processes so that the capillary wall is covered by large swollen processes or segments of podocyte cytoplasm (Arakawa and Tokunaga, 1972; Lehtonen *et al.*, 1973; Buss and Lamberts, 1975). In view of this it would be better to use the noncommital phrase 'loss of foot processes' instead of 'fusion of cell processes' as some more meticulous students of the subject have already done.

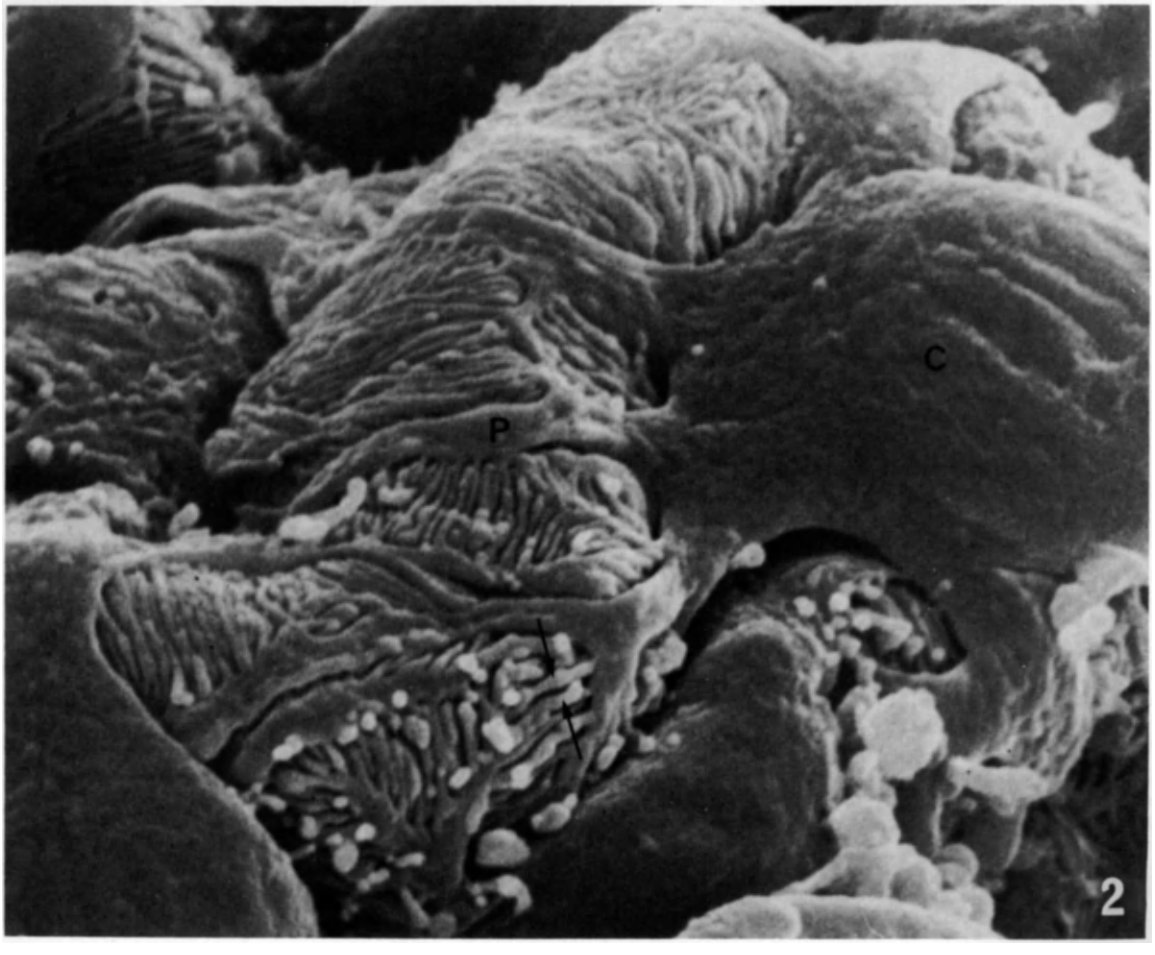
★An exception to this are the fenestrated capillaries of the human renal glomerulus, where pores with and without diaphragms occur.

Plate 496

Fig. 1. Glomerulus of rat kidney. Note the podocyte (C), primary processes (P), foot process (F), filtration slits (S), basal lamina (B) and endothelium of fenestrated capillary (arrow). ×16 500

Fig. 2. Scanning electron micrograph of a glomerulus of rabbit kidney showing a podocyte (C), primary processes (P), foot processes (arrows) and filtration slits between them. ×8000


P
P
P
C
B
F
S
B
1


C
P
2

Cilia, flagella and sperm tails

Certain cylindrical, hair-like motile processes first observed on some protozoa and later on certain epithelia of higher animals, have long been known to light microscopists as cilia and flagella. It has been customary to call relatively short numerous processes arising from a cell 'cilia' while the term 'flagella' has been reserved for longer, solitary or not too numerous processes arising from the cell. According to Fawcett (1966), 'The shaft of the cilium is 0.2–0.25 μm in diameter and 5–10 μm long. Flagella range from this length to 150 μm or more'. Ultrastructural studies have shown that the basic structure of cilia and flagella is identical. Many cell processes which in the past were classified as flagella are now called cilia and the term 'cilia' is often used in a collective sense when referring to both cilia proper and flagella.

In suitable preparations examined with the light microscope, a small granular body can be seen in the cell close to the base of the cilium, as can an axial filament (axoneme) which arises from this structure and runs along almost the entire length of the cilium. Ultrastructural studies show that the granular body referred to as a 'basal body', 'basal corpuscle', 'basal granule', 'kinetoplast' or 'blepharoplast' is in fact a hollow cylinder with the same structure as a centriole. The homology of these two structures, recognized long ago by cytologists (Henneguy, 1897; Lenhossek, 1898), has now been confirmed by electron microscopists (*see* page 181). On the basis of fine structural variations of morphology, about six types of basal bodies have been identified (Fawcett, 1961; Randall and Hopkins, 1962). Striated rootlets* (usual periodicity about 70 nm), apparently serving to anchor the basal bodies, are often seen associated with most types of cilia, as are microtubules or filaments connected to the basal bodies and rootlets (*Plate 362, Fig. 1*). Whether these filaments or microtubules are anchoring devices or play a role in the coordination and propagation of the ciliary wave has been frequently debated (Parducz, 1967).

The electron microscope shows that the axial filament of the light microscopist is not a single filament but a bundle of microtubules. This structure, now referred to as the 'axoneme' or 'axial microtubule complex', shows nine pairs of microtubules arranged in a circle around the periphery of the cilium, and two microtubules in the centre. This is referred to as the '9+2' pattern of organization. The significance of this pattern, which is remarkably constant for many cilia and flagella throughout the plant and animal kingdoms has yet to be elucidated. Although numerous variations of the 9+2 pattern have been seen, most of these can be looked upon as variations of the same master plan. It is the universality and constancy, rather than the variations of the 9+2 pattern, which are intriguing. What advantage such a plan of organization has in evolutionary terms that it should have been adopted by such diverse forms of life remains unexplained.

*For a note on the nomenclature of rootlets, *see* footnote on page 1004.

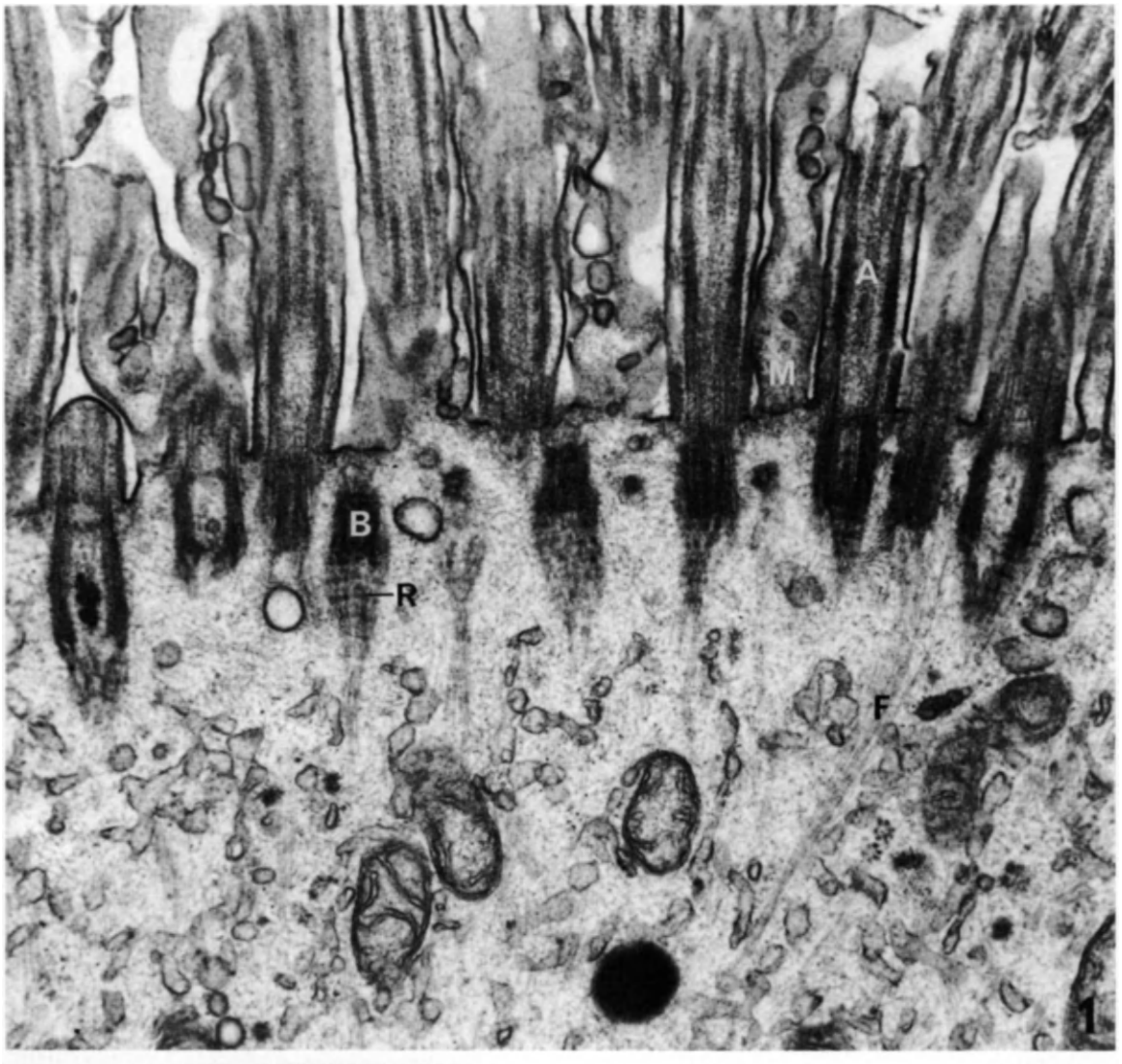
Plate 497

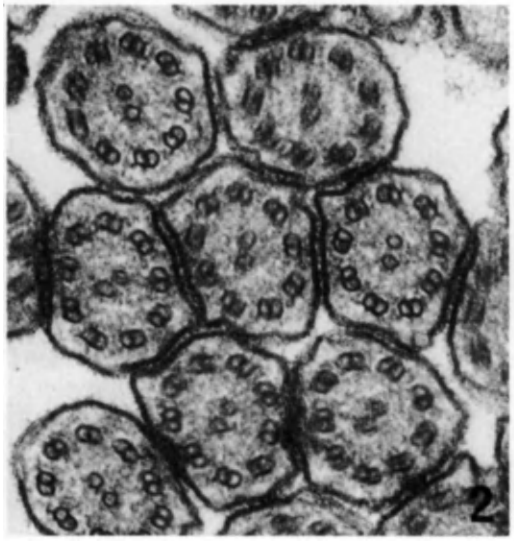
Fig. 1. The proximal portions of cilia from human bronchial mucosa are depicted here. (For a low-power view, *see Plate 80.*) Besides the cilia with their axial microtubule complex (A), some microvilli (M) are seen, springing from the cell surface. Also seen are basal bodies (B), striated rootlets (R) and associated filaments (F). ×40 000

Fig. 2. Transversely cut cilia from the bronchial mucosa of a cow, showing the characteristic 9+2 pattern of microtubules. ×89 000

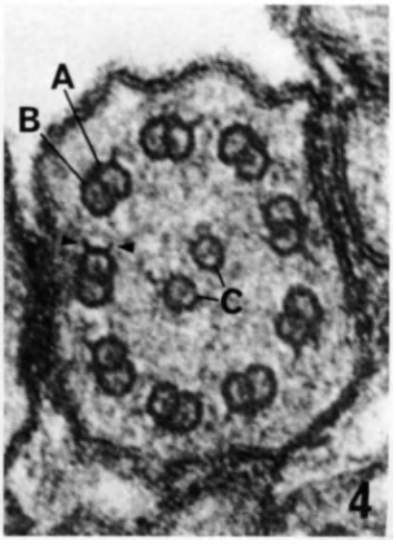
Fig. 3. Transverse section of cilia near their terminal part. The microtubules terminate at variable distances as the tip of the cilium is approached so that fewer microtubules are seen and doublets are replaced by singlets (arrow). ×83 000

Fig. 4. A 9+2 cilium from human bronchial mucosa. Note the trilaminar structure of the ciliary membrane, the central microtubules (C) and the peripheral doublets comprising microtubules A and B, and the dynein arms (arrowheads) arising from microtubule A. ×160 000


A
M
B
R
F
1


2


3


A
B
C
4

In transverse sections of cilia (*Plate 497, Figs. 2–4*), the central microtubules present discrete circular profiles, but the peripheral doublets show a figure-of-eight configuration. One of these microtubules, which at times appears solid or filament-like and bears the outer and inner dynein arms is designated 'microtubule A' or 'A-microtubule'. The other microtubule is called 'microtubule B' or 'B-microtubule'*. The direction of the ciliary beat occurs in a plane perpendicular to the plane joining the two central microtubules.

The two central microtubules have often been considered essential for motility and such motile cilia (9+2) are at times referred to as kinocilia. This contrasts them from cilia which are not motile, such as the connecting cilium of the vertebrate retinal rod cell (De Robertis, 1956), which has a 9+0 pattern of organization. Ultrastructural studies have shown that a large variety of cells possess an occasional or a few cilia (page 1180) and that many such cilia show the 9+0 pattern, but not much is known about their motility. However, the motile sperm tails of *Myzostommium cirriferum* have a 9+0 pattern of organization†. Their motion, although sluggish compared to 9+2 sperm tails, is not restricted to one plane (Afzelius, 1961, 1962). One may therefore argue that the central microtubules determine the direction of the beat, rather than the motility or otherwise of the cilium.

The tails of many but not all non-mammalian vertebrate spermatozoa are remarkably similar to the cilia and flagella described above. The tail of the mammalian sperm is more complex, but it too bears an axial microtubule complex with the 9+2 pattern. The main difference here is the occurrence of a further set of dense rods or fibres around the axial microtubule complex (*Plate 498*).

The term 'stereocilia' has been used in classic histology to describe certain long slender cell processes such as those seen on the pseudostratified columnar epithelium of the epididymis and the hair cells of the vestibular labyrinth (Bloom and Fawcett, 1969; Lentz, 1971). These processes do not contain an axial microtubule complex, nor are they associated with basal bodies, and as such they are no longer considered to be cilia but a variety of microvilli (page 1160).

*For further details about the nomenclature of the components of the axoneme, *see Plate 507, Fig. 2.*
†In Gregarine, motile flagella with a 6+0 and a 3+0 pattern have been described (Schrevel and Besse, 1975; Prensier *et al.*, 1980).

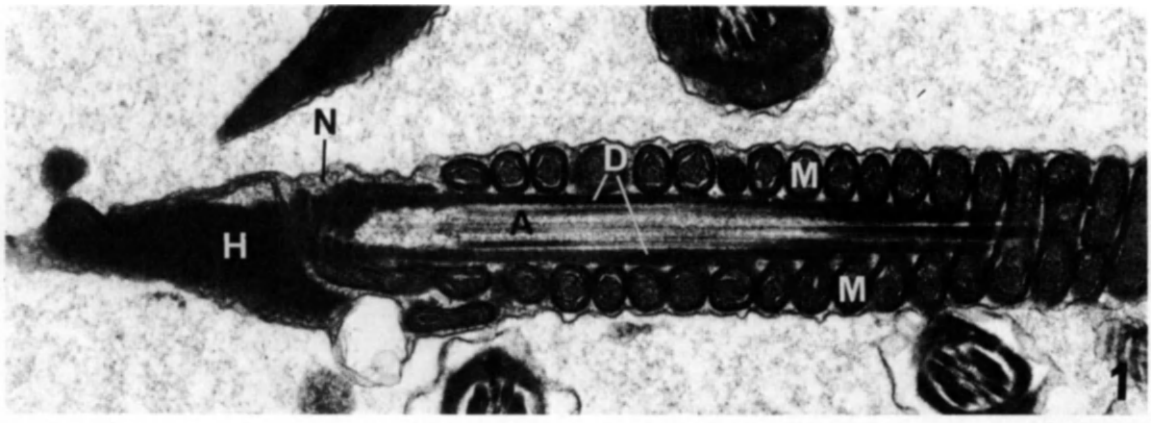
Plate 498

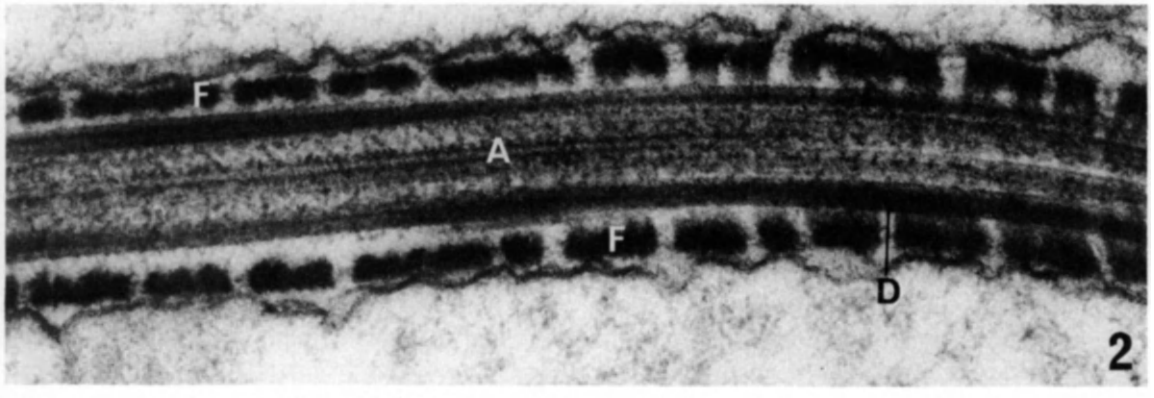
Sections through mouse spermatozoa cut in various planes. (The sperm has a head and a tail; the tail is further subdivided into the neck, middle piece, principal piece and end piece (*Bloom and Fawcett, 1969*).)

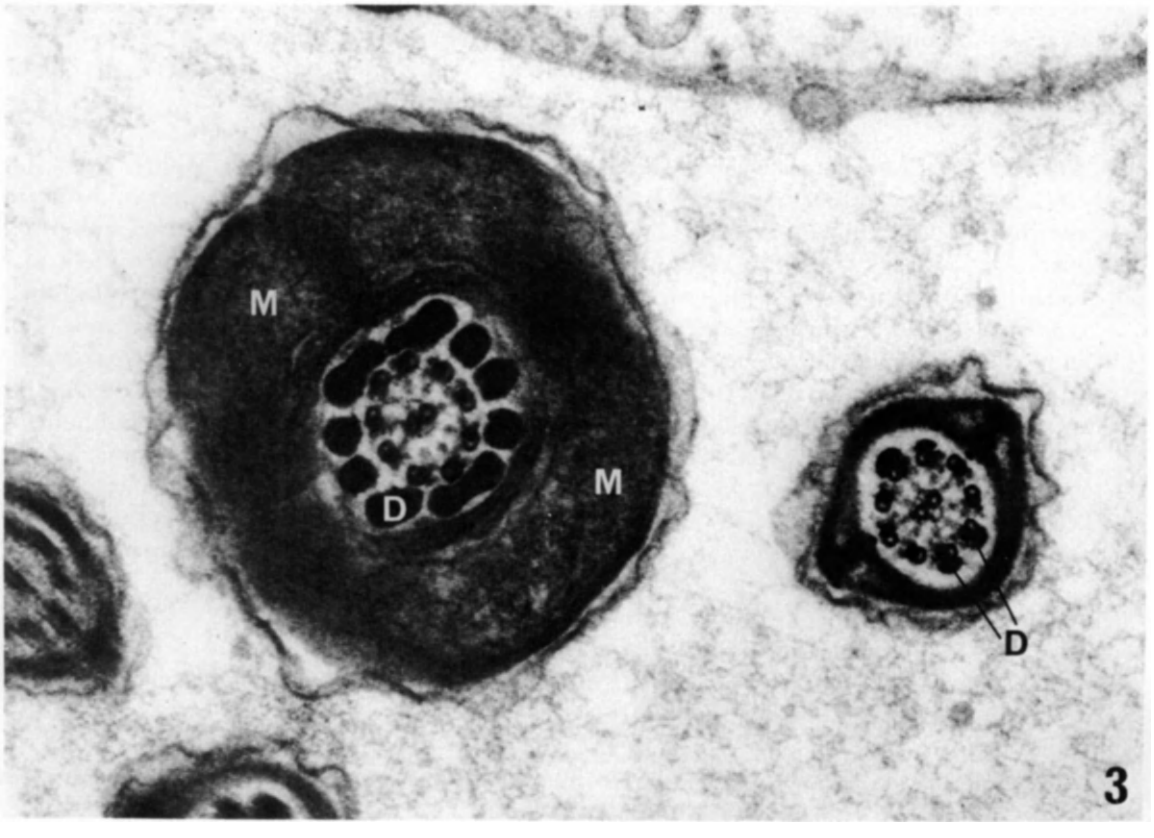
Fig. 1. Longitudinal section showing the head (H), neck (N) and middle piece with its gyres of helically arranged mitochondria (M), axial microtubule complex (A) and the outer dense rods of fibres (D). ×28 000

Fig. 2. Longitudinal section through the principal piece, showing the ribbed fibrous sheath (F), outer dense fibres (D) and the central microtubules of the axial microtubule complex (A). ×81 000

Fig. 3. Transverse sections through sperm tails. The one on the left, passing through the middle piece, shows the mitochondrial sheath (M), nine peripheral dense fibres (D) and the 9+2 microtubule complex. The one on the right passing through the distal part of the principal piece, shows only four dense fibres (D), the others having terminated higher up the tail. The outer and inner dynein arms and the spokes and spoke heads are discernible in the axoneme of the sperm tails (*for nomenclature see Plate 507, Fig. 2*). ×81 000


N
D
M
A
H
M
1


F
A
F
D
2


M
D
M
D
3

Single, primary or oligocilia

Electron microscopy has now revealed the presence of single cilia or a few cilia on a large variety of cells (*Plates 499–502*). However, these structures, whose function and significance still elude us, had been noted on a few cells with the light microscope, the first such report being by Zimmermann in 1898. In light microscopic studies and also in some early electron microscopic studies these structures were referred to as 'central flagella' or 'isolated flagella'. In later studies these structures have been called 'solitary cilia', 'single cilia' or 'primary cilia'. None of these terms is particularly suitable. It is clear now that in some examples of so-called 'single cilia', two or more cilia may be found on a single cell, while other cells in the same sample may show only a single cilium. Such variations reflect the difficulty of ascertaining the number of cilia per cell from a random ultrathin section, and also, no doubt, the biological variation in the number of cilia truly present.

The term 'primary cilia' was coined by Sorokin (1968) to describe cilia with a 9+0 structure which develop in fetal rat lung, long before the abundant 9+2 cilia form in the bronchial mucosa. Since then, some authors have tried to extend this term to cover various examples of single or few cilia seen in other tissues. This seems undesirable, for such a sequence of formation of primary (9+0) cilia followed by the formation of secondary (9+2) cilia has been demonstrated only in the lung. It should be noted that primary cilia as reported by Sorokin had a 9+0 structure. While this is true of many of the single or few cilia seen in other sites, one can also cite examples where 9+2 and other patterns of microtubular arrangement have been found (*see below* for further discussion on primary cilia).

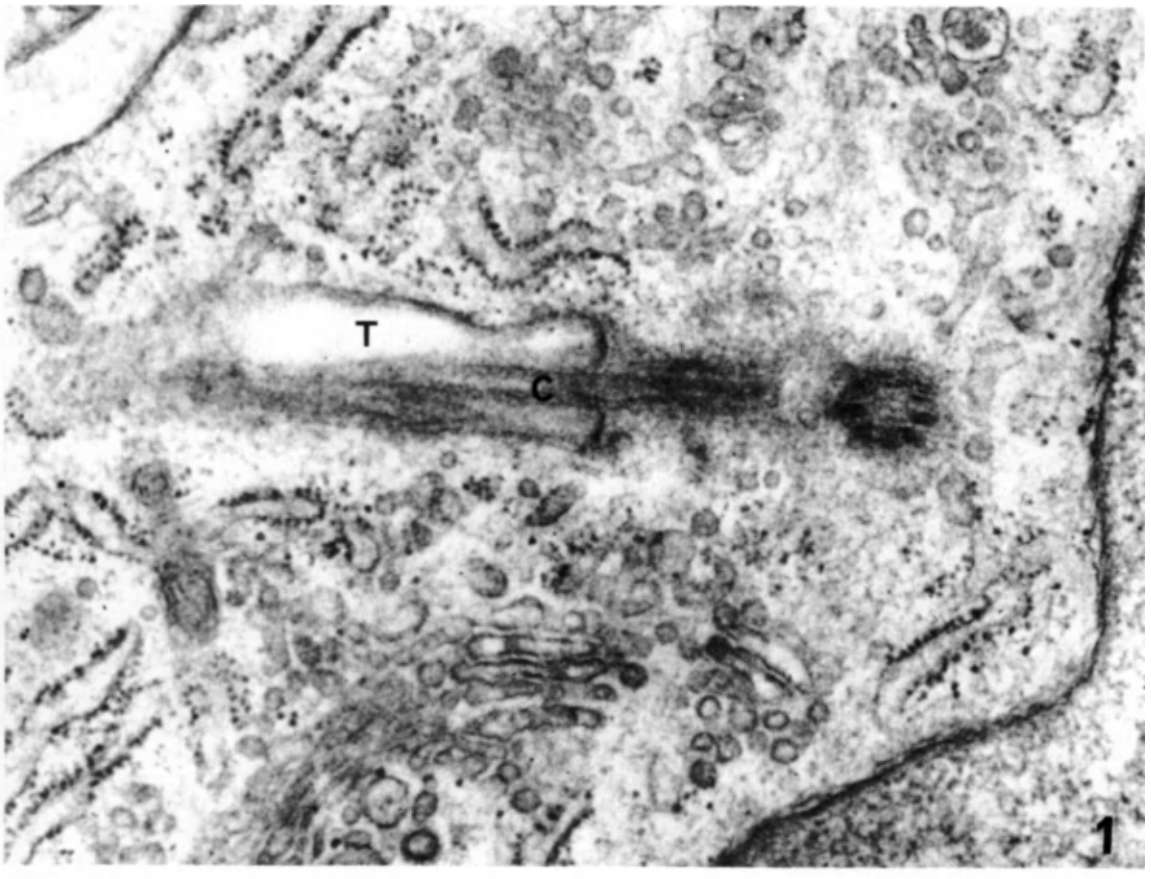
Indeed, the only consistent difference between the classic cilia of ciliated epithelia and the ones considered here is that, while in the former instance there are hundreds of cilia per cell, in the latter their numbers are quite small. Hence it seems appropriate to coin a new term 'oligocilia' (meaning 'few cilia') to describe them. Under this term shall be described various examples of single or few cilia, wherever found and whatever their structure.

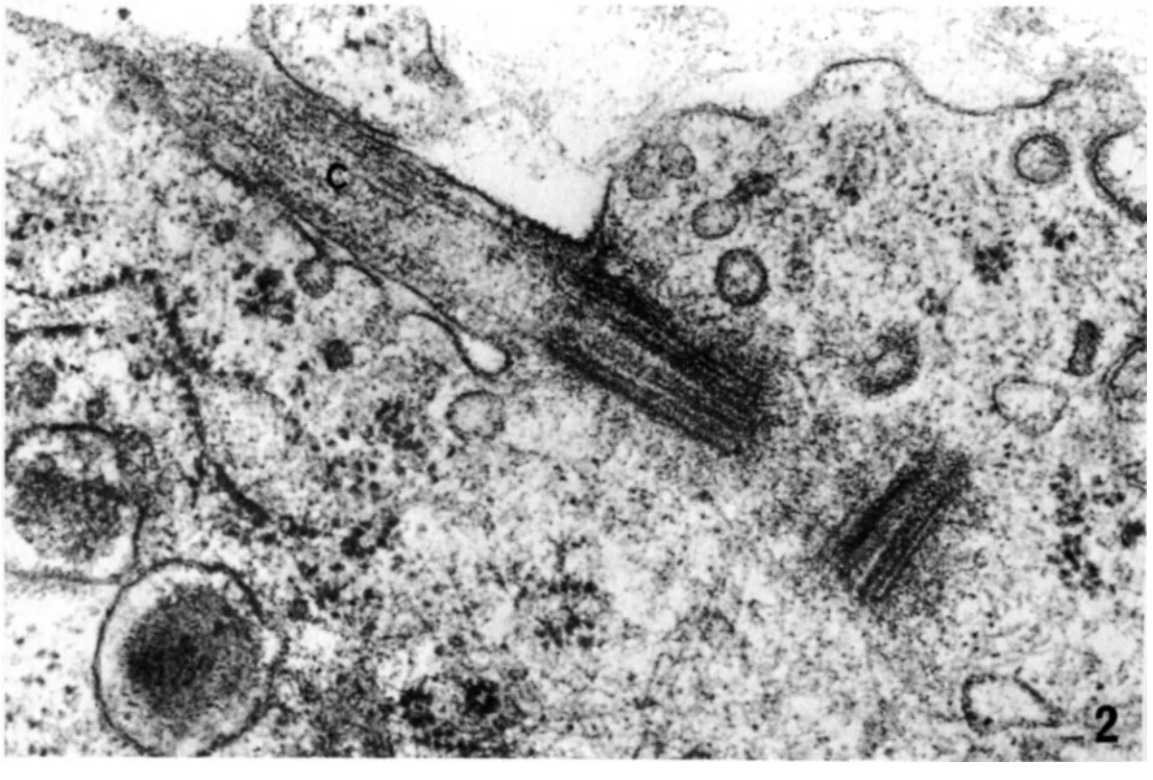
Oligocilia have now been observed in the cells of a wide variety of tissues and organs. These include: (1) mouse, rat, rabbit, dog and human adenohypophysis (Barnes, 1961; Salazar, 1963; Zeigler, 1963; Kagayama, 1965; Wheatley, 1967; Dingemans, 1969; Dustin *et al.*, 1979); (2) adrenals of rat and hamster (De Robertis and Sabatini, 1970; Propst and Muller, 1966); (3) chicken, dog and human thyroid (Kano, 1952; Fujita, 1963); (4) mouse, deer and human parathyroid (also human parathyroid adenoma) (Munger and Roth, 1963; Stoeckel and Porte, 1966; Black *et al.*, 1970; Roth, 1970; Altenähr and Seifert, 1971) (*Plate 502, Fig. 3*); (5) follicular epithelium of rat and guinea-pig ovary (Björkman, 1962; Adams and Hertig, 1964); (6) stromal cells of rat uterus (Tachi *et al.*, 1969); (7) rete testis of rat (Leeson, 1962); (8) seminal vesicles of mouse (Deane and Wurzelmann, 1965); (9) pancreas of chicken, mouse and rat (exocrine, endocrine and ductular cells) (Munger, 1958; Zeigel, 1962; Baradi and Brandis, 1969); (10) intrahepatic bile duct of normal rat, bat and humans (adult and embryo) and rat and humans with biliary obstruction (Steiner *et al.*, 1962; Grisham, 1963; Grisham and Porta, 1963; Steiner and Carruthers, 1963; Motta and Fumagalli, 1974; Grisham *et al.*, 1975; Tanuma and Ohata, 1978); (11) fat storing cells (Ito cells) in the liver of many vertebrates including humans (for references *see* Tobe *et al.*, 1985); (12) kidney tubules of mammals, including humans (Leeson,

Plate 499

Fig. 1. A human synovial cell showing a cilium (C) arising from one member of a centriolar pair. The cilium is seen lying in an obliquely sectioned tunnel (T) or vacuole. ×55 000 (*From Ghadially and Roy, 1969*)

Fig. 2. A chondrocyte from rabbit articular cartilage, showing a cilium (C) similar to that in *Fig. 1.* ×71 000


T
C
1


C
2

1960; Latta *et al.*, 1961; Myers *et al.*, 1966; Tisher *et al.*, 1966)*; (13) human fetal kidney (Zimmermann, 1971); (14) Wilm's tumour but not other human kidney tumours (Tannenbaum, 1971); (15) experimentally produced kidney tumours in hamsters (Mannweiler and Bernhard, 1957); (16) cultured monkey kidney cell infected with herpesvirus (*Plate 501, Fig. 3*); (17) renal cells from various nephropathies (Latta *et al.*, 1967; Rossmann and Galle, 1968; Larsen and Ghadially, 1974; Katz and Morgan, 1984; Lungarella *et al.*, 1984) (*Plate 500, Fig. 2*); (18) basal cells of human epidermis and basal cell carcinoma (Wilson and McWhorther, 1963; Elofsson *et al.*, 1984); (19) melanocytes in the epidermis (Flood and Totland, 1977); (20) human gingiva and oral mucosa of rat (Nikai *et al.*, 1970); (21) embryonic nervous tissue of mouse (Cohen and Meininger, 1987); (22) sensory cell of inner ear of guinea-pig (Wersall, 1956; Engstrom and Wersall, 1958); (23) choroid plexus of rabbit (Millen and Rogers, 1956); (24) guinea-pig, cat and human retina (Sjöstrand, 1953; De Robertis, 1956; De Robertis and Lasansky, 1958; Tokuyasu and Yamada, 1959; Allen, 1965); (25) a variety of cells from the nervous system of goldfish, rat, mouse, rabbit and humans (Palay, 1961; Taxi, 1961; Dahl, 1963; Grillo and Palay, 1963) (*Plate 501, Figs. 1 and 2; Plate 502, Fig. 5*); (26) fibroblasts (in culture and from various normal and pathological tissues) of chicken, mouse, rat, Chinese hamster and humans (Sorokin *et al.*, 1962; Schuster, 1964; Wilson and McWhorther, 1963; Stubblefield and Brinkley, 1966; Wheatley, 1969; Busuttil *et al.*, 1976) (*Plate 500, Fig. 1; Plate 502, Figs. 1, 2 and 4*); (27) meningiomas (Cervos-Navarro and Vazquez, 1966); (28) smooth muscle (Sorokin, 1962); (29) reticular cells and unidentified cells from the spleen of rat, rabbit and chicken and haemopoietic cells from vitelline sac of embryos (De Harven and Bernhard, 1956; Bernhard and De Harven, 1960; Roberts and Latta, 1964; Abdel-Bari and Sorenson, 1965; Breton-Gorius and Stralin, 1967); (30) chondrocytes from epiphyseal and articular cartilage of mouse, rabbit and dog† (Scherft and Daems, 1967; Wilsman, 1978) (*Plate 499, Fig. 2*); (31) guinea-pig, rabbit and human synovial cells (Wyllie *et al.*, 1964; Ghadially and Roy, 1969; Campbell and Callahan, 1971) (*Plate 499*, Fig. 1); (32) osteocytes in femoral cortical bone of mice (Tonna and Lampen, 1972); (33) pathologically altered human gastric mucosa (Okuda and Ogata, 1976; Kawamata *et al.*, 1986; Rubio and Kato, 1986; Rubio and Serck-Hanssen, 1986); (34) smooth muscle cells in experimentally produced rabbit aortic atherosclerotic lesions (Haust, 1984); (35) smooth muscle cells and endothelial cells from human aortic atherosclerotic lesions (Haust, 1987); (36) human ameloblastoma (Lee *et al.*, 1972); (37) Brenner's tumour of ovary (Klemi and Nevalainen, 1977); (38) serous cystadenoma of ovary (Fenoglio *et al.*, 1977); and (39) pineoblastoma (Kline *et al.*, 1979).

Certain generalizations have frequently been made regarding oligocilia. It has been said that these cilia are solitary or few, that they have a diplosomal basal organization (i.e. they arise from one member of a pair of centrioles comprising the diplosome), a 9+0 axial microtubule complex and that they lie in a vacuole or a tunnel or invagination of the plasma membrane. It will be recalled that the usual variety of cilia is numerous, has a single centriole as a basal body, a 9+2 axial microtubule complex and arises near the cell surface.

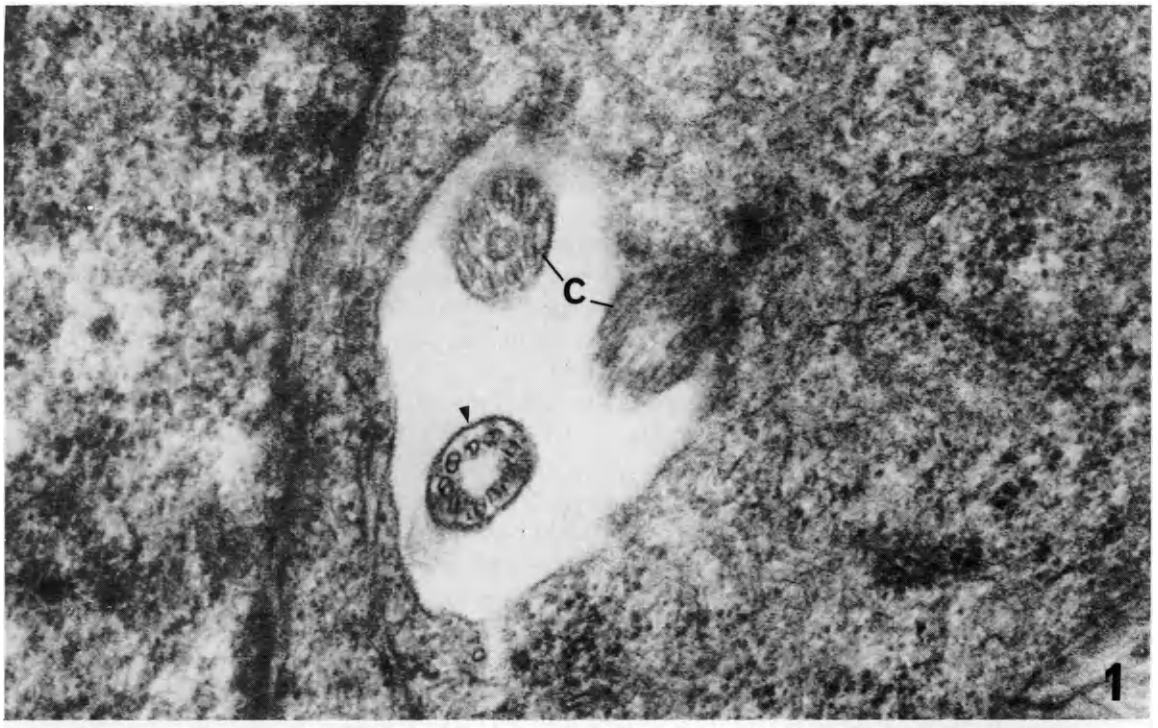
*Kidney tubules of many lower vertebrates are endowed with numerous cilia. They cannot be accepted as examples of oligocilia. (For a review and references, *see* Larsen and Ghadially, 1974.)

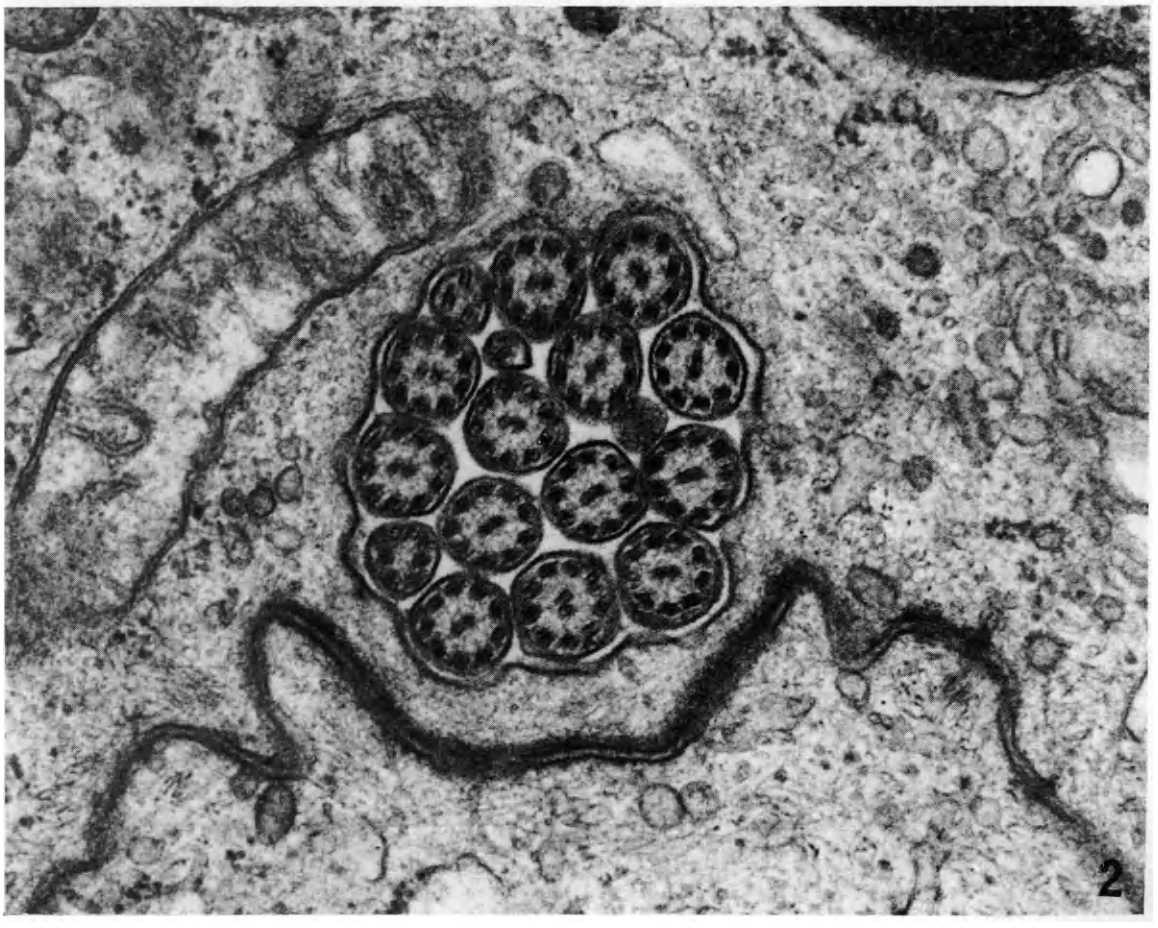
†The studies of Wilsman (1978) utilizing serial sections and transmission electron microscopy show that virtually every chondrocyte in neonatal and adult canine articular cartilage bears a 9+0 cilium.

Plate 500

Fig. 1. Evidence of more than one cilium per cell is seen here in a cultured mouse fibroblast. In a vacuole or transversely sectioned tunnel or invagination are seen the bases of two cilia (C) cut obliquely. The transverse section of the cilium with the 9+0 structure (arrowhead) could be a resectioning of a cilium folded back on itself or a third example. ×60 000 (*Wheatley, unpublished electron micrograph*)

Fig. 2. Some 15 or more sections of cilia (9+2 structure) are seen within a traversely sectioned tunnel lying within a human kidney tubular epithelial cell. From a case of lupus nephritis. ×60 000 (*From Larsen and Ghadially, 1974*)


C
1


2

Exceptions and amplifications of the generalizations made above are as follows. Firstly, regarding the number of cilia per cell, one may observe that between the two extremes of single cilia and the hundreds of cilia per cell in typical ciliated epithelia lie examples where some cells with two (Stubblefield and Brinkley, 1966; Roth, 1970) or three or four (Millen and Rogers, 1956; Wheatley, 1969) cilia per cell have been seen, and in *Plate 500, Fig. 2*, is shown an example where it would appear that 15 or more cilia are arising from a human kidney tubular epithelial cell.

It is worth noting that, while many oligocilia have shown a 9+0 pattern, there are examples where the familiar 9+2 pattern has been observed (e.g. Wersall, 1956; Millen and Rogers, 1956; Grisham and Porta, 1963). In the neurons of the rat, Dahl (1963) observed cilia which near the base showed the 9+0 pattern but higher up along the shaft one of the doublets was displaced to the centre (*Plate 502, Fig. 5*). An 8+1 pattern was noted by Munger and Roth (1963) for the cilia in parathyroid gland, and Stoeckel and Porte (1966) reported that the peripheral microtubular pairs may vary from 6 to 9. In pathological renal tissue the commonest type of cilium encountered is the 9+2 cilium, but rare examples of 9+0, 9+1, 8+0, 8+1, 8+2 and 7+2 cilia have also been reported to occur (for references, *see* Larsen and Ghadially, 1974). In some studies microtubular complexes were found to be completely or almost completely lacking in the oligocilia (Sorokin, 1962; Adams and Hertig, 1964; Scherft and Daems, 1967). In many studies the microtubular pattern is not reported simply because the number of cilia seen was too few and transversely sectioned shafts were not encountered.

Reports of oligocilia arising from a single centriole or basal body rather than one member of a pair of centrioles may also be found in the literature. In some instances, particularly when but a single cilium has been seen, it seems highly probable that this was due to one member of the pair of centrioles not being included in the section rather than being truly absent. Where two cilia have been seen it is at times evident that both members of the diplosome have formed cilia.

Oligocilia often, but not invariably, appear to lie in a vacuole or a tunnel. It is conceivable that in some instances the 'vacuole' is no more than an oblique section through an invagination of the plasma membrane communicating with the exterior. In other instances, however, it is likely to be a stage in the growth of the cilium, for a vesicle is known to be associated with early stages of ciliary development in many sites (Sotelo and Trujillo-Cenóz, 1958).

The function and significance of most oligocilia remain obscure*. Since often the two central microtubules of the complex are absent, it is assumed that these cilia are not motile. In fact, proof regarding the motility of virtually all oligocilia is lacking, for they have been visualized in

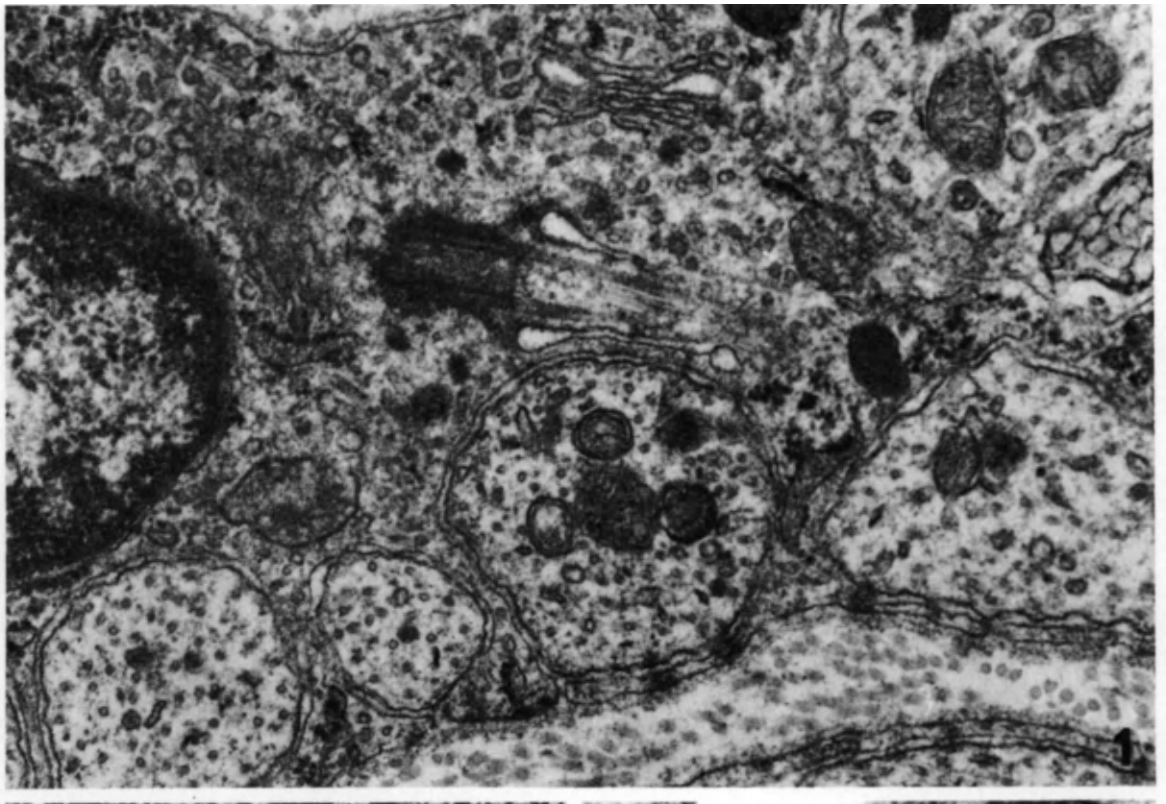
*However, the function of some of these cilia is well established. For example, the 9+0 cilium of the vertebrate retinal rod cell has a photoreceptor function. In certain lower animals, rare examples of 9+2 and 8+1 cilia with photoreceptor function are also known to occur. The solitary 9+2 cilium on the sensory cells of the inner ear is considered to have a mechanoreceptor function. (*See* Afzelius, 1969, for further examples and references.)

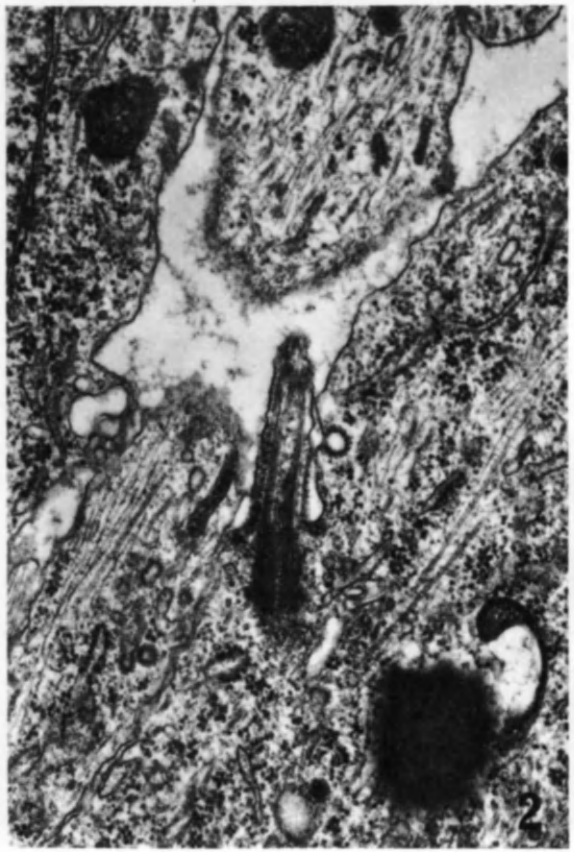
Plate 501

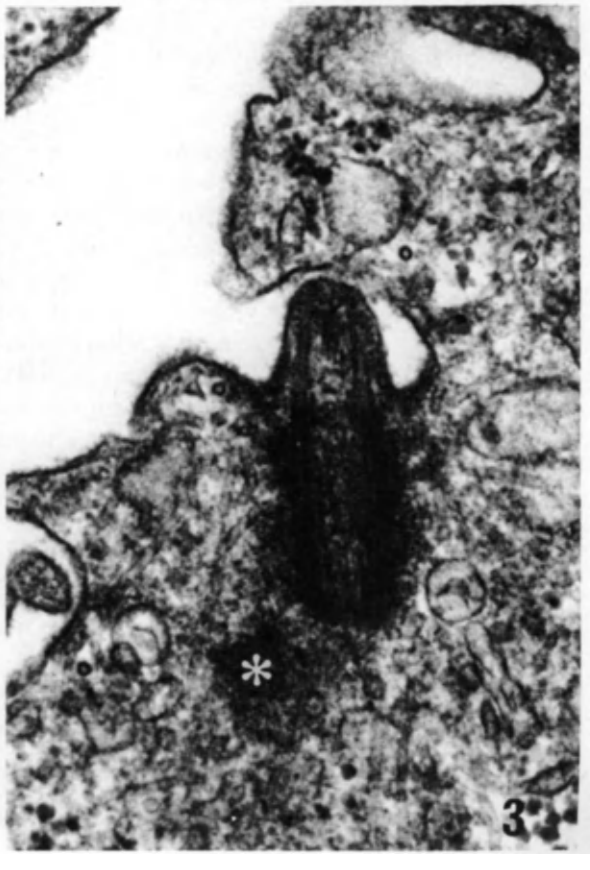
Fig. 1. A cilium found in a Schwann cell from the superior cervical ganglion of a 32 day old mouse. ×45 000 (*Newstead, unpublished electron micrograph*)

Fig. 2. A cilium found in a principal neuron from a four day old culture of superior cervical ganglion from a 20 day old fetal mouse. ×32 000 (*Newstead, unpublished electron micrograph*)

Fig. 3. A small superficial cilium found in a cultured monkey kidney cell infected with herpesvirus. Only one centriole forming the basal body of the cilium is clearly seen but the presence of the other member of the pair can just be discerned (★). ×59 000







electron micrographs and not in living cells. An exception to this are the oligocilia produced in cultured fibroblasts exposed to Colcemid. Here Stubblefield and Brinkley (1966) found with the phase-contrast microscope that a few of the cilia were beating erratically.

Munger (1958) proposed a chemoreceptor function for cilia in endocrine organs, while the absence of the central microtubules led Barnes (1961) to conclude that oligocilia may have a sensory function. However, this view was not supported by Grillo and Palay (1963), who recalled that 'most of these examples do not involve known receptor cells', and by Sorokin (1962), who remarked that he could see 'no compelling reason for assigning a sensory function to these structures'.

From their study of cilia in the rat nephron, Latta *et al.* (1961) suggested that 'these structures may be viewed as an evolutionary remnant because cilia are found much more frequently in the excretory ducts of lower animals'. Similarly, Grisham and Porta (1963) and Grisham (1963) have pointed out that biliary epithelial cells of several non-mammalian vertebrates bear cilia and that their rare occurrence in rats and humans 'may indicate a reversion to a more primitive condition'.

In some instances, such as thyroid follicular cells in the fowl and the oral epithelium of the rat (Fujita, 1963; Nikai *et al.*, 1970), oligocilia have been noted to be more abundant in young or embryonic tissues as compared to the corresponding tissue in the adult, but in mouse osteocytes (Latta *et al.*, 1961; Tonna and Lampen, 1972), the converse appears to be the case. Hence the often expressed idea that these cilia are primitive or embryonal structures deserving to be called primary cilia (in analogy with the situation in the lung) is not supported by available evidence.

Ciliation has been induced in: (1) cultured fibroblasts treated with Colcemid or cytochalasin B (Stubblefield and Brinkley, 1966, 1967; Krishan, 1971); (2) neural and glial cells of cat brain by pargyline (Milhaud and Pappas, 1968); and (3) sea-urchin embryos by a variety of agents (Lallier, 1964). On the basis of such observations, Milhaud and Pappas (1968) have suggested that 'stimulation of centriolar reproduction without subsequent mitosis may lead to ciliary formation'. A negative correlation between mitosis and cilia production is also thought to occur in the adenohypophysis. Oligocilia are, relatively speaking, of fairly common occurrence in this region, but it is said that they are of less frequent occurrence in experimental situations where mitotic activity is enhanced, and Dingemans (1969) explains the situation by stating that 'centrioles cannot simultaneously be involved in mitosis and constitute a part of the basal structure of a cilium'. However, Peterson and Berns (1980) state that 'the centriole can serve both functions at the same time' and they quote an unpublished observation by Jensen and Rieder who found that 'some PTK cells in culture may have a cilium growing out of one of the centrioles at the pole while the cell is in mitosis'.

As stated earlier, no unifying hypothesis regarding the significance of these cilia has been proposed and their function, if any, is unknown. The collective evidence, however, indicates that the ability of centrioles to form cilia exists and persists in virtually all cells, and that they can be provoked to produce cilia by a variety of natural and experimental stimuli.

Plate 502

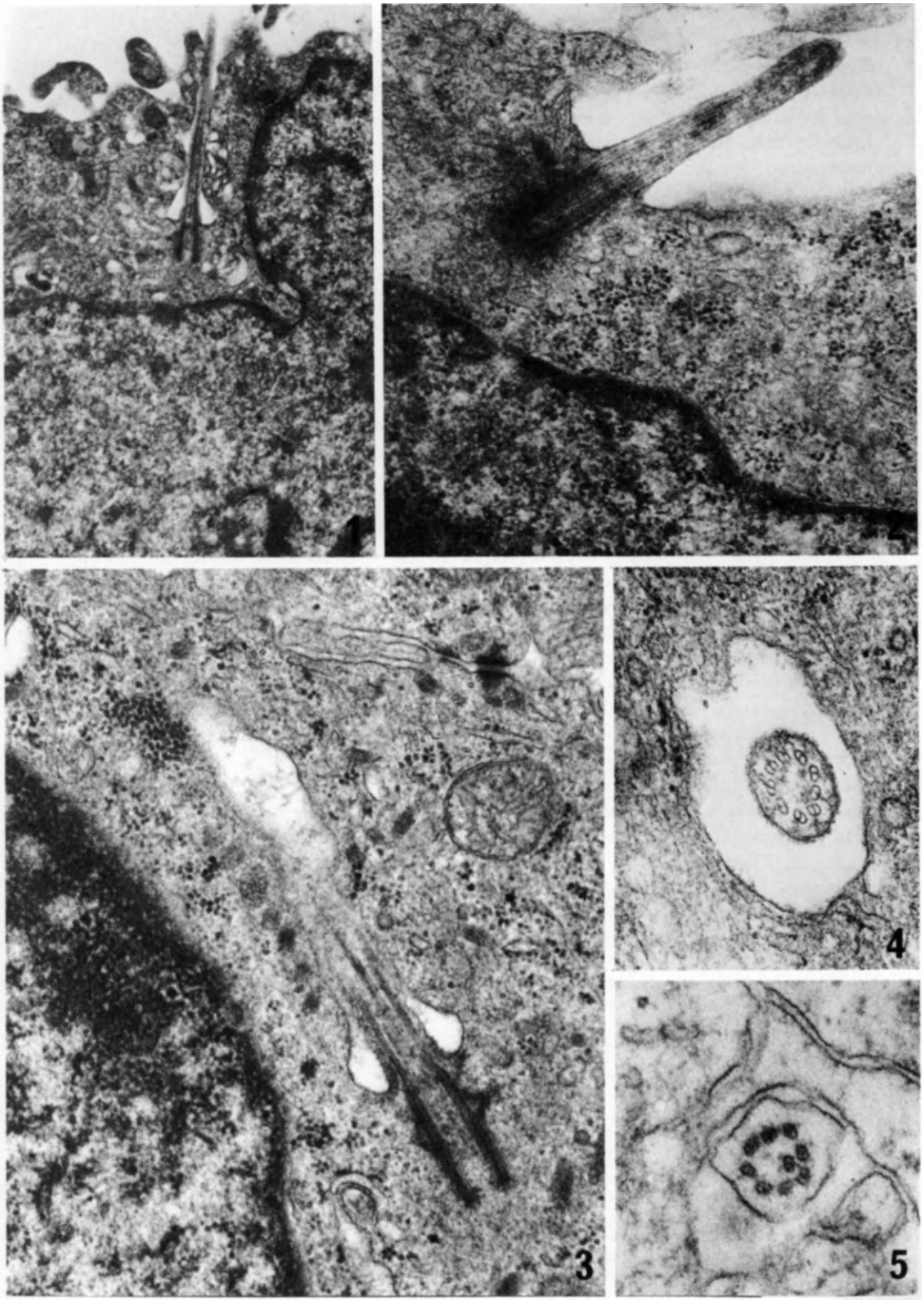
Fig. 1. A fibroblast in culture, showing a cilium lying at the bottom of a deep invagination. ×16 000 (*Wheatley, unpublished electron micrograph*)

Fig. 2. A fibroblast in culture, showing a cilium arising from a centriole near the surface of the cell. ×38 000 (*Wheatley, unpublished electron micrograph*)

Fig. 3. A cell from a human parathyroid adenoma, showing a cilium lying in a characteristic vacuole or invagination. ×41 000 (*Larsen and Ghadially, unpublished electron micrograph*)

Fig. 4. Transverse section of a 9+0 cilium from a mouse fibroblast in culture. ×65 000 (*Wheatley, unpublished electron micrograph*)

Fig. 5. A cilium from the rat cerebral cortex showing eight peripheral doublets. The ninth doublet is displaced towards the middle. ×75 000 (*From Dahl, 1963*)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Atypical cilia

Atypical or pathologically altered cilia have been seen in the ciliated epithelium of the respiratory passages and also in a few other tissues. For descriptive purposes such altered cilia may be divided into five main groups: (1) compound cilia, that is to say, cilia containing multiple axial microtubule complexes set in a common matrix and enclosed in a single membrane (*Plate 503* and *Plate 504, Figs. 1 and 2*); (2) swollen cilia, where the prominent feature is an abundance of matrix as compared with the normal state (*Plate 504, Fig. 3*); (3) cilia showing various fine alterations* of structure such as disorganization and/or loss of some of the microtubules from the complex (Ailsby and Ghadially, 1973), vesiculation of the ciliary membrane (Friedmann and Bird, 1971; Denholm and More, 1980); (4) intracytoplasmic cilia where the axial microtubule complex is deviated and follows an intracytoplasmic course rather than emerging as a free cilium from the surface (Wong and Buck, 1971; Larsen and Ghadially, 1974) (*Plate 504, Fig. 4*); and (5) cilia with atypical basal bodies†. Needless to say, such categories are not clear-cut, and examples of cilia showing more than one of the above-mentioned morphological alterations may be found.

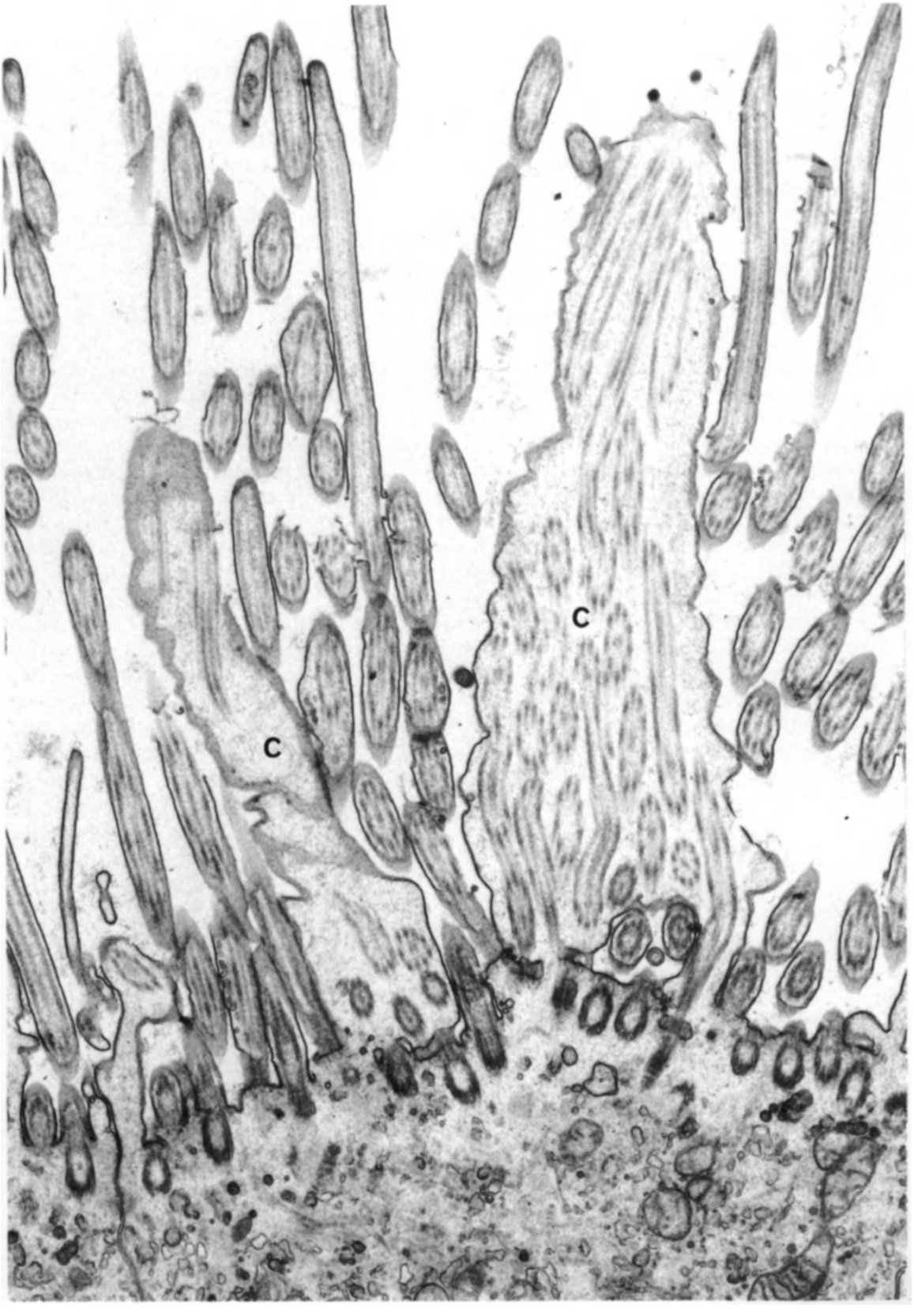
It is worth noting that the term 'compound cilia' has been used to describe the membranelle (composed of rows of cilia) and the cirrhus (a collection of cilia resembling a water-colour brush) of protozoa. In these instances the cilia are set in a common matrix (extracellular) to form what is called a 'compound motile organelle' (Fawcett, 1961). Such cilia beat in unison as if 'fused'. However, the individual cilia in these so-called 'compound cilia' can be separated by microdissection (Afzelius, 1969). It seems to me that these structures should be called compound motile organelles, as suggested by Fawcett, and not compound cilia so as to avoid confusion with cilia more deserving this appellation such as those shown in *Plate 503*. In order to avoid confusion some authors refer to such compound cilia as giant cilia. This term, however, is unsuitable for it does not indicate the main feature of this change, namely the presence of multiple axial microtubule complexes within a single ciliary sheath, but stresses the size, which can be quite variable. For example, many compound cilia contain only two or three axial microtubule complexes (*Plate 504, Fig. 1*) and are not particularly large. These can hardly be accepted as giant cilia.

Compound cilia (i.e. cilia containing multiple axial microtubule complexes within a single ensheathing ciliary membrane) have been found in: (1) epithelium lining of colloid cyst of the third ventricle (Coxe and Luse, 1964); (2) human malignant polycystic teratoma of the ovary (Luse and Vietti, 1968); (3) human nasal papilloma (Gaito *et al.*, 1965); (4) human ovarian carcinoma (*Plate 504, Fig. 2*); (5) bronchial mucosa of a heavy smoker with bronchial carcinoma (Ailsby and Ghadially, 1973) (*Plate 503*); (6) antral mucosa of maxillary sinuses in allergic conditions and ventricular mucosa of the human larynx (Friedmann and Bird, 1971); (7)

*Alterations of the axoneme producing the immotile cilia syndrome are discussed on pages 1196–1200.
†Atypical basal bodies and centrioles are dealt with on pages 1194 and 1195.

Plate 503

From the bronchial mucosa of a man who had smoked 25 cigarettes per day over a period of 46 years and developed a bronchial carcinoma. The cilia shown here were found in a segment of apparently normal-looking mucosa collected well away from the tumour site. This electron micrograph shows two compound cilia (C) containing transversely and longitudinally sectioned axonemes and numerous normal-looking cilia sectioned in various planes. ×22 000 (*From Ailsby and Ghadially, 1973*)


C
C

middle-ear mucosa of a deaf patient and an adult guinea-pig (Kawabata and Paparella, 1969); (8) embryonic chick otocyst in culture (Friedmann and Bird, 1971); (9) tracheal epithelium of the fowl infected with laryngotracheitis virus (Purcell, 1971); (10) human endometrial epithelium (Denholm and More, 1980), and (11) main excretory duct of human submandibular gland in cases of obstructive sialadinitis (Riva *et al.*, 1987).

In many of the above-mentioned instances only one or a few compound cilia with 2–4 axial microtubule complexes were seen. The really large compound cilia have been seen mainly in the respiratory passages and in some tumours.

For example, in the bronchial mucosa of the patient reported by us (Ailsby and Ghadially, 1973) there were innumerable compound cilia containing 2–4 axial microtubule complexes. The largest compound cilia seen in transverse section probably contained about 27 axial microtubule complexes (*see Fig. 2* in Ailsby and Ghadially, 1973). Moreover, it was often observed that the microtubules within some of the complexes were disarranged. In other instances the central pair of microtubules was absent (9+0 pattern). Absence of the central microtubules was also quite frequently observed in otherwise normal-looking cilia. Failure to visualize these microtubules did not appear to be due to obliquity of sectioning or poor fixation, for peripheral microtubules were well resolved, and numerous cilia with the normal 9+2 pattern were also present, in close proximity to those showing a 9+0 organization.

Another somewhat rare abnormality was the occurrence of moderately to markedly swollen cilia containing only a single axial microtubule complex but more than the usual amount of matrix (*Plate 504, Fig. 3*). Swollen cilia have been produced in the gill tissues of marine bivalves (*Mytilus edulis*) by subjecting them to high pressure (700 atmospheres). This produces a large oedematous expansion of the cilium followed by a disintegration of the microtubules of the axoneme (Fritsch, 1973). Swollen cilia of a somewhat different morphology have been reported by Duncan and Ramsey (1965) in the porcine nasal mucosa in *Bordetella-bronchiseptica*-induced rhinitis (*Plate 505*). In this example the swelling affected the distal part of the ciliary shaft so that an appearance akin to a balloon on a string, or a table tennis racket, was created. The magnitude of the swelling was also quite remarkable, the swollen portion of the cilium measuring approximately $1.5 \times 2.2\,\mu m$. The axial microtubule complex showed the characteristic 9+2 arrangement in the relatively unaltered stem of the cilium, but became disorientated and coiled in the distal swollen portion of the cilium.

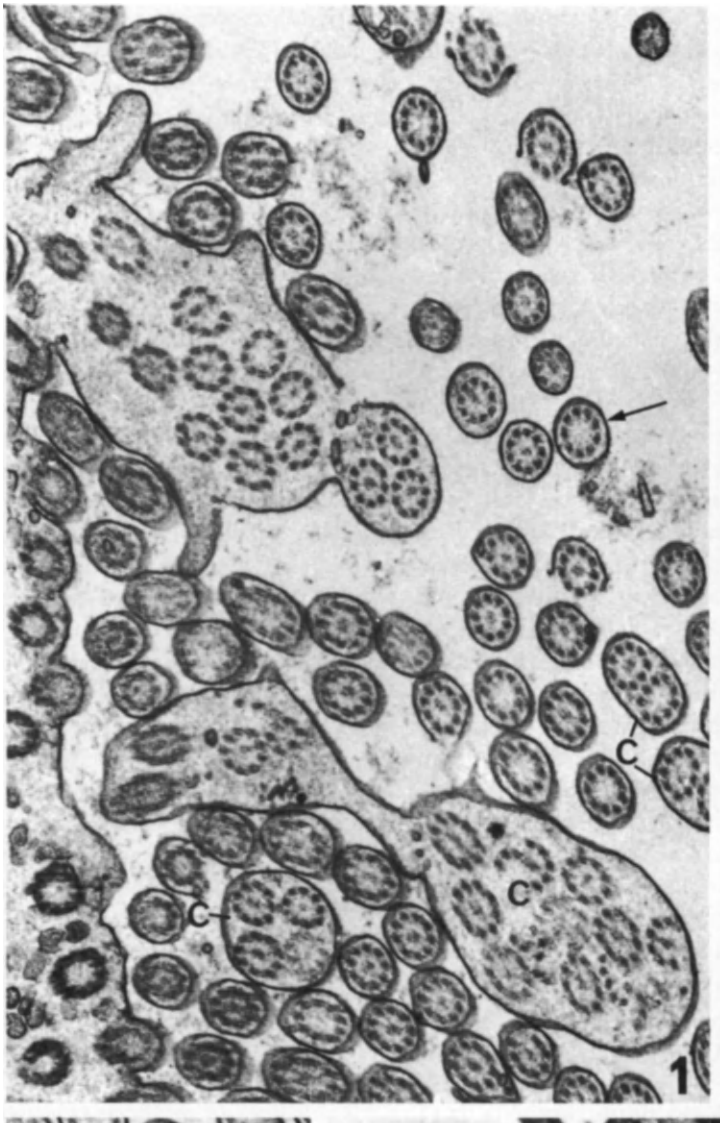
Plate 504

Fig. 1. Compound cilia (C) containing two or more axial microtubule complexes are seen here, as are numerous normal-looking cilia and some (arrow) where the central microtubules are missing. Note also the missing central microtubules and disorganized complexes in the compound cilia. From the same case as *Plate 503*. ×25 000 (*Ailsby and Ghadially, unpublished electron micrograph*)

Fig. 2. A compound cilium seen in a tumour cell obtained from a malignant ascitic effusion. From a case of ovarian carcinoma. ×29 000

Fig. 3. A transversely cut swollen cilium. There is an excess of matrix but only a single axial microtubule complex is present. From the same case as *Plate 503*. ×34 000 (*From Ailsby and Ghadially, 1973*)

Fig. 4. A ciliated cell from the bronchial mucosa of a vitamin-A-deficient rat, showing intracytoplasmic cilia. A deviated axial microtubule complex (C) and attached basal body (B) are clearly seen in the cytoplasm. ×22 000 (*From Wong and Buck, 1971*)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C
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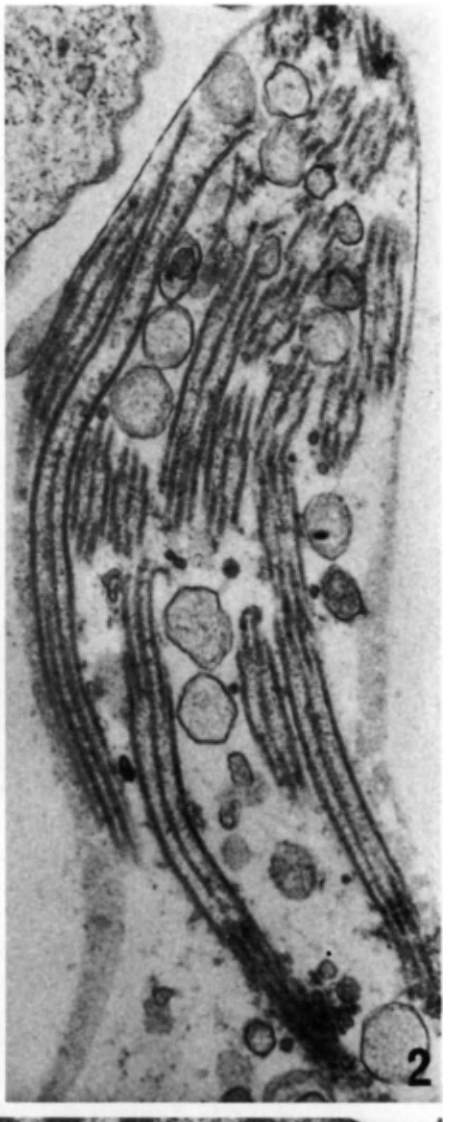
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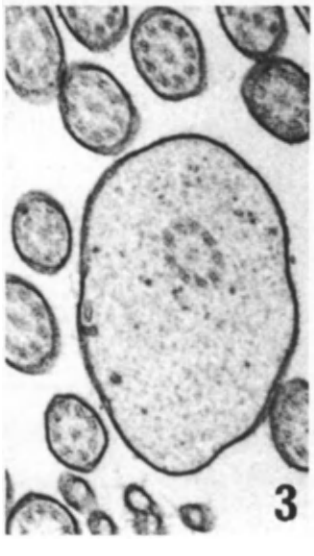
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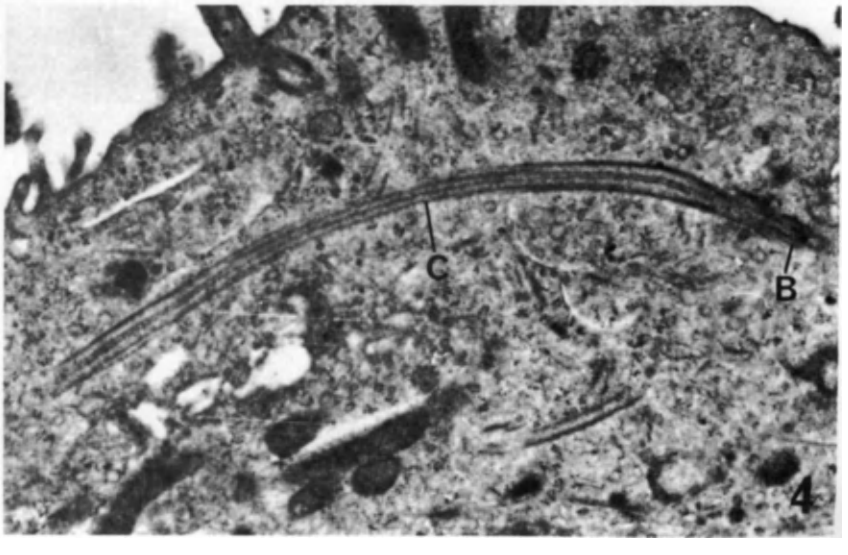
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B
4

The significance and mechanism of production of swollen cilia are not evident, but it would seem that such cilia may be functionally incompetent, for the axial microtubule complex may be inadequate to move the increased mass of the swollen cilium effectively.

Little is known about the manner in which compound cilia are produced, but one could propose that they are either produced by a fusion of pre-existing cilia, or arise as a result of multiple axial microtubule complexes entering a single large evagination of the plasma membrane. Whether such cilia are capable of beating is also not known. In the large compound cilia seen by us, in the bronchial mucosa, the haphazardly distributed and poorly orientated axial microtubule complexes do not inspire confidence in the ability of such cilia to assist in the movement of the mucus blanket over the bronchial epithelium.

It has long been known that the ciliated epithelium of the respiratory tract may be damaged and undergo squamous metaplasia in a variety of chronic inflammatory states and in vitamin A deficiency (Straub and Mulder, 1948; Wilhelm, 1954; Wong and Buck, 1971). It has also been established that various noxious agents such as cigarette smoke, sulphur dioxide, formaldehyde and ammonia impair the movement of the mucus blanket (mucus-escalator) over the respiratory epithelium, by altering the viscosity of the mucus and causing a disturbance or paralysis of the ciliary beat (Hilding, 1956; Falk *et al.*, 1959; Kensler and Battista, 1963, 1966; Dalhamn, 1964). Light microscopic studies failed to show any overt morphological changes in the cilia themselves, but the electron microscope shows that many and varied are the morphological changes that can occur in the cilia of the respiratory passages. It would therefore appear that pathologically altered cilia may constitute yet another factor responsible for the sluggish movement of mucus and the pulmonary pathology which results from an impairment of this vital cleansing mechanism which helps to rid the respiratory tract of irritants, carcinogens and pathogenic organisms.

In the foregoing discussion attention has been concentrated on morphologically altered cilia. It is, however, worth noting that the various noxious agents which damage the respiratory epithelium also produce a widespread destruction and loss of cilia. One of the questions that has been asked is whether such damage can be repaired by the production of new cilia by the surviving deciliated cell.

According to Burian and Stockinger (1963) and Burian (1966), regeneration of cilia does occur after deciliation by various chemical agents, but Hilding (1965) reported that, in the calf trachea, deciliated cells produced by mild mechanical trauma were exfoliated within about one hour after injury, and were later replaced by immature cells from the deeper layers which then proceeded to form cilia. A similar experiment was performed on rabbits by Hilding and Hilding (1966) and they stated that, 'Removing cilia from rabbit cells by our methods resulted in so much disruption that it seems unlikely that the remaining fragments would survive'.

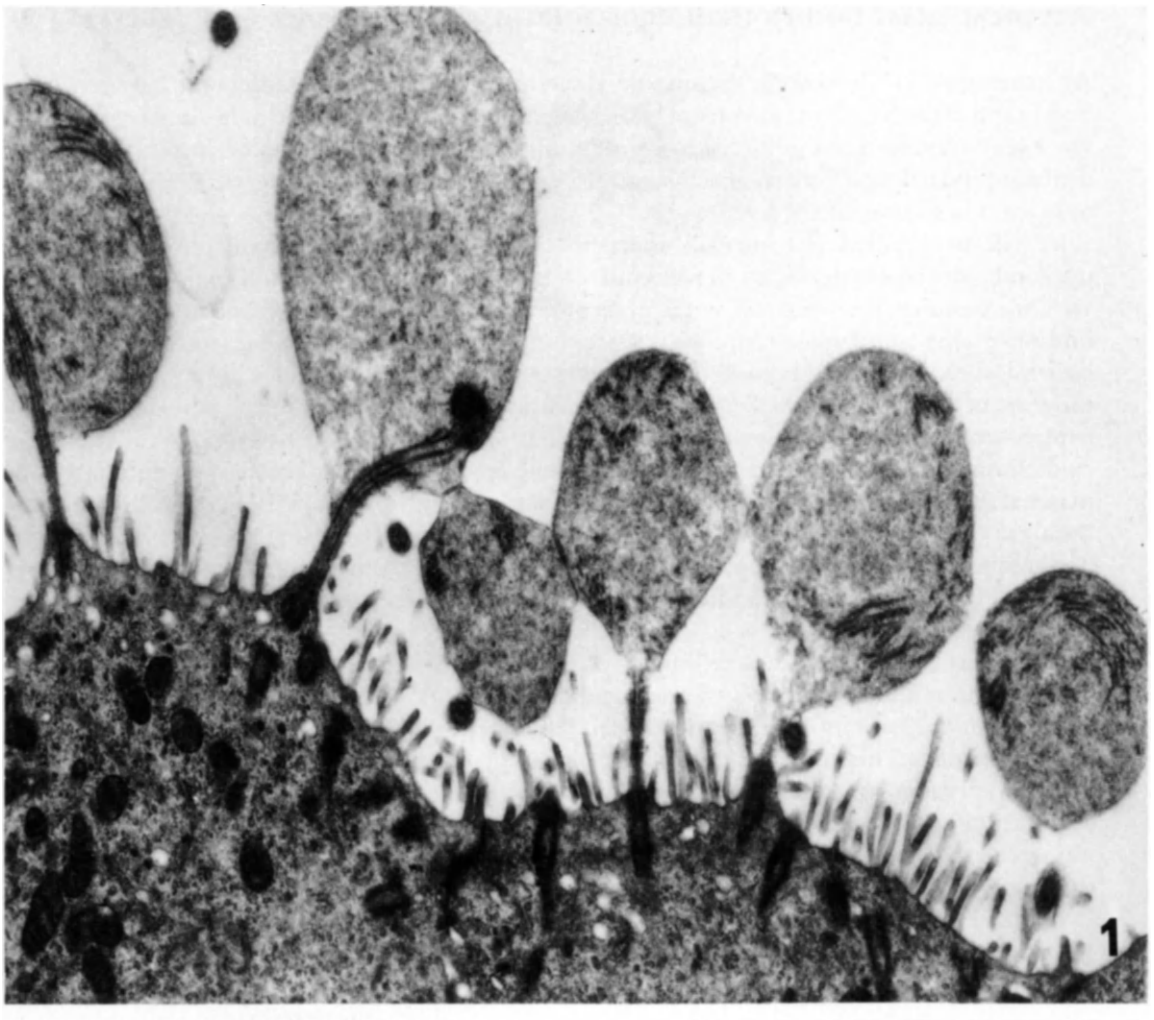
It would appear that there is as yet no compelling reason to believe that cilia destroyed by pathological agents can regenerate. Indeed, in view of the known rapid turnover of cells in most epithelia, one would imagine that a deciliated cell would be discarded and replaced by another cell.

Plate 505

Swollen cilia from the nasal mucosa of a pig with experimentally produced *Bordetella bronchiseptica* rhinitis. (*From Duncan and Ramsey, 1965*)

Fig. 1. Section through swollen cilia, showing the swollen distal portion and the normal-looking proximal portion of the ciliary shaft. ×18 000

Figs. 2 and 3. Coiling of the axial microtubule complex within the swollen portion of the cilia. ×44 000; ×49 000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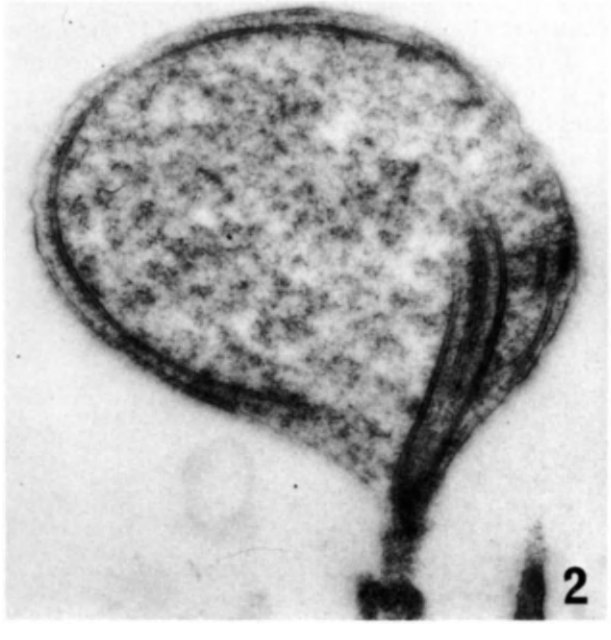
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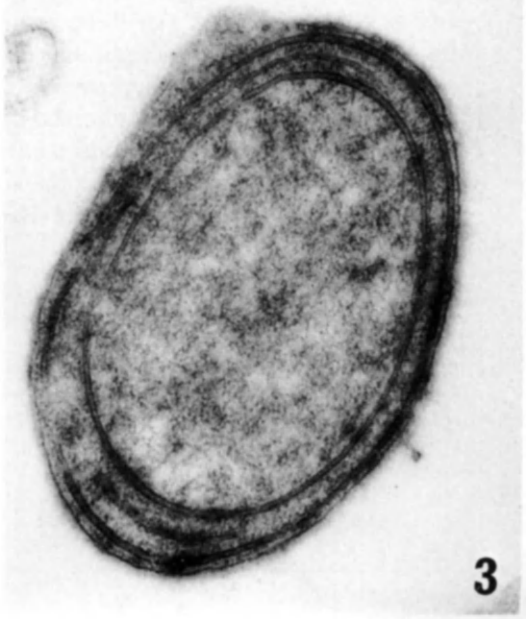
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Atypical basal bodies (half centrioles, giant centrioles and others)

As mentioned in Chapter 2, there is no discernible morphological difference between basal bodies and centrioles, and often basal bodies are referred to as centrioles in the literature (but not *vice versa*), therefore one is forced to consider them together. These bodies show a remarkable degree of constancy in structure and location, the few exceptions to this generalization form the topic of this section of the text.

It will be recalled (Chapter 2) that the classic basal body or centriole contains nine microtubular triplets deployed in a circular fashion, but in certain atypical centrioles this is not so. For example: (1) in ciliated thymic cysts of nude mice (Cordier, 1974) and in human cases of immotile cilia syndrome (Afzelius, 1983; Lungarella *et al.*, 1985) structures called 'half centrioles' (actually basal bodies) have been described. The half centrioles show a semi-circle of three to six microtubular triplets; (2) in the tracheal mucosa of a guinea pig a basal body with 14 triplets arranged in an S-shaped configuration has been seen (Dalen, 1981); (3) in the bronchial epithelium of a dog with immotile cilia syndrome an expanded basal body with homogeneous material in the centre surrounded by 9 or 10 triplets was seen (Afzelius, 1983); (4) in a mutant of the algae *Chlamydomonas rheinhardtii* basal bodies with a ring of nine singlets (instead of triplets) has been seen (Goodenough and St Clair, 1975); and (5) in the male-sterile mutant of the fern, *Ceratopteris thalictroides* basal bodies with disorganized triplets have been found (Duckett *et al.*, 1980).

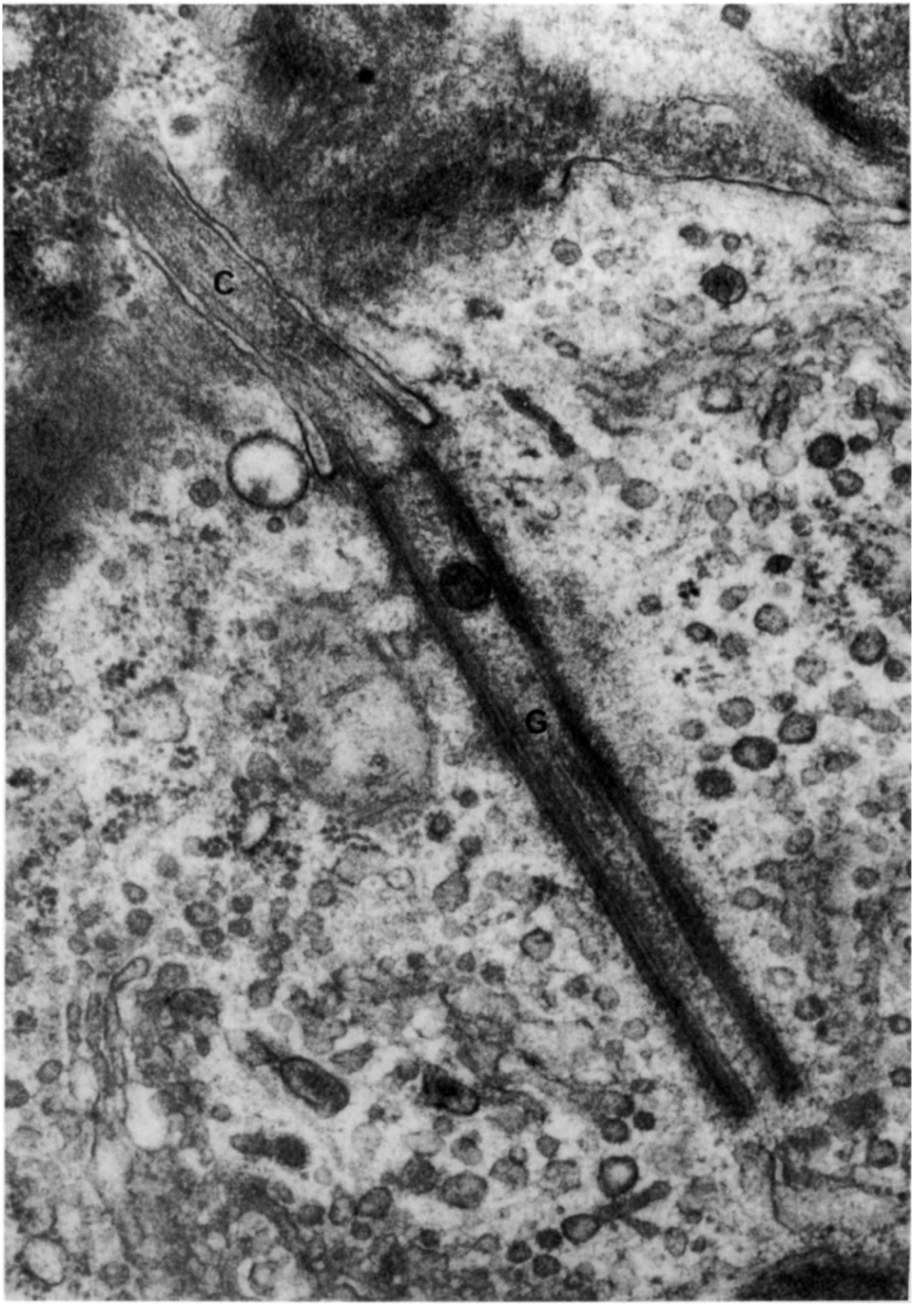
Centrioles and basal bodies in human cells are about 0.4 μm long and 0.25 μm in external diameter. In a lupus kidney we (Larsen and Ghadially, 1974) found a giant centriole or basal body about 0.22 μm in diameter and about 2 μm long (i.e. about five times the length of the usual centriole). This seems to be the only human giant centriole that has been reported in the literature. It lay in an unidentifiable cell in a zone of markedly disorganized renal tissue adjacent to a pathological glomerulus. We presume that this is a renal tubular epithelial cell because numerous 9+2 cilia and intracytoplasmic cilia were found in the renal tubular epithelial cells in this specimen.

Giant centrioles measuring up to 8 μm in length occur in testes of neuropteran insects (Friedlander and Wahrman, 1966). These giant centrioles develop in the meiotic prophase, whereas only minute centrioles are found in spermatogonia. Because of their large size giant centrioles are easily detected with the light microscope. They were described in lepidopterans as early as 1897 by Meves. Friedlander and Wahrman (1966) review this old literature and point out that giant centrioles have been seen in: (1) maturing germ cells (usually male, sometimes female) of invertebrates and vertebrates; (2) protozoa; (3) algae; and (4) fungi.

The reason why germ cells of certain species have such large centrioles is obscure, but clearly this is a physiological situation. In contrast to this, the giant centriole in lupus kidney can only be described as pathological. No theory has been advanced to explain why giant centrioles develop, what purpose they serve or what function they perform.

Plate 506

A cilium (C) is seen arising from a giant (G) centriole or basal body. ×64 000 (*From Larsen and Ghadially, 1974*)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Immotile cilia syndrome

In 1975, two reports described the occurrence of flagellar mutants in sterile men. They had live but immotile spermatozoa (Afzelius *et al.*, 1975; Pedersen and Rebbe, 1975). Ultrastructural studies show that paralysis of the sperm tail was due to a defect in the axoneme, namely the lack of dynein arms on the A-microtubules of the peripheral doublets. Further studies (Afzelius, 1976; Eliasson *et al.*, 1977; Pedersen and Mygind, 1976) showed that the cilia in the respiratory tract were also defective in that they lacked dynein arms. This produces the immotile cilia syndrome which is characterized by extremely slow mucociliary clearance with respiratory tract disease in childhood and sterility* in the adult male. (*Plates 507* and *508*.)

The term immotile cilia syndrome has been criticized on the grounds that in some cases the cilia are not totally immotile but show poor or defective motility (Pedersen and Mygind, 1980; Rossman *et al.*, 1980; Rutland and Cole, 1980). Therefore, various alternative terms have been suggested such as 'ciliary dysfunction syndrome', 'ciliary dyskinesis', 'dyskinetic cilia syndrome' and 'primary dyskinetic cilia syndrome' (Pedersen and Mygind, 1980; Rossman *et al.*, 1980; Sturgess *et al.*, 1980; Veerman *et al.*, 1980), but these terms have not gained general acceptance perhaps because there are several precedents to this situation (e.g. in anaemia there is not a total absence of blood as the name implies and amelanotic melanomas are not totally devoid of melanin). In any case whether the cilia are totally immotile† or beat feebly or erratically, the result is the same—a failure of adequate mucus transport which leads to pulmonary pathology. Thus according to Afzelius (1981) such cilia may be regarded as 'functionally immotile'.

About half the cases of immotile cilia syndrome can also be classified as Kartagener's syndrome (situs inversus, chronic sinusitis and bronchiectasis). It is thought that situs inversus probably results from loss of ciliary activity within the archenteron which is necessary for the clockwise rotation of the viscera in the embryo (Camner *et al.*, 1975). Conversely, not all individuals classifiable as Kartagener's syndrome show cilia with ultrastructural abnormalities with the transmission electron microscope (Herzon and Murphy, 1980). It is possible that in such cases there occurs a more subtle abnormality which has not been detected or is undetectable by the transmission electron microscope.

Several reports now indicate that a variety of alterations of the morphology of the axoneme is associated with immotility or poor or defective motility. This includes: (1) absence of both dynein arms (Afzelius *et al.*, 1975; Afzelius, 1976; Bleau *et al.*, 1978; Rossman *et al.*, 1978; Waite *et al.*, 1978; Veerman *et al.*, 1980); (2) absence of inner dynein arms (Afzelius and Eliasson, 1979); (3) absence of outer dynein arms (Afzelius and Eliasson, 1979). The spermatozoa were motile but progressive motility was reduced; (4) decreased length of outer arms (Fischer *et al.*, 1978); (5) defective or absent spokes and/or spoke heads (called 'radial spoke defect') (Eliasson *et*

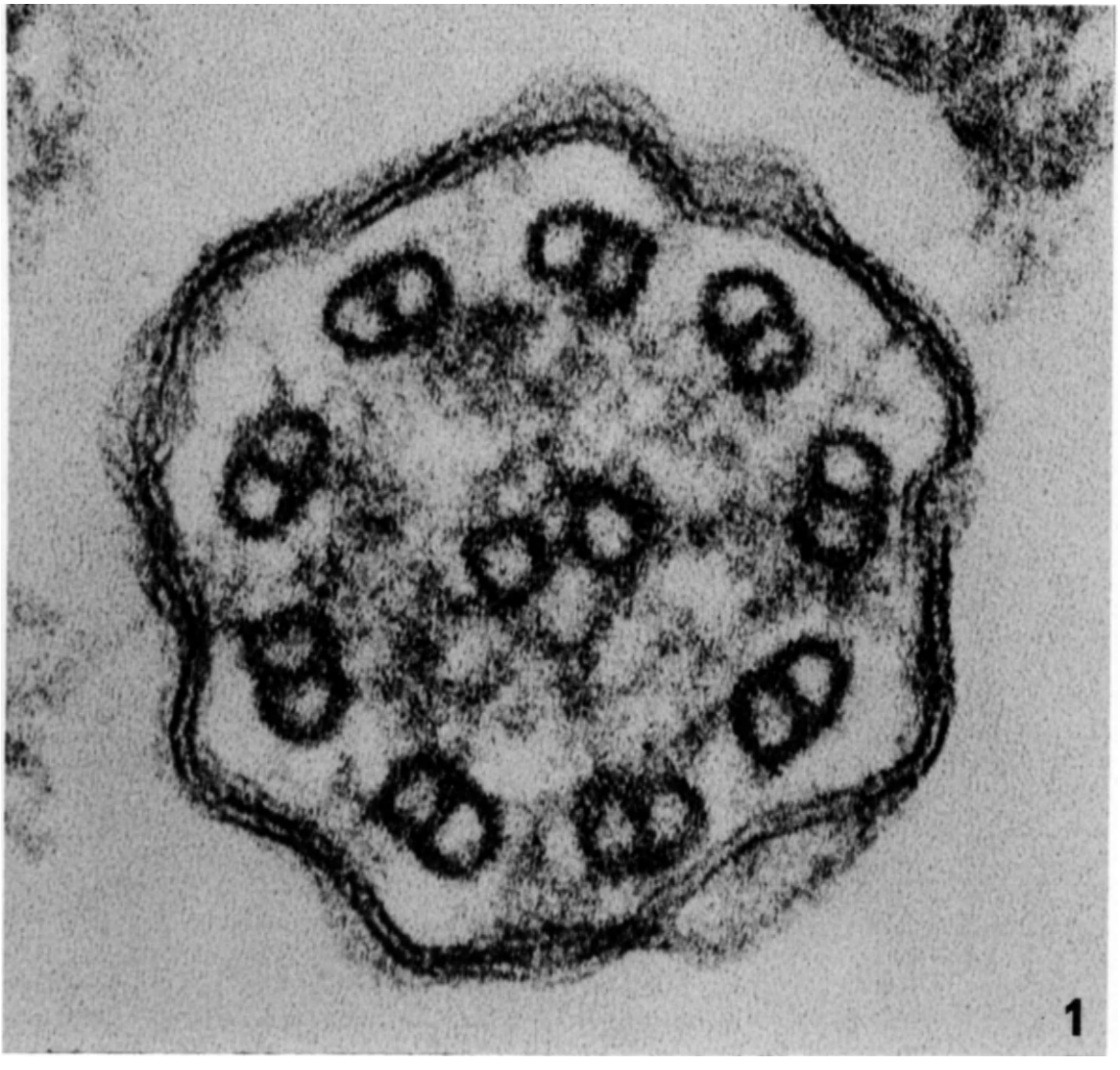
*Cilia in the fallopian tube are also affected, but the ovum manages to traverse the fallopian tube (presumably by peristalsis of the tube) and sterility does not result.

†Likewise the tails of spermatozoa from some of these individuals are totally immotile while in others the tail oscillates but is unable to effectively drive the sperm forwards. It matters not from the point of the patient or the clinician because in either case the patient is sterile.

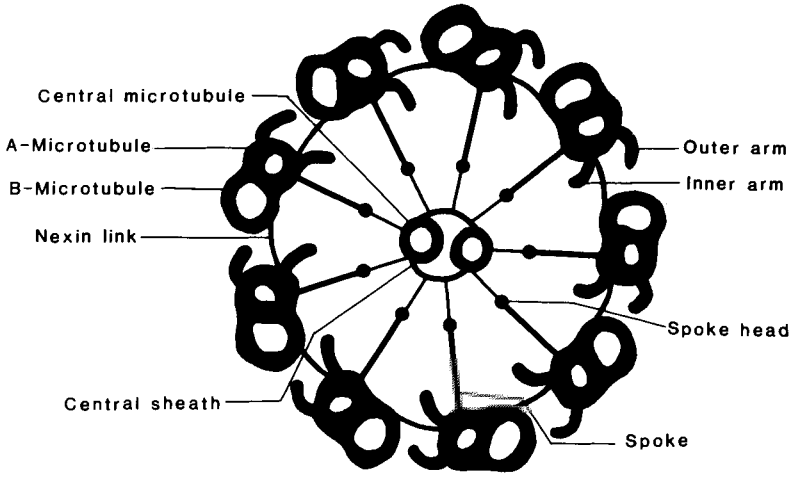
Plate 507

Fig. 1. A cilium from the nasal mucosa of a patient with Kartagener's syndrome. Outer and inner dynein arms are absent. ×370 000 (*Tandler, unpublished electron micrograph*)

Fig. 2. Diagram illustrating the structure and nomenclature of the components of the normal axoneme



1


Central microtubule
A-Microtubule
B-Microtubule
Nexin link
Central sheath
Outer arm
Inner arm
Spoke head
Spoke

2

al., 1977; Sturgess *et al.*, 1979; Grimfield *et al.*, 1980; Perrin *et al.*, 1981; Antonelli *et al.*, 1981). The absence of spokes leads to a displacement of one of the central two microtubules and may be accompanied by displacement of one of the outer microtubular doublets to the centre of the cilium. A case with radial spoke defect and lack of inner dynein arms has been described by Schneeberger *et al.* (1980); and (6) transposition of microtubules. The cilia lack central microtubules and one of the outer doublets is transposed to the central region. Thus, the cilia have an 8+1 pattern of doublet microtubules (Sturgess *et al.*, 1980; Sturgess and Turner, 1984).

Defective cilia of the type described above have been seen in other species besides man. These include: (1) a male-sterile strain of mice: *hyp* mutant (hydrocephalic polydactyl) with dynein-deficient sperm tails (Bryan, 1977; Bryan and Chandler, 1978); (2) male-sterile strains of *Drosophila* with abnormal axoneme patterns in sperm tails (Wilkinson *et al.*, 1974; Rungger-Brändle, 1977); (3) dogs (Carrig *et al.*, 1974; Stowater, 1976); (4) mutant strains of algae (*Chlamydomonas*). One strain lacks dynein arms and in another the spokes are missing (Huang *et al.*, 1979; Luck *et al.*, 1977); and (5) a ciliate (*Paramecium*) and a fern (*Ceratopteris*) (Afzelius, 1981).

As noted earlier, not all cases which show the clinical signs and symptoms of immotile cilia syndrome (including Kartagener's syndrome) show defective axonemes. It would appear that other abnormalities may also produce the clinical picture of immotile cilia syndrome. For example, cases have been reported where: (1) the cilia and basal bodies were absent in cells that by all other ultrastructural criteria resembled ciliated cells (Jahrsdoerfer *et al.*, 1979; Fonzi *et al.*, 1982); (2) the ciliated cells were replaced by brush cells (Gordon and Kattan, 1984); (3) the basal bodies were defective (basal bodies with missing triplets, some look like half centrioles) and the accessory structures were also abnormal (e.g. giant rootlets and two instead of one basal foot) (Lungarella *et al.*, 1985); and (4) the cilia were randomly orientated* but there was no detectable defect in the axoneme (Lupin and Misko, 1978). This was in two cases of Kartagener's syndrome. Randomly oriented cilia have been quite frequently observed in patients with immotile cilia syndrome (including Kartagener's syndrome) but this has been in association with other abnormalities such as the absence of dynein arms.

There are several cilial changes (pages 1188–1193) which are not associated with the immotile cilia syndrome even though they are at times seen in patients with this condition. Most, perhaps all of them, seem to be secondary changes engendered by disease and noxious agents (e.g. smoking, carcinogens, asthma, bronchitis and viral and bacterial infections). Such changes include: (1) compound cilia; (2) swollen cilia; (3) internalized cilia; and (4) cilia with absent central microtubules†.

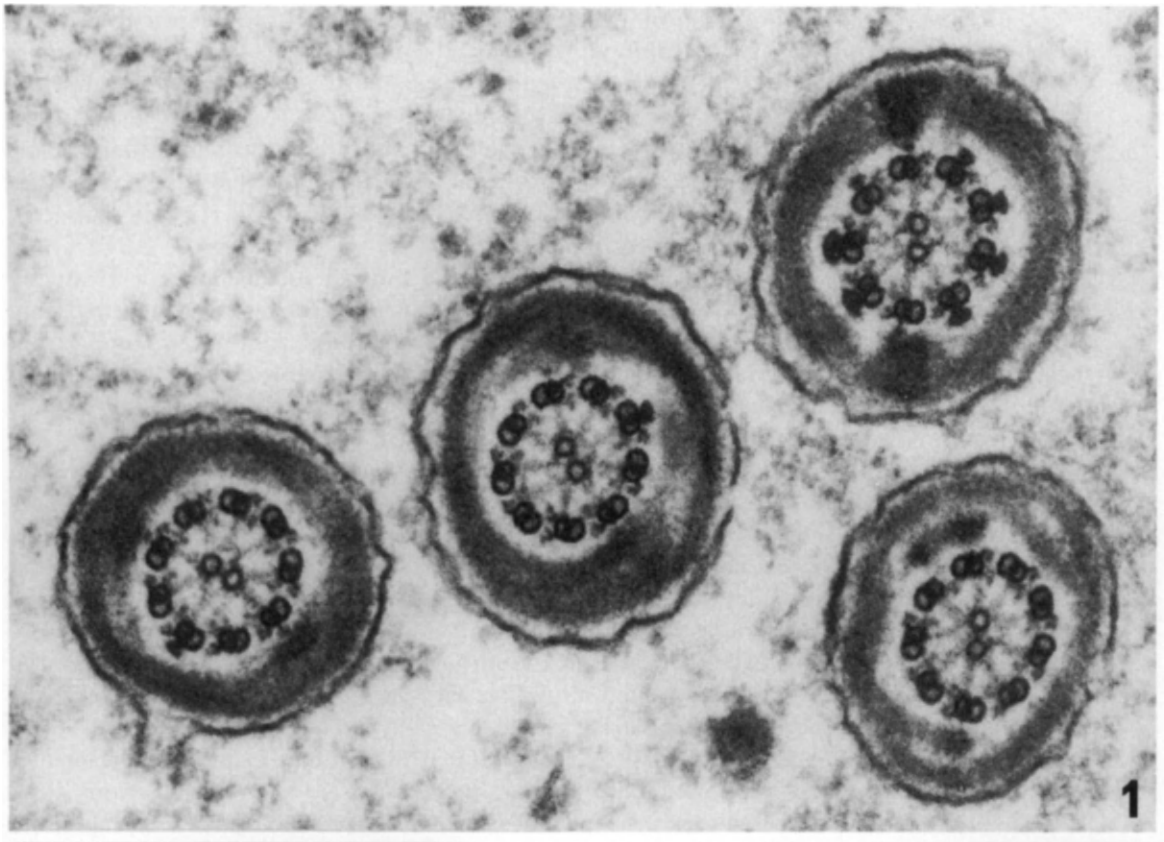
*The cilial beat occurs in the direction perpendicular to the plane or line (in sectioned material) joining the two central microtubules. When sections of a group of cilia on a normal ciliated cell are examined, the line joining the central microtubules in any one cilium is as a rule more or less parallel to similar lines drawn in other cilia. This is spoken of as the 'fixed orientation'. It tells us that the cilia are all beating in the same direction. When the lines are not parallel to each other (some may even be at right angles to others) the situation is referred to as 'random orientation'. Cilial orientation can also be deduced by the position of the basal feet on a group of basal bodies. It takes little imagination to see that randomly-oriented cilia beating in different directions would be partially or totally ineffective in moving mucus over the bronchial mucosa.

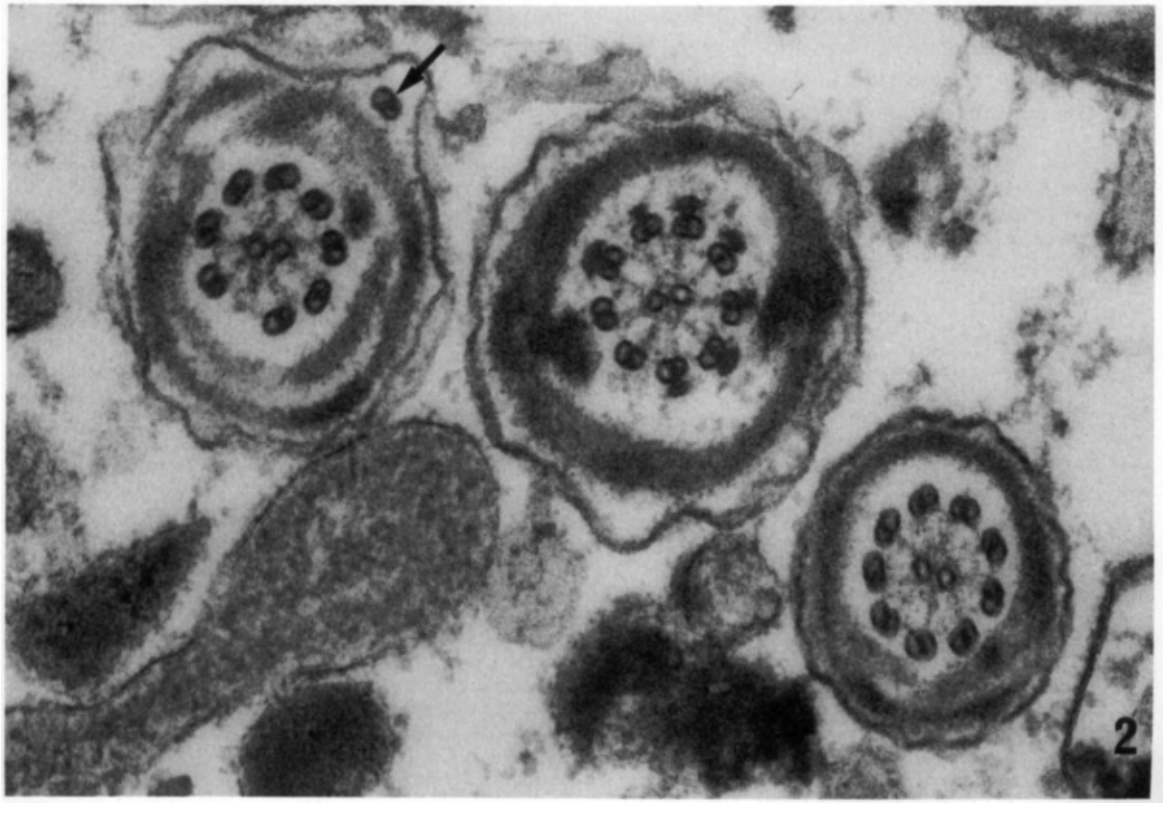
†The direction of the ciliary beat is lost when the central microtubules are absent. Hence, one would imagine this would be yet another way in which the immotile cilia syndrome is produced. However, absence of one or both central microtubules does not appear to be specifically associated with the immotile cilia syndrome. Cilia with this abnormality have been found not only in the immotile cilia syndrome but also in various other conditions such as asthma, cystic fibrosis, bronchitis, bronchiectasis, and in the lungs of smokers.

Plate 508

Fig. 1. From semen of a normal sperm donor. Seen here are four normal sperm tails where the details of the axoneme are well visualized. ×105 000

Fig. 2. From semen of a case of immotile cilia syndrome. (The sperms showed very little motility.) Profiles of three sperm tails clearly showing a virtually total absence of dynein arms. In one of them a microtubule doublet (arrow) is displaced. ×105 000


1


2

Let us now consider the practical problems faced by the electron microscopist when presented with a biopsy from a patient thought to have the immotile cilia syndrome. The most common procedure for ultrastructural investigation of cilia is to study a biopsy of nasal or tracheobronchial mucosa. It is my experience and that of others (e.g. Sturgess and Turner, 1984) that nasal biopsies are a poor source of ciliated cells for ultrastructural* diagnostic purposes. Frankly, I think they are a waste of time and effort, for the few cilia that may be present are often poorly preserved, squashed or tangled so that only a rare accurately transected cilium (where one can study the axoneme) is encountered.

Properly collected and processed biopsies of bronchial mucosa usually but not invariably provide an adequate number of cilia for ultrastructural evaluation. At least two biopsies (from different sites in the bronchial tree) are needed to mitigate sampling problems stemming from focal inflammatory and metaplastic changes in the respiratory tract. It is now well known that numerous non-specific cilial abnormalities are seen even in apparently normal individuals. Further, not every cilium shows a full complement of dynein arms; and other structures like spokes are also not constantly and consistently demonstrated. An occasional cilial section showing a lack of dynein arms is only to be expected because in the transition zone (i.e. where the doublets turn into singlets. (*See Plate 497, Fig. 3*) no dynein arms are present (Rautiainen *et al.*, 1984). Conversely, in the immotile cilia syndrome occasional cilia containing some dynein arms (usually rather short ones) are seen, and as mentioned before cases have been reported where no defect in the axoneme is detectable.

In view of such problems, it has been suggested (Sturgess and Turner, 1984) that 'At least 200 sections of cilia should be examined at high resolution with the sections oriented to visualize the microtubules and their accessory structures'.

Wherever possible (i.e. the patient has to be an adult male) one should examine a specimen of semen, because immotility or poor or abnormal motility is easily seen in wet preparations and it is my experience that the presence or absence of dynein arms and spokes are as a rule more clearly and unequivocally demonstrated in sperms than in bronchial cilia, perhaps because the procurement and processing of sperms is far simpler and less prone to error and artefacts. For example, the possibilities of crushing, dragging and drying artefacts are totally avoided as also the noxious effects of anaesthetic agents.

*However, nasal brushings and scrapings may provide useful material for studying cilial motility by phase contrast microscopy and other means. According to Sturgess and Turner (1984) this is 'a useful screening method to identify individuals for further investigations'.

Plate 509

Fig. 1. A normal sperm tail where the structure of the axoneme is singularly well visualized. Compare with diagram in *Plate 507*. ×230 000 (*Electron micrograph supplied by Dr B.A. Afzelius*)

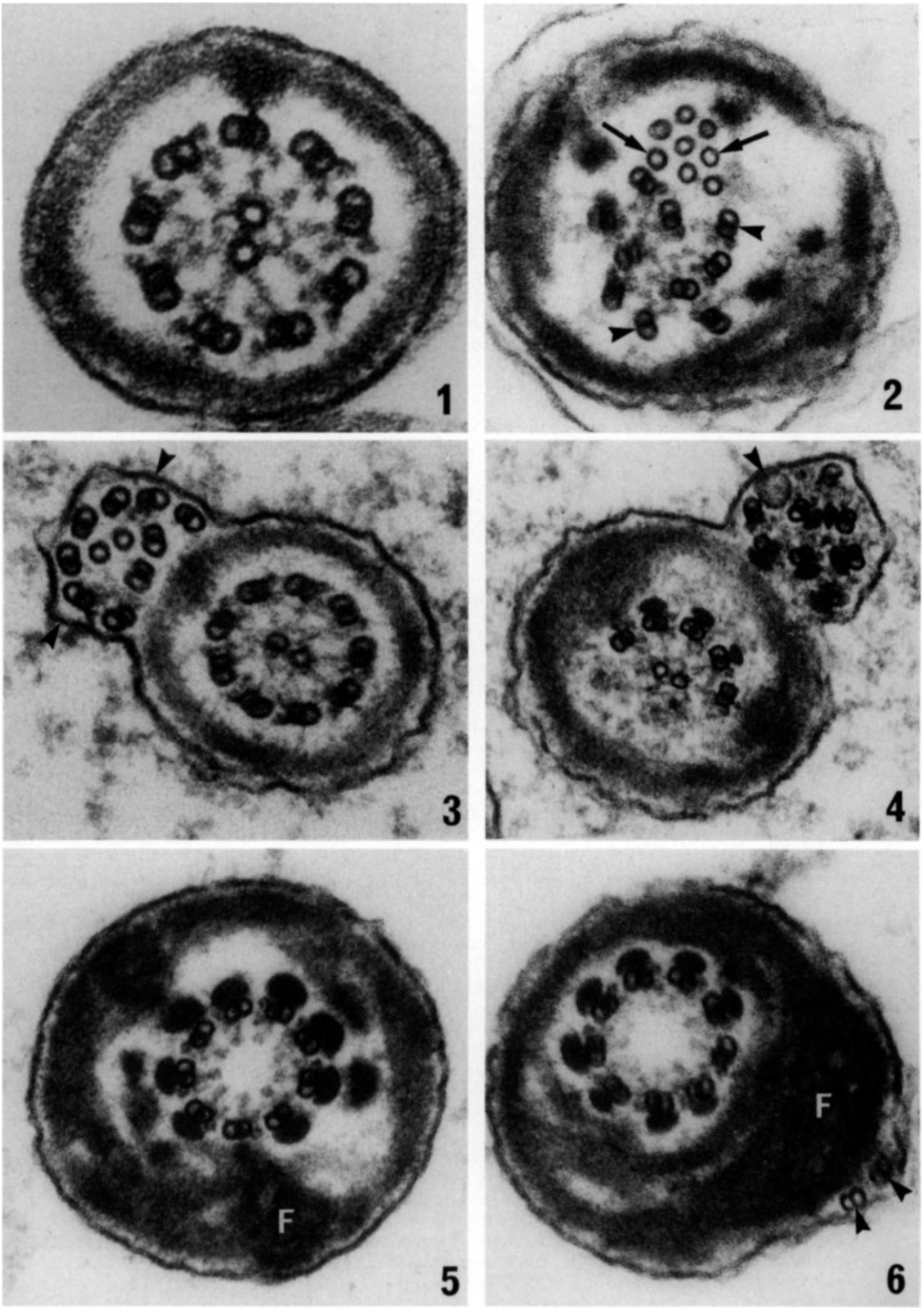
Fig. 2. Sperm from a case diagnosed as 'infertility secondary to complete necrospermia' (no respiratory tract symptomatology). In fact, the sperms were immotile, but not necrotic. No normal sperms were detected, they showed various abnormalities. Seen here is an abnormal sperm tail with an axoneme composed of eight singlets (arrows) and ten doublets (arrowheads). ×125 000

Fig. 3. From semen of a normal donor. Large numbers of normal sperms were present but some showed various abnormalities. Seen here is a sperm tail with a normal axoneme bearing a hump (between arrowheads) containing two singlets and nine doublets showing a reversed orientation as compared to the doublets in the sperm tail. A plausible explanation here would be that the axoneme is bent back on itself within the membranous envelope and has hence been cut twice. ×131 000

Fig. 4. From the same case as *Fig. 3.* A sperm tail with four missing doublets, and a hump containing doublets and a vesicle (arrow). One may speculate that perhaps fusion of two atypical sperm tails occurred. ×106 000

Fig. 5. Same case as *Fig. 2.* Sperm tail lacking central microtubules and central sheath. The fibrous sheath (F) shows patchy thickening. ×123 000

Fig. 6. Same case as *Fig. 2.* Sperm tail lacking central microtubules. Note the marked thickening of the fibrous sheath (F) and two microtubular doublets (arrowheads) outside the sheath. ×117 000


1
2
3
4
F
5
F
6

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17

Extracellular matrix (extracellular components)

Introduction (classification and nomenclature of fibrous components)

Where exactly the cell ends and the extracellular compartment begins was a subject of controversy in the past, but it is now widely accepted that this boundary is the cell membrane with its coat. As is well known the cells of connective tissues (e.g. connective tissue proper, cartilage and bone) are separated by an abundant matrix but the cells of epithelia are not, the 'cement substance' between them being the attenuated cell coat.

In this chapter we deal primarily with the two main components of the connective tissue matrix, namely: (1) the fibrous component (collagen fibres and elastic fibres), sometimes referred to as the 'fibrous matrix' or 'fibrillary matrix'; and (2) the ground substance or the interfibrillary matrix which contains mainly proteoglycans, solutes and water (*Plate 510*).

The nomenclature of the various 'thread-like' structures which comprise the fibrous component of the matrix is now so confusing that it has become difficult to comprehend the literature on this subject, or communicate one's thoughts clearly. This confusion stems mainly from two sources: (1) improper use of classic terms such as 'fibres', 'fibrils', and 'filaments'; and (2) introduction of superfluous terms such as 'microfibrils', 'tubulofilaments' and several others. It is possible to describe adequately and unambiguously, on almost all occasions, all thread-like structures, be they intracellular or extracellular with just the three classic terms, 'filament', 'fibril' and 'fibre' and one relatively new one called 'protofilament'.

A filament may be defined as a thread-like structure about 2–20 nm in diameter. It may occur singly (solitary filament) or a group of filaments may aggregate to form a fibril. The low figure of 2 nm reflects the thinnest filament one may hope to see with the kind of resolution attainable in ultrathin sections of biological tissues examined with the electron microscope. It is now common knowledge that in the above mentioned circumstances the attainable resolution is only 2–3 nm even with the finest electron microscope. The better than 0.2 nm resolution of such microscopes is demonstrable only in special preparations such as carbon foil or gold microcrystals.

This range of thickness: 2–20 nm, covers virtually every filament one can think of, like, myofilaments, intermediate filaments, collagen filaments, elastic fibre filaments and amyloid filaments. The term 'tubulofilaments' is used by some authors to describe filaments presumed to have a hollow centre. This is a peculiar term at best for a structure is either tubular (i.e. hollow) or filamentous (i.e. solid), it cannot be both. Examples of this dilemma are the electron-dense filaments of elastic fibres (discussed in greater detail on page 1252). Virtually all workers believe that their occasional 'hollow' appearance stems from a staining artefact

whereby only the periphery of the filament is stained but Inoué and Leblond (1986) have brought forth evidence which suggests that they are hollow, in which case they would qualify as 'electron-dense microtubules'. To adopt this term now would be very confusing, hence for the moment I will retain the term electron-dense filaments.

Despite the massive literature on the structure of collagen fibrils, there are some who believe that at times collagen fibrils are hollow and terms such as 'hollow collagen' and 'tubular collagen' are used to describe what is no more than a common staining artefact whereby only the periphery of the fibril is stained (*Plate 511*). A more pronounced version of the same artefact is the so-called 'negatively stained collagen' where the collagen fibrils have failed to stain and appear quite lucent against a darker matrix. It is possible that, in some circumstances, 'negatively stained collagen' and 'tubular collagen' may reflect a pathologically altered collagen or an altered interfibrillary matrix which hampers the staining of collagen, but to the best of my knowledge no proven example of this exists.

Some filaments (and microtubules) have been found to be composed of more slender thread-like structures which are best referred to as 'protofilaments' (0.15–0.3 nm in diameter); an important proviso being that a protofilament must be a part of a filament or a microtubule; the protofilament when isolated by experimental manoeuvres loses its thread-like form. For example, the protofilaments of actin filaments (and tubulin microtubules) dissociate into globular subunits when isolated.

A fibril is defined as an aggregate of filaments. Fibrils range in size from about 20 nm to 2 μm*. Some fibrils, such as myofibrils and many but not all tonofibrils are visualized with the light microscope but the mature collagen fibril with its well known periodic structure† is not, because it is usually less than 0.1 μm in diameter. The so-called 'collagen fibril' of the light microscopist is, in fact, a slender collagen fibre (i.e. a collection of collagen fibrils). To make this distinction some workers prefer to call collagen fibrils seen with the electron microscope, 'collagen microfibrils'. This, however, is undesirable for it brings in its wake a host of useless and confusing terms with the prefix 'micro'. Nomenclature and classification are more properly based on properties and characteristics of the structure itself and not on the instrumentation needed to reveal it.

A fibre may be defined as an aggregate of fibrils. The thickness of fibres is quite variable. For example, skeletal muscle fibres range in size from 10–100 μm, while collagen fibres usually range from about 1–10 μm (Bloom and Fawcett, 1975). Larger rope-like collagenous structures are best referred to as 'bundles of fibres'. Not all accumulations of fibrils necessarily form fibres. For example, collagen fibrils can also aggregate to form sheet-like or lamellar structures (e.g. the collagen lamellae of the cornea and intra-articular menisci).

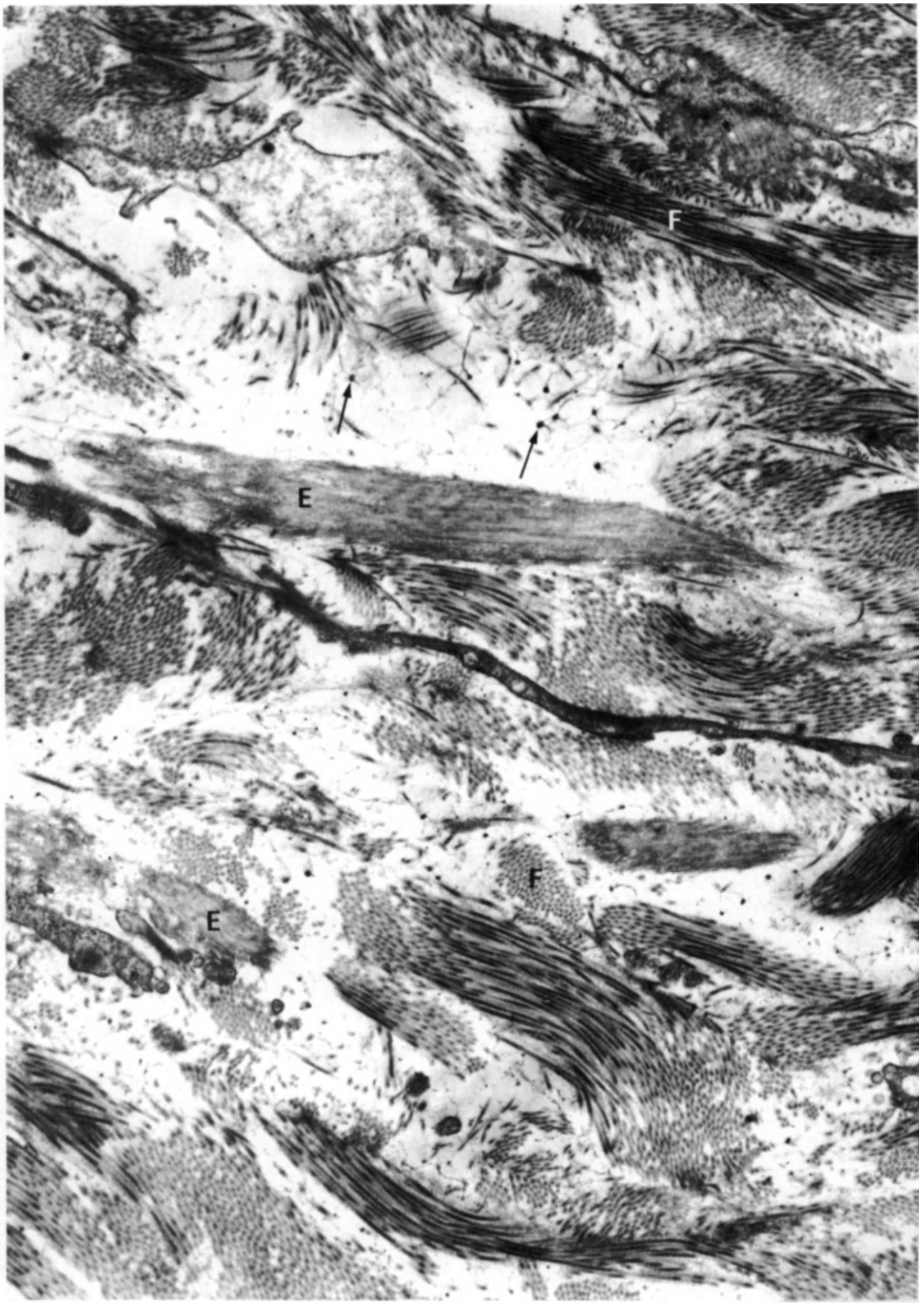
The terminology outlined above is not new, it follows long established principles of nomenclature and rejects some more recently introduced terms which have failed to prove their worth. In order to ensure clarity of presentation, some of the points made here are repeated and amplified in appropriate sections of this chapter. The terminology adopted will be the one outlined above, but various other terms that have been used to describe each structure will also be mentioned so as to facilitate perusal of the literature on these subjects.

*This range covers most fibrils. The light microscopist's collagen fibril is said (Ham, 1969) to be about 0.3–0.5 μm thick. The top figure of 2 μm refers to myofibrils.

†However, not all collagen fibrils show the expected banded cross-striated pattern. Very slender or young fibrils may show only a vague suggestion of periodic structure, and cross-striations are often difficult to stain and visualize in hyaline cartilage because of interference from the glycosaminoglycan-rich interfibrillary matrix.

Plate 510

The three major extracellular components identifiable by the electron microscope are depicted in this electron micrograph from human skin. They are: collagen fibres (F) composed of collagen fibrils, elastic fibres (E) composed of elastic fibre filaments and elastin and proteoglycan particles (arrows) which present as electron-dense particles and associated filaments. × 12 000


F
E
F
E

Collagen filaments, fibrils and fibres

The collagens are macromolecular proteins of approximate molecular weight 300 000, composed of three helical polypeptide chains. The amino acid composition of these chains is characterized by a high content of glycine, proline and alanine, absence of cysteine and tryptophan, low content of tyrosine and the presence of unique hydroxylated amino acids (hydroxyproline and hydroxylysine) (Perez-Tamayo and Rojkind, 1973).

It is essential for the electron microscopist to be cognizant of the various types and levels of organization of collagenous structures formed by collagen molecules as noted in the Introduction (page 1215). Three types of fibres* have long been recognized in connective tissues by light microscopists; collagen fibres (white fibres), elastic fibres (yellow fibres) and reticular fibres (argyrophilic fibres). However, ultrastructural studies have shown that reticular fibres are collagenous in nature†. The collagen fibres are wavy fibres of variable length measuring about 1–10 μm in diameter. In suitable situations and preparations the light microscope shows that collagen fibres are composed of finer thread-like structures (0.3–0.5 μm in diameter) called 'fibrils'. However, the electron microscope shows that the fibril of the light microscopist is not the true subunit of the collagen fibre. The true fibril as seen with the electron microscope is a structure measuring about 20–120 nm in diameter in most connective tissues, but it can be up to about 160 nm thick in certain sites such as the semilunar cartilages of the knee (Ghadially *et al.*, 1980). Such native collagen fibrils are readily identified in electron micrographs by their banded or cross-striated appearance. They exhibit, both by low-angle x-ray diffraction and electron microscopy, a marked axial periodicity. In suitably stained preparations each period is seen to consist of a light and a dark band (sometimes referred to as the major bands) and one can also discern some 10–13 fine dark cross-striations (which delineate nine or more minor bands) within each period. The length of each period (i.e. one dark band + one light band)‡ depends on the state of hydration of the collagen fibril. In fresh wet collagen (measured by x-ray diffraction) the periodicity ranges from 64–70 nm. In ultrathin sections it ranges from 52–62 nm.

*One of the lay meanings of the term 'fibre' is 'a thread-like body'. For example in the textile industry the term 'fibre' refers to any slender thread-like structure that can be woven into a fabric, there is no precise limit as to how thick or long a structure has to be to be called a 'fibre'. This must not be used as an excuse to call all thread-like structures seen with the light and electron microscope 'fibres'.

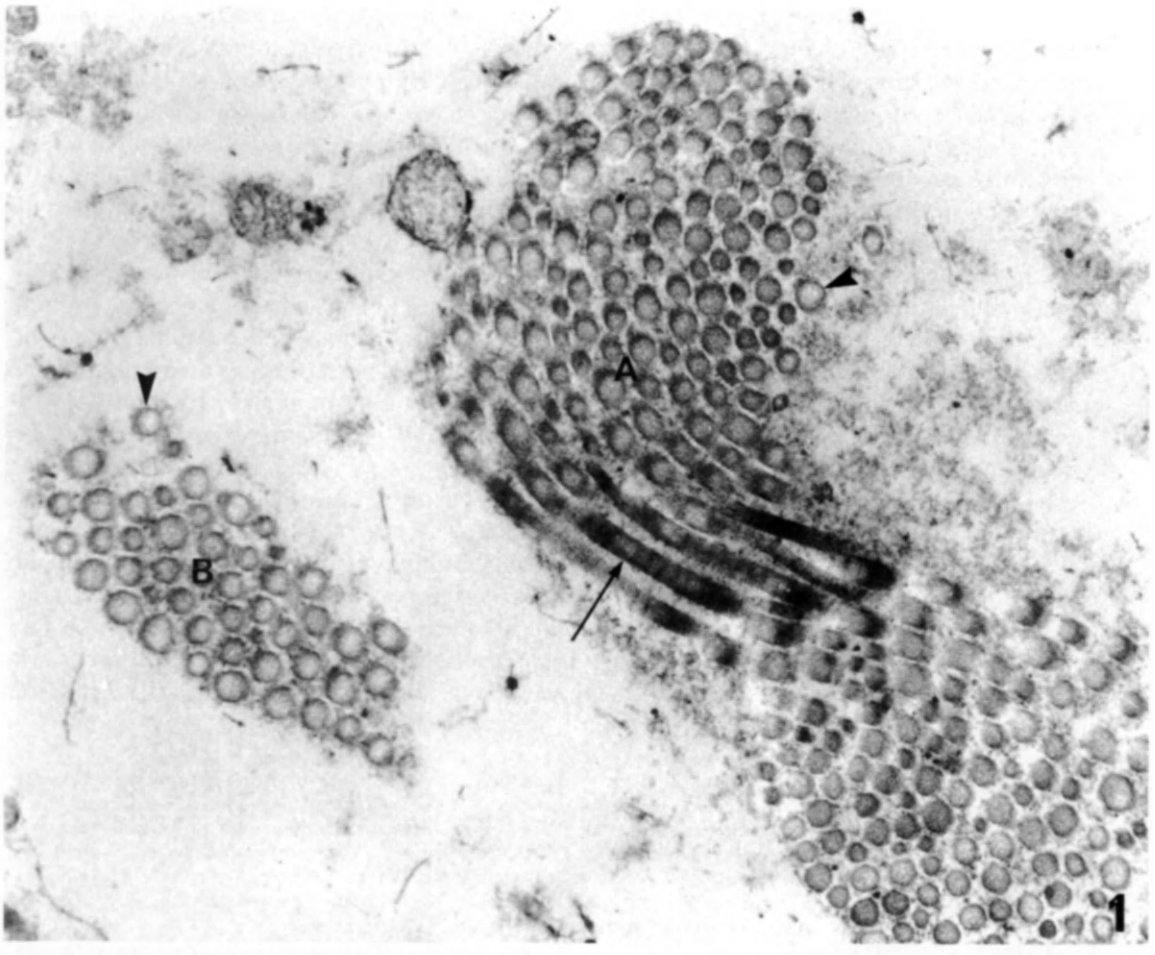
†The electron microscope shows that both collagen fibres and the much more slender reticular fibres are composed of fibrils with the periodic structure typical of collagen. It appears that the difference in staining reaction with silver (collagen, brown or grey; reticulum, black) is due to physical rather than fundamental chemical differences.

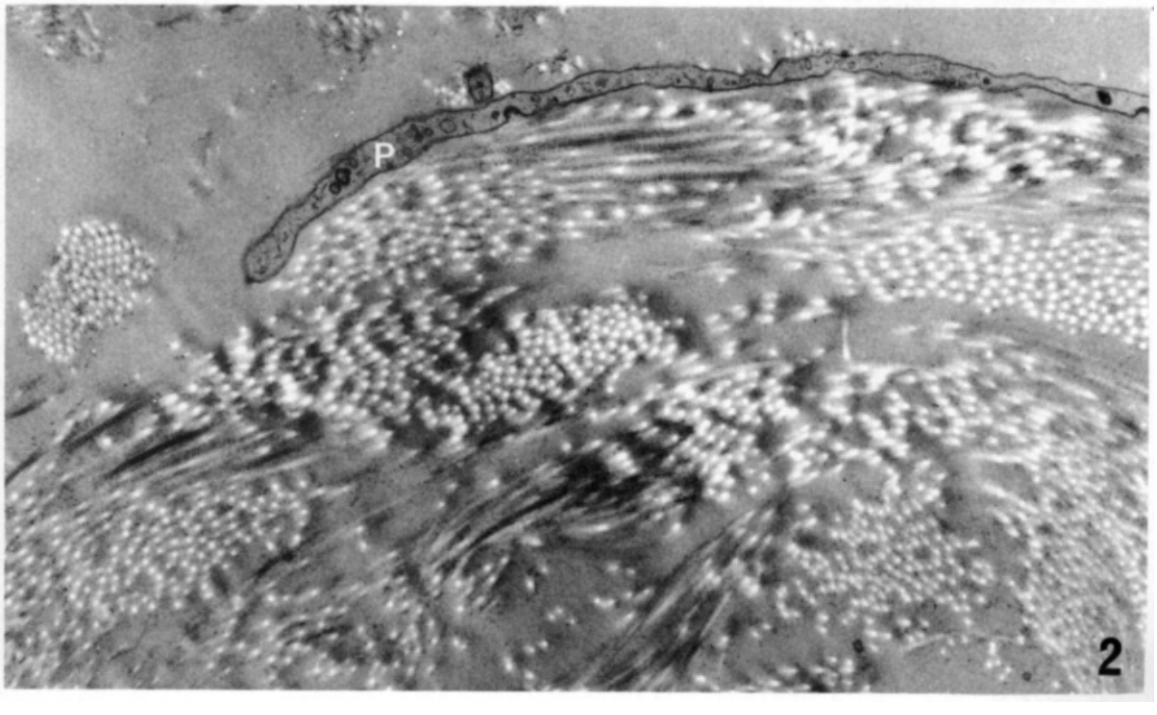
‡The length of a period can also be determined by measuring the distance between the middle of a dark band and the middle of the next dark band. In actual practice it is better to measure the distance across several periods (ten if possible) so that an average figure for the periodicity may be obtained. Obliquely cut fibrils (identified by poor resolution or 'absence' of minor bands) should not be used to calculate the period.

Plate 511

Fig. 1. Two collagen fibres from the skin of a normal rabbit. The fibrils comprising one of the fibres (A) are cut in various planes, because of the wavy tortuous nature of the fibre. In portions of the fibrils cut longitudinally (arrow) the cross-striated pattern characteristic of collagen fibrils is seen. Some of the transversely cut fibrils (arrowheads) in this fibre (A) and virtually all the fibrils in the neighbouring fibre (B) show a tubular appearance, because only the periphery of the fibril is well stained. × 37 000

Fig. 2. Collagen fibres in the subsynovial tissue of a normal rabbit. The fibrils comprising the fibres have almost completely failed to stain. Hence they appear very electron-lucent. This appearance is at times referred to as 'negatively-stained collagen'. Interestingly enough the cell process (P) of a fibroblast present here appears to be correctly stained. × 12 000


A
B
1


P
2

Thus, for the electron microscopist the hallmark of collagen is the characteristically banded fibril. However, some workers use the term 'microfibril' to describe this structure, but it is difficult to see any real merit in this. It seems far better to correct the wrong impression engendered by the limits of resolution of the light microscope than to commemorate it by creating a new term. Besides, such a practice is out of line with nomenclature of other 'thread-like structures' found elsewhere. For example we speak of: (1) myofibres composed of myofibrils, and these in turn are composed of myofilaments; and (2) tonofibrils composed of tonofilaments.

Such a sequence (i.e. filaments, fibrils and fibres) can be demonstrated in collagen fibres also because there is little doubt that collagen fibrils are composed of collagen filaments even though this is not readily apparent in ultrathin sectioned material (Gross and Schmitt, 1948). In shadowed preparations and negatively-stained preparations collagen fibrils exhibit longitudinal striations suggesting that the fibril comprises filaments about 3–5 nm in diameter (Smith, 1968; Piez and Trus, 1977). X-ray diffraction studies confirm this by showing filament spacings of 3.8 nm (Miller and Wray, 1971; Miller and Parry, 1973).

The collagen filaments are made up of rod-like tropocollagen molecules* which are about 300 nm long and 1.5 nm wide in the wet state and about 240–280 nm in length in the dehydrated state. The tropocollagen molecule consists of three helical polypeptide α-chains wound round each other to form a triple helix which extends co-linearly throughout the length of the tropocollagen molecule. The tropocollagen molecules are arranged in what is known as 'an approximately quarter staggered arrangement' and this is what gives the native collagen fibril its cross-striated appearance (i.e. produces the major and minor bands or striations) (*Plates 512* and *513, Fig. 1*).

*According to the Smith (1968) model each filament is thought to be built up of five rows of approximately quarter-staggered tropocollagen molecules. Thus the collagen filament may be regarded as a five-stranded 'rope'. Each of the five strands would then have to be regarded as collagen protofilaments. Another way of looking upon this situation would be that a single row of end-to-end aligned tropocollagen molecules comprise a collagen filament and that lateral aggregation of these filaments (with tropocollagen molecules of adjacent filaments deployed in a quarter-staggered arrangement) comprises a collagen fibril. Details of the molecular organization of collagen and its biological and pathological significance are beyond the scope of this chapter; for this the reader should consult Ramachandran (1967); Traub and Piez (1971); Perez-Tamayo and Rojkind (1973); Lapiere (1973); Mathews (1975); and Mayne and Burgeson (1987).

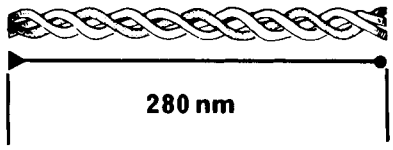
Plate 512

Diagrams showing the organization of collagen. (*A slightly modified version constructed from Figs. 3 and 4 from de Bont, 1985*)

Fig. 1. Three helical polypeptide alpha chains forming a rod-like or filamentous tropocollagen molecule about 280 nm long and 1.5 nm thick.

Fig. 2. Depicted here (A) are end to end rows of tropocollagen molecules forming a collagen fibril. The tropocollagen molecules are aligned in staggered fashion overlapping by a quarter of their length to form the characteristic cross-striated collagen fibril (B) with a banding periodicity of about 64 nm as seen in negatively stained preparations. The dark band occurs where the stain penetrates into what is called the 'hole zone' (★). The light band occurs in the overlap zone where the stain is largely excluded. In positively stained material the density of the bands is reversed, i.e. the band which appeared light in negatively stained preparation presents as a dark band in positively stained preparation (and *vice versa*) because there is more stainable mass in the overlap zone than in the hole zone.

Fig. 3. Depicted here are: a collagen fibril (A); a group of fibrils forming a collagen fibre (B); and a group of fibres forming a collagen bundle (C).



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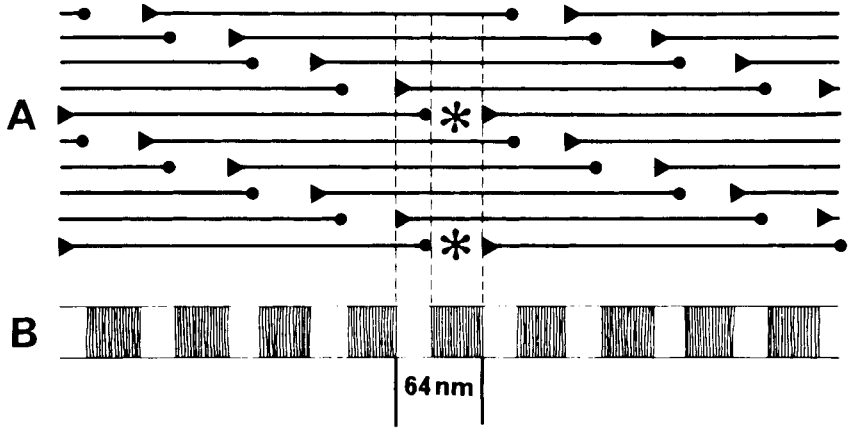

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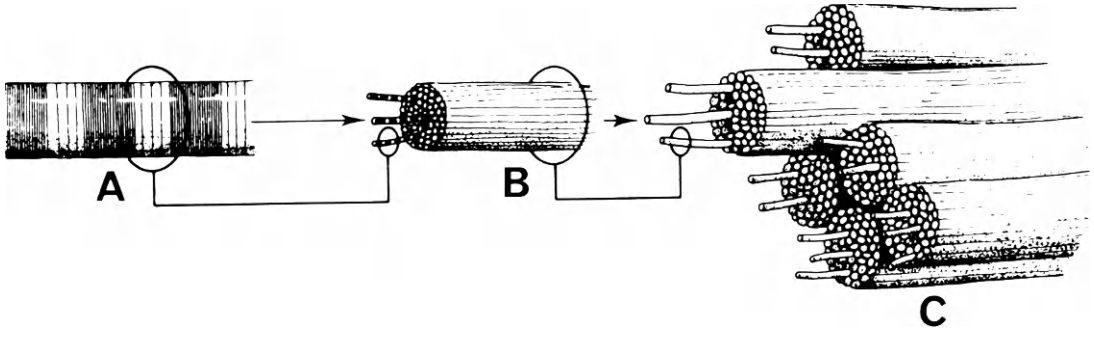


3

During fibrillogenesis covalent bonds develop between the three α-chains of each tropocollagen molecule. In mature collagen, very stable irreducible cross linkages occur which confer great strength to collagen fibrils. Collagen fibrils and fibres are flexible, hence they are of little value in resisting a compressive load, but they offer great resistance to a pulling force. The breaking point of human collagen fibres is several hundred kilograms per square centimetre and their extension at this point is minute. The virtual inextensibility and high tensile strength (about 15–30 kg mm^{-2}) weight for weight is comparable to steel (Harkness, 1968).

As noted earlier collagen fibrils form rope-like structures which we call 'fibres'. They also form collagen sheets or lamellae as in aponeuroses, cornea and menisci and three-dimensional nets or a meshwork as in articular cartilage*. In most mature tissues, most of the fibrils constituting a fibre or lamella are of a fairly uniform diameter, only a few fibrils much larger or smaller than the average are seen. In the cornea the thin collagen lamellae are composed of very regularly spread collagen fibrils of a remarkably uniform diameter (about 25 nm) (*Plate 513, Fig. 2*). The transparency of the cornea is largely due to this, for scattered rays of light cancel each other by destructive interference. However, in intra-articular discs and menisci collagen fibrils of markedly different thickness (20–160 nm) are found (*Plate 514*) mingled together to form fibres and lamellae (Silva, 1969, 1970; Ghadially *et al.*, 1978). Such an arrangement makes these structures efficient broad-band shock absorbers, capable of absorbing energy over a wide range of vibration frequencies.

It has long been recognized that the structural units of collagen are synthesized intracellularly, but polymerization to form collagen fibrils occurs extracellularly. The first step in this process is the synthesis of polypeptide α-chains by the polyribosomes of the rough endoplasmic reticulum to form procollagen. There has been much controversy regarding the intracellular transport and secretion of procollagen. The suggested possible routes of egress from the cisternae of the rough endoplasmic reticulum include: (1) into the cytoplasmic matrix and thence to the exterior (Cooper and Prockop, 1968; Reith, 1968; Salpeter, 1968); (2) via fusion of cisternae with the cell membrane or via transport vesicles derived from the cisternae fusing with the cell membrane and discharging their contents to the exterior (Ross and Benditt, 1965); and (3) the conventional route of secretion through the Golgi complex similar to that adopted by various secretory cells (Revel and Hay, 1963).

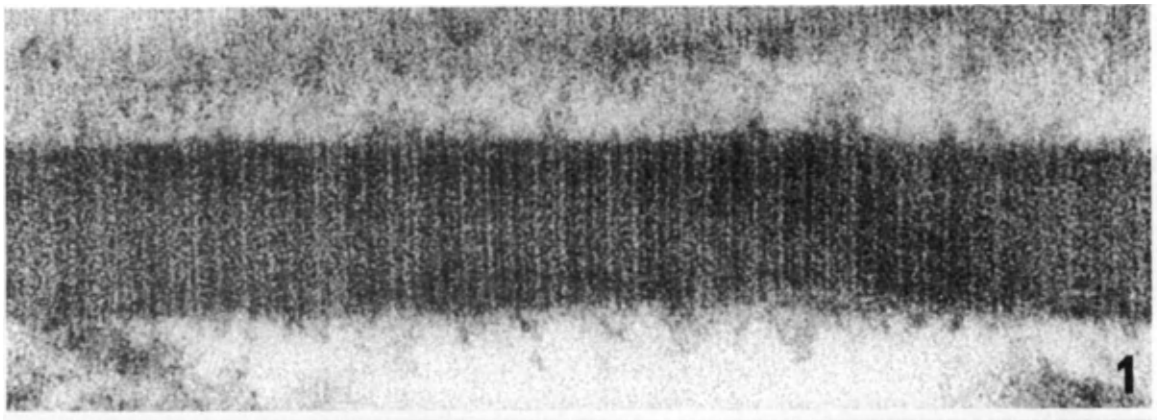
However, there is now a considerable body of evidence supporting the idea that the Golgi complex is involved in procollagen secretion. For example, this has been shown to be so in the case of odontoblasts with tritium-labelled proline. The label appears first in the rough endoplasmic reticulum then in the Golgi complex and secretory vacuoles whence it is discharged into the predentine matrix (Weinstock and Leblond, 1974). Furthermore, with the ferritin-labelled antibody technique procollagen has been demonstrated in the rough endoplasmic reticulum and Golgi complex of corneal and tendon fibroblasts (Olsen *et al.*, 1975; Nist *et al.*, 1975).

*The matrix of articular cartilage consists essentially of a collagenous framework (called the 'fibrillary matrix') within which is entrapped the ground substance (called the 'interfibrillary matrix') rich in water and proteoglycans. As mentioned before, collagen fibrils and fibres have great tensile strength but a fibril or fibre cannot support a compressive load; it would just crumple or fold. It is the osmotic pressure (swelling pressure) of water imbibed by the proteoglycans retained and restrained by the collagen meshwork which gives cartilage its resilience and its load-bearing properties. Thus cartilage may be described as a tissue under tension, the full tensional stresses being borne by the collagen fibrils when the cartilage is in the non-loaded state. These tensile stresses are relaxed or relieved as soon as the tissue is loaded (e.g. in weight bearing) (for references and details *see* Ghadially, 1983).

Plate 513

Fig. 1. A collagen fibril from the nucleus pulposus of a human intervertebral disc showing alternating dark and light bands and fine cross striations. The dark and light bands are more easily appreciated at lower magnifications (*see* for example *Plates 514* and *Plates 523–525*). × 190 000

Fig. 2. The stroma (substantia propria) of a human cornea. Note alternating lamellae composed of uniformly thick collagen fibrils cut transversely (T) and longitudinally (L). × 30 000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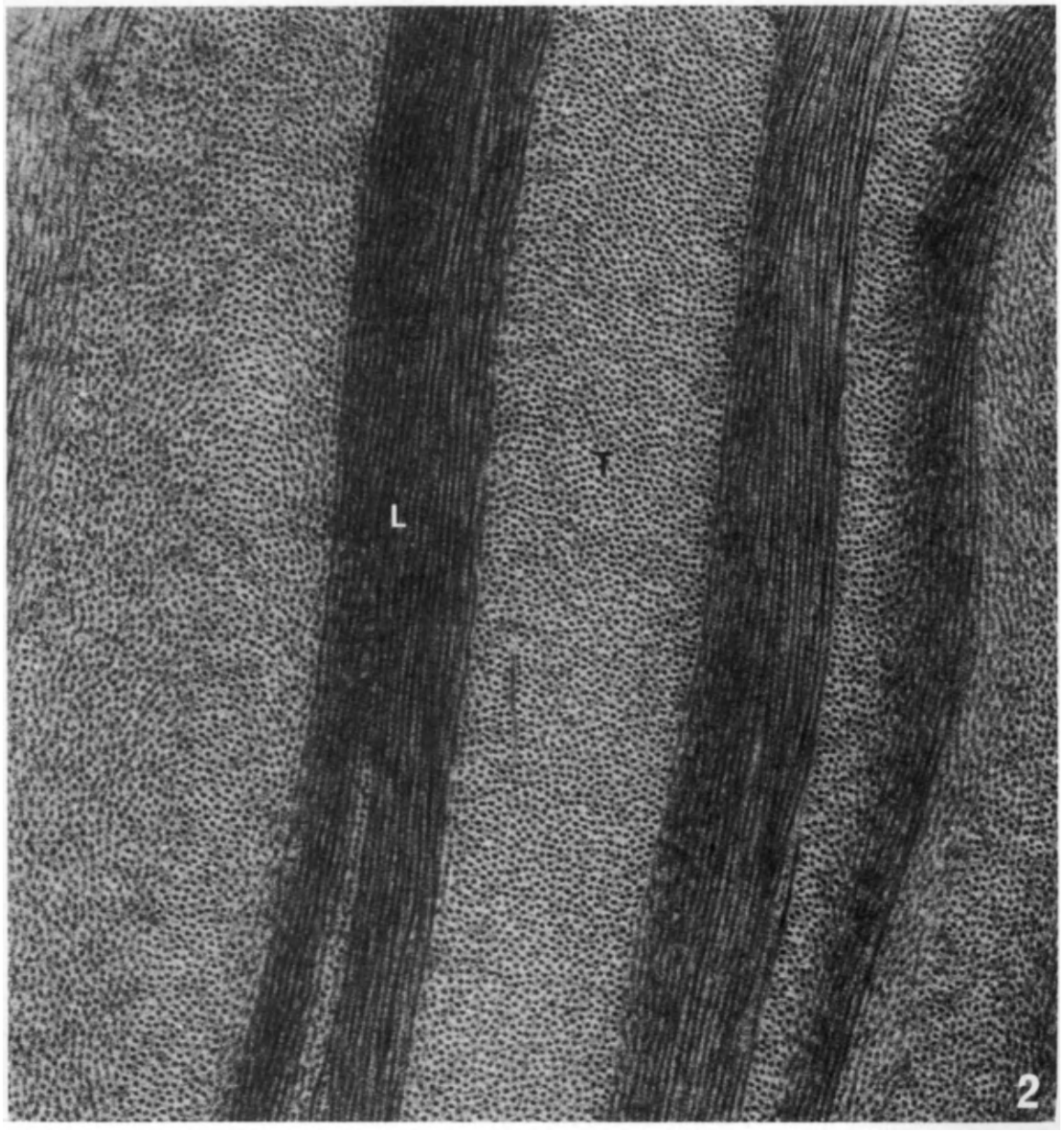

L
T
2

The procollagen molecule is larger than the tropocollagen molecule since the α-chains of procollagen possess random coil extensions (telopeptides) at both ends of the central helical section. These telopeptides are referred to as 'N' (amino) and 'C' (carboxyl) terminal non-helical regions. Conversion of procollagen to tropocollagen necessitates the proteolytic cleavage and removal of most of the non-helical terminal extensions. Although some degree of modification of procollagen may occur intracellularly (Bornstein, 1974) the cleavage of most of the extension peptides is not completed until after the procollagen is secreted because the required enzyme (procollagen peptidase) is located extracellularly (Layman and Ross, 1973). This is probably the main reason why collagen fibrils do not form in an intracellular location (except on rare occasions; *see* page 1000). Once the tropocollagen molecules are formed they aggregate into native fibrils of characteristic periodicity if the conditions are physiological*.

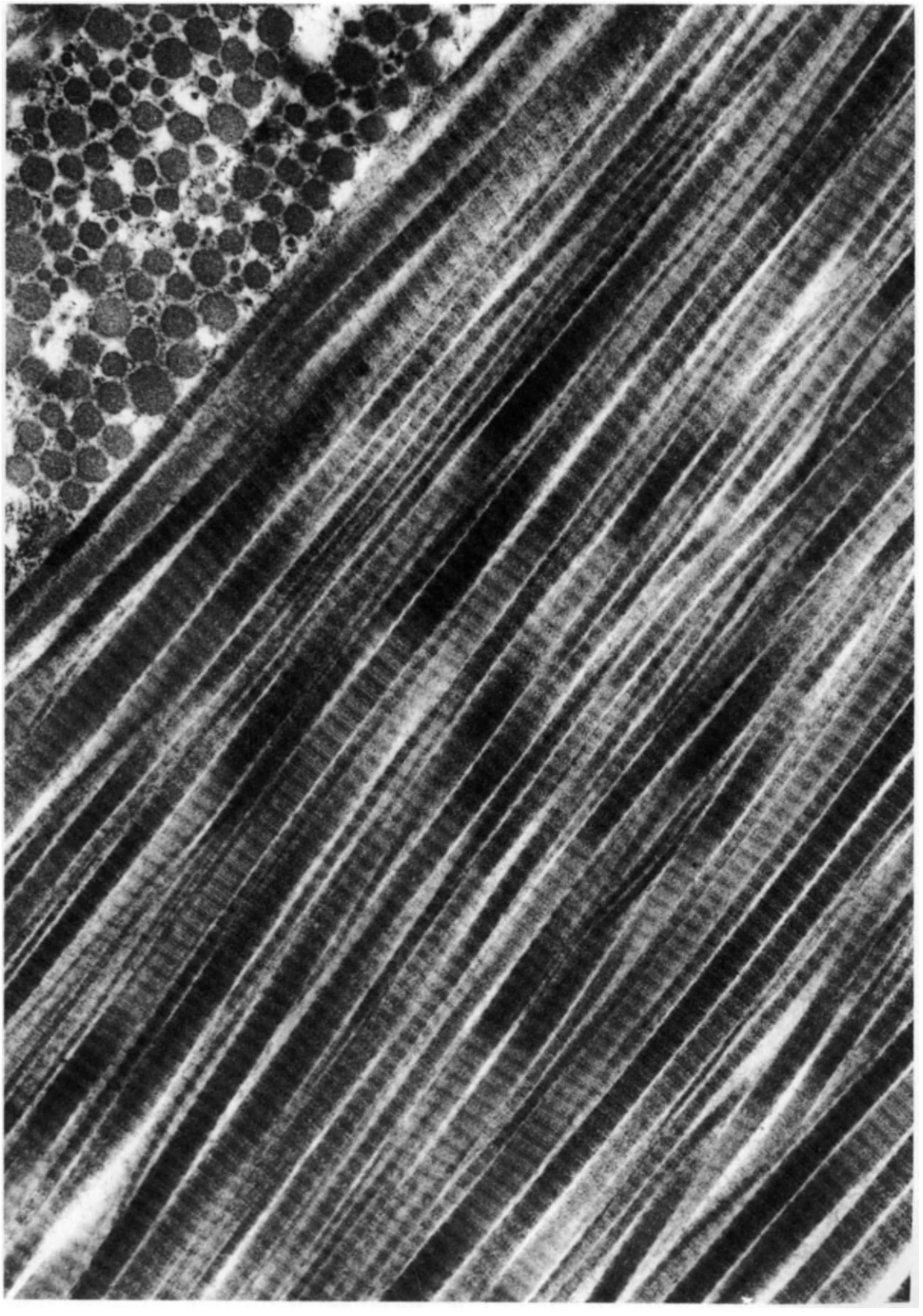
Several chemically and genetically distinct collagens are found in mammals (for references supporting statements made in this and subsequent paragraphs *see* Mayne and Burgeson, 1987). It was the studies of Miller and Matukas (1969) and Miller *et al.* (1971) that first showed that the characteristically banded collagen fibrils seen with the electron microscope are formed by three different but homologous types of collagen. These collagens which are at times collectively referred to as the 'classic collagens' are: type I collagen which is the most abundant and widely distributed variety of collagen found in many tissues (e.g. skin, bone, tendon); type II collagen which is the principal collagen in hyaline and elastic cartilage (fibrocartilage, fibrillated articular cartilage and 'dedifferentiated' chondrocyte cultures contain also some type I collagen); and type III collagen which occurs in almost all tissues in varying ratios with type I collagen.

Besides the classic collagens we have: type IV collagen found in basal lamina and external lamina (normally does not form filaments and fibrils, *see* page 1056); type V collagen occurs as unbanded slender (12 nm) fibrils in association with the stromal surface of some basal laminae and classic collagen fibrils; type VI collagen is of ubiquitous occurrence (presents as slender fibrils with a banding periodicity of 100 nm); type VII collagen is the major or perhaps the sole component of anchoring fibrils (*see* page 1228); type VIII collagen is the major component of Descemet's membrane (also found in cultures of some endothelial cells and cultures of some tumour cells such as human astrocytoma cells); type IX collagen occurs in cartilage matrix (probably preferentially located (probably as slender fibrils) in the pericellular matrix); type X collagen is found in hypertrophic cartilage, calcified cartilage and chondrocyte cultures; and type XI collagen is found in the matrix of cartilage of various species including humans, probably as slender fibrils.

*This is confirmed by *in vitro* studies using solutions of tropocollagen, *see* pages 1234–1240.

Plate 514

Rabbit semilunar cartilage. Portions of two collagen lamellae; one where the constituent fibrils are cut transversely (top left of picture) and the other where the fibrils are cut longitudinally. Note the marked variations in the diameter of the collagen fibrils. × 50 000



Collagen in fossils and mummies

It is now well known that in mummies the connective tissues (particularly collagen) are frequently well preserved but epithelial tissues are less often recognizable (Williams, 1927; Sandison, 1963; Reyman *et al.*, 1976; Montes *et al.*, 1985). Collagen fibrils seem to be particularly long-lasting in that they have also been detected in fossils millions of years old. It is beyond the scope of this text to review the voluminous literature on fossils and mummies. We will confine our attention to ultrastructural studies where banded collagen fibrils have been demonstrated in ancient specimens.

Collagen fibrils have been found in: (1) approximately 450 million-year-old fossils of the dendroid graptolite (*Dictyonema* sp.) from the Upper Ordovician (banding periodicity about 80 nm) (Towe and Urbanek, 1972); (2) ground sloth, bison, lion-like cat, wolf and gopher fossil dentine of Pleistocene age (banding periodicity 64 nm) (Doberenz, 1967); (3) decalcified Jurassic fossil bone of *Pholidophorus bechei* (banding periodicity 62 nm) (Doberenz and Lund, 1966); (4) fossil bone of genus *Equus* from Pleistocene deposits (banding periodicity 60 nm) (Wyckoff *et al.*, 1963); (5) approximately 80 million-year-old fossilized phalangeal bone of dinosaurs from the Upper Cretaceous (banding periodicity about 60 nm) (Pawlicki *et al.*, 1966); (6) approximately 40 000 year-old soft tissue from a woolly mammoth calf entombed in permafrost (banding periodicity about 64 nm (Goodman *et al.*, 1980); and (7) approximately 1526 year-old trachea of Saint Cyriacus* (banding periodicity about 56 nm) (*Plate 515*).

The periodic banding of collagen fibrils in fossils and mummies is as a rule difficult to demonstrate in ultrathin sections because these biochemically altered fibrils† stain poorly or not at all with phosphotungstic acid or uranyl acetate. In the trachea of Saint Cyriacus (item 7), the collagen was very difficult to stain and only in a few zones some fibrils showing periodic banding were demonstrated. In the frozen woolly mammoth (item 6), however, the periodic banding was very well visualized. In keeping with this the amino acid composition of extracted polypeptides from the woolly mammoth were found to closely resemble those obtained from the modern African elephant tendon (Goodman *et al.*, 1980). Why the morphology of collagen fibrils is so well preserved even at the ultrastructural level over such long periods of time is not clear but one may argue that the tight packing, multiple hydrogen bonding, staggering and cross-linking between tropocollagen molecules, played an important role in maintaining the morphological integrity of these fibrils.

*Historical records show that in the year 362–363 AD, the Christian martyr Saint Cyriacus was killed by pouring molten lead into his oral cavity. As a bishop he spent part of his life in Ancona, Italy where his remains are still venerated. Professor G. Mariuzzi informs me that an autopsy performed in 1979 confirms this and also shows that Saint Cyriacus was starved (numerous prominent skin folds) and tortured, as evidenced by unrepaired fracture of the skull and a 30–40 day-old (at the time of death) fracture of the right femoral neck. Light microscopic studies by Professor Mariuzzi show that the trachea was well preserved and that mucosa, submucosa and ossified cartilage could easily be identified. Histochemically, lead was demonstrated on the tracheal surface and atomic absorption spectroscopy showed a 40 times higher concentration of lead in the trachea than in the skin (used as a control). Ultrastructural and electron-probe x-ray analytical studies carried out by Professor S. Cinti and myself also confirm the presence of lead in the trachea.

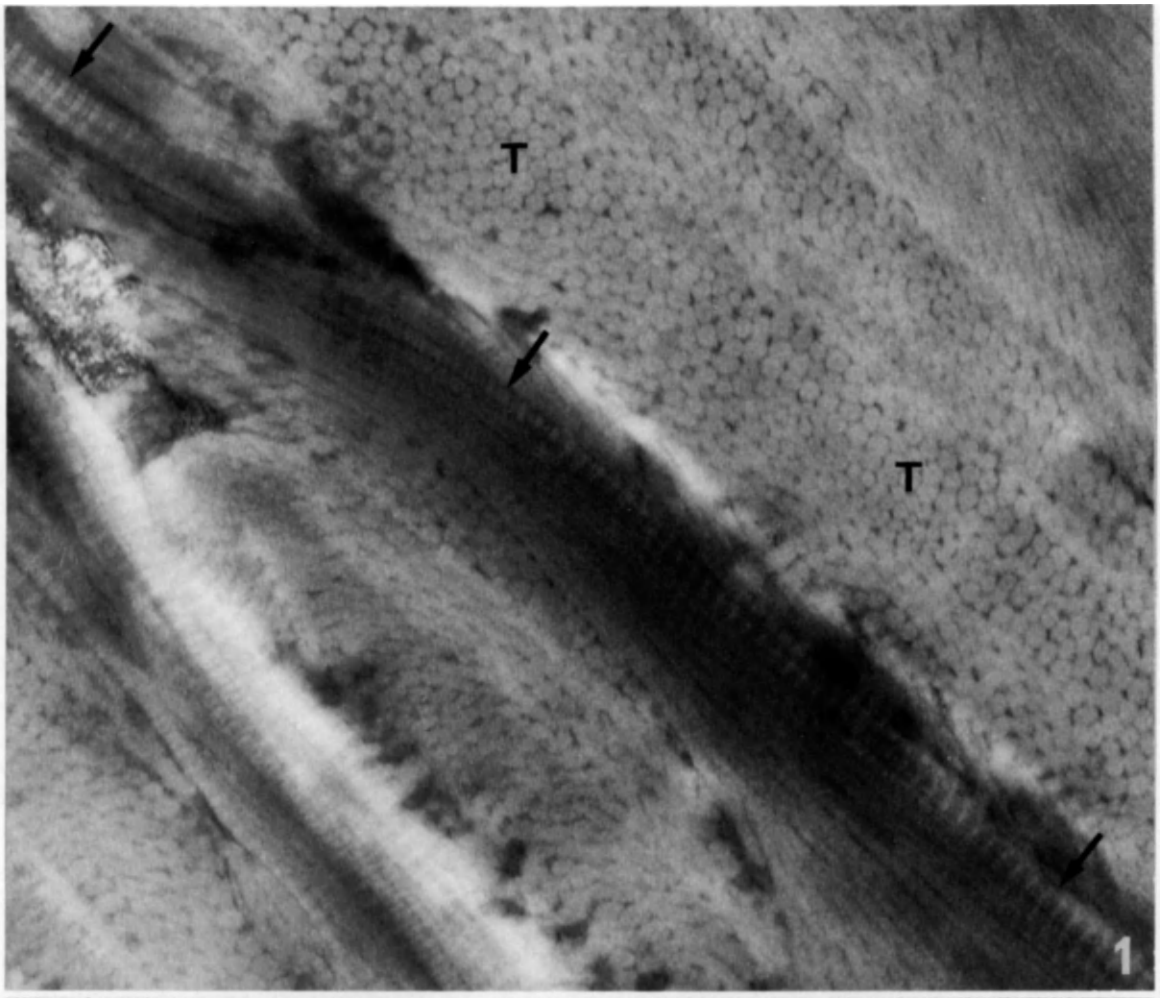
†Biochemical preservation is poorer than fine structural preservation, even so small amounts of hydroxyproline and hydroxylysine have been demonstrated in fossil bone from pleistocene deposits (Wyckoff *et al.*, 1963).

Plate 515

(*From a piece of mummified trachea of Saint Cyriacus supplied by Professors G. Mariuzzi and S. Cinti*)

Fig. 1. Collagen fibrils cut in various planes. Note the circular profiles of transversely cut fibrils (T) and the periodic banding (about 57 nm) of fibrils cut longitudinally. × 44 000

Fig. 2. The periodicity of banding of the collagen fibrils seen here is about 55 nm. × 72 000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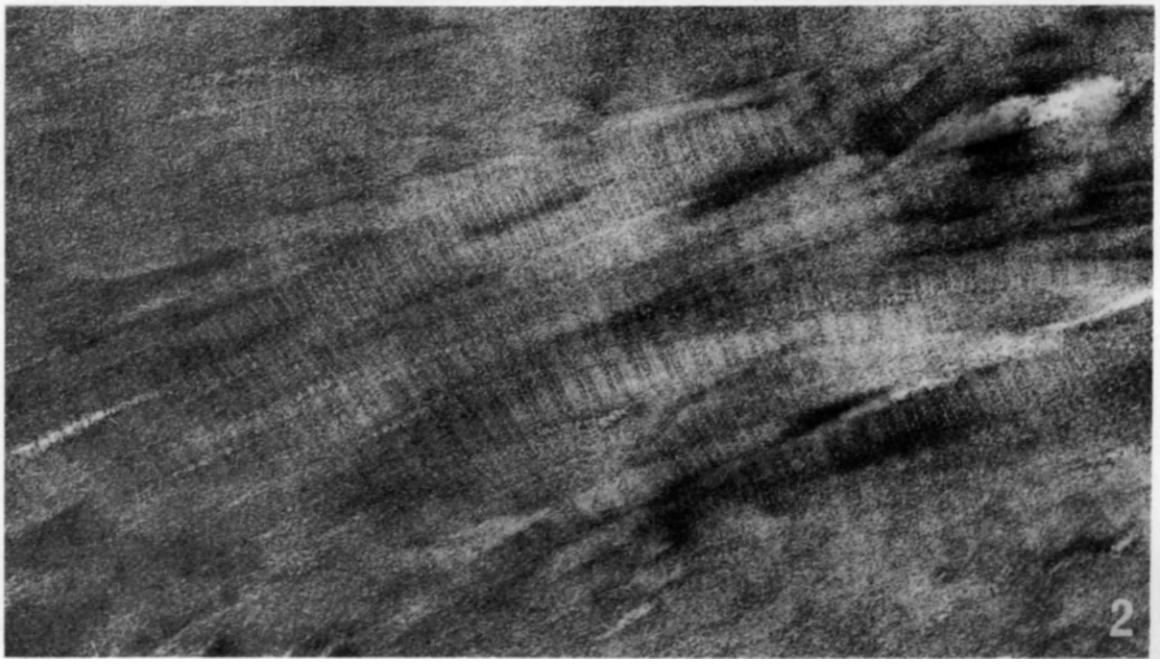
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Anchoring fibrils

The term 'anchoring fibrils'* is used to describe fibrils that tether the basal lamina (and hence indirectly the overlying epithelium) to the underlying connective tissue (*Plate 516*). In sectioned material they present straight, J-shaped or U-shaped profiles. The U-shaped profile is rarely seen because the chances of a given section cutting through both limbs of a U-shaped fibril are rather small. It is not too clear whether all the J-shaped and straight profiles are sections through portions of fibrils that are actually U-shaped. The J-shaped and U-shaped profiles of anchoring fibrils appear to hook or loop around a collagen fibril or fibrils.

In the skin anchoring fibrils have also been found attached to plaques or linear segments of dense material lying in the connective tissue under the basal lamina (*Plate 516*). This material is morphologically indistinguishable from the lamina densa of the basal lamina and like the lamina densa contain type IV collagens (Burgeson, 1987). These plaques have been regarded (Palade and Farquhar, 1965; Ghadially, 1982) as no more than profiles of tangentially cut undulating basal lamina at the dermoepidermal junction. However, it seems that this may not be the full explanation and it is claimed that many of these plaques are in fact discrete structures (i.e. not continuous with the basal lamina) lying free in the connective tissue matrix and that they should be called 'anchoring plaques' (Burgeson, 1987). From this stems the hypothesis that a network of anchoring fibrils interconnecting plaques and the lamina densa of the basal lamina is what anchors the epidermis to the dermis.

Anchoring fibrils were first described by Brody (1960) in the normal human skin. He called them 'reticular filaments'. Anchoring fibrils have been noted in: (1) normal human skin (Brody, 1960; Kobayasi, 1968; Swanson and Helwig, 1968; Briggaman and Wheeler, 1968, 1975a; Hashimoto, 1970; Briggaman *et al.*, 1971; Yaoita and Katz, 1976; Caputo and Peluchetti, 1977); (2) dermatitis herpetiformis (Yaoita and Katz, 1976); (3) gold rash from a case of rheumatoid arthritis (*Plate 516*); (4) cylindroma (Hashimoto and Lever, 1969); (5) skin of amphibians (*Rana*, *Bufo*, *Xenopus* and *Amblystoma*) and rat (Farquhar and Palade, 1965; Palade and Farquhar, 1965; Bruns, 1969; Bruns and Gross, 1970; Banks *et al.*, 1984); (6) notochord of *Rana* (Bruns, 1969; Bruns and Gross, 1970); (7) human oral mucosa (normal hyperplastic and carcinomatous) (Stern, 1965; Susi *et al.*, 1967; Frithiof, 1969); (8) normal oral mucosa of rat (Shimono and Clementi, 1976); (9) human gingiva (Schroeder and Theilade, 1966; Frank and Cimasoni, 1970; Nikai *et al.*, 1971; Sugimoto *et al.*, 1973; Takarada *et al.*, 1974); (10) human cervix and vagina (Younes *et al.*, 1965; Hackemann *et al.*, 1968; Rupec and Hoffmeister, 1969; Laguens, 1972); (11) normal mouse uterus (Rowlatt, 1969); and (12) human lung in severe pulmonary fibrosis

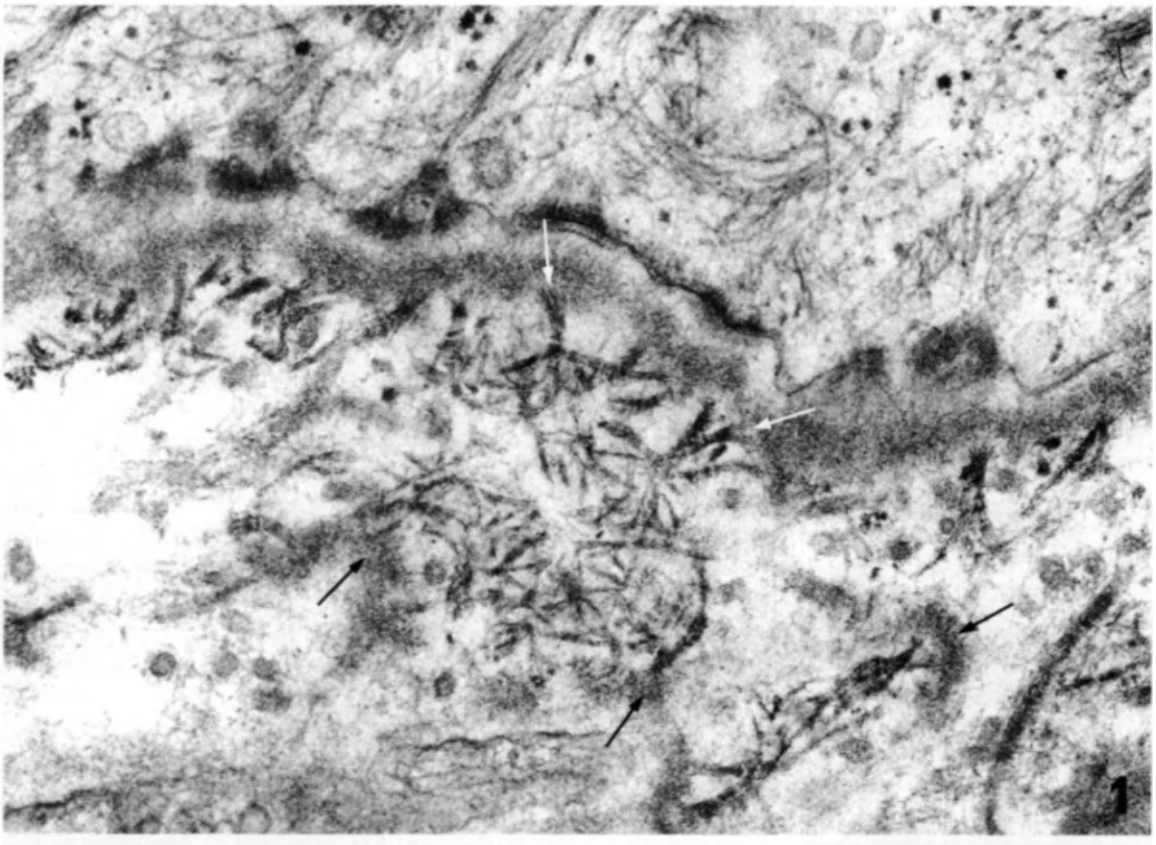
*At the dermoepidermal junctions, fine filaments extend from the plaques of hemidesmosomes, traverse the lamina lucida and terminate in the lamina densa. These filaments, which are presumably the equivalent of the link filaments which traverse the intercellular cleft of desmosomes (*see* page 1108), no doubt 'anchor' the cell to the lamina densa. Even so it is best not to call them 'anchoring filaments' as some authors have done because, as their name implies, anchoring fibrils (which form the subject of this section of the text) are composed of a bundle of anchoring filaments. To compound the chaos 'anchoring fibrils' have at times been called 'anchoring filaments', 'bundles of anchoring filaments' and 'anchoring fibres'. I would suggest that the term 'link filaments' be used to describe the filaments attaching or linking the cell to the lamina densa and anchoring fibrils (composed of anchoring filaments) for the fibrils attaching or anchoring the lamina densa to the underlying connective tissue.

Plate 516

Skin biopsy from an area of cutaneous rash which developed in a case of rheumatoid arthritis after chrysotherapy.

Fig. 1. Numerous anchoring fibrils are seen attached to the lamina densa of the basal lamina. Note how some of the fibrils (white arrows) fan out at the site of attachment. Also seen are collections of anchoring fibrils attached to structures (black arrows) which may be regarded as tangentially cut basal lamina belonging to epithelium not included in the section or as anchoring plaques lying free in the matrix. ×48000

Fig. 2. High-power view of anchoring fibrils. A banded anchoring fibril (arrow) is hooking around a transversely cut collagen fibril (arrowhead). × 88000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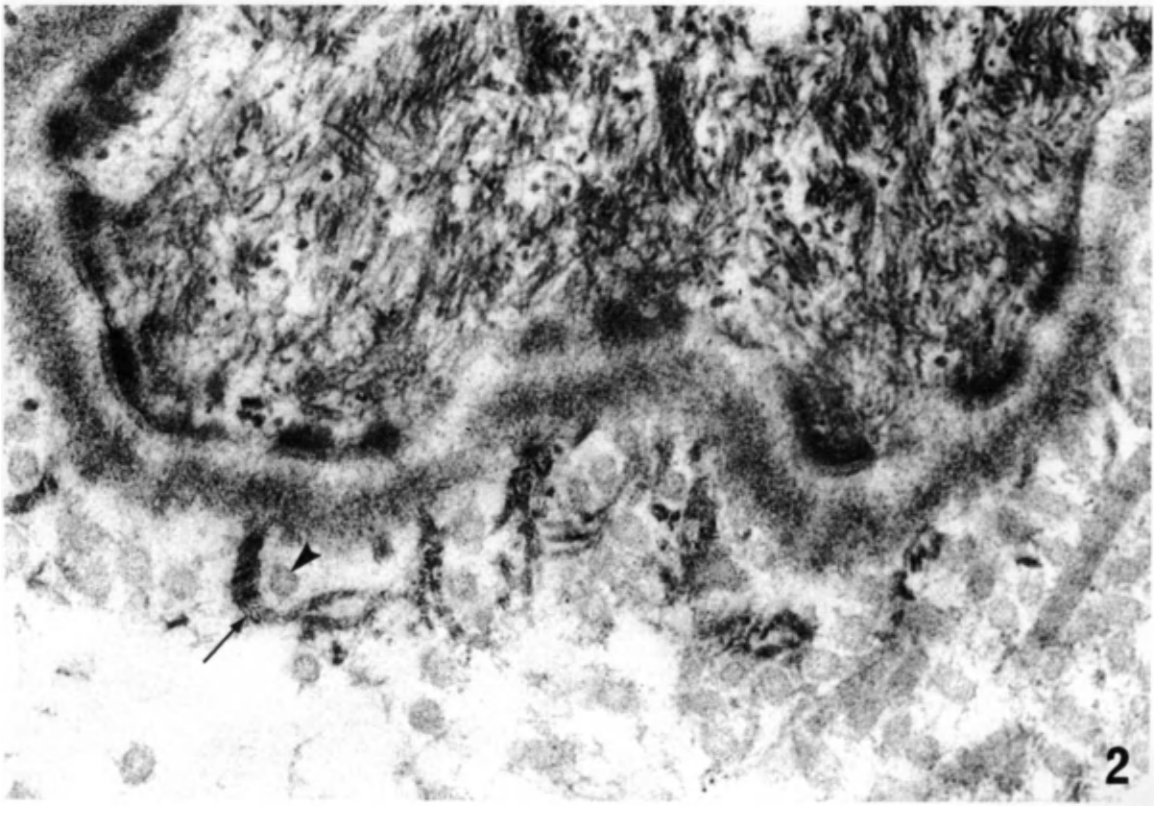
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(Kawanami *et al.*, 1978); and (13) malignant spindle cell tumour* (*Plate 517*).

A review of the above-mentioned literature shows that the anchoring fibrils in human material measure 20–60 nm in diameter and that they have a length of about 200–750 nm. Filaments forming these fibrils are at times clearly visualized, particularly at the expanded ends where they fan out to attach to the basal lamina. Based on their susceptibility to bacterial collagenase it has long been speculated that anchoring fibrils are essentially collagenous in nature, even though their banding pattern is different from that of native collagen fibrils (Briggaman and Wheeler, 1975a,b; Kawanami *et al.*, 1978). This suspicion has now been confirmed by the demonstration of type VII collagen in anchoring fibrils by immunofluorescence and ultrastructural immunolocalization techniques, and it has been shown that the length and banding pattern of anchoring fibrils is similar to that of segment long-spacing collagen crystallites produced *in vitro* from type VII collagen. (For reference *see* Mayne and Burgeson, 1987.)

As is well known (*see* page 1056) the basal lamina is formed in part or exclusively from epithelium, while the matrical collagen fibrils are usually formed by fibroblasts. Experiments (Briggaman *et al.*, 1971) utilizing recombinant skin grafts where epidermis separated from dermis (by trypsinization) is recombined with inverted dermis, confirm the epidermal origin of basal lamina and show that the anchoring fibrils are of dermal origin (presumably produced by fibroblasts).

Anchoring fibrils appear to be a morphologically distinct type of connective tissue fibril associated with the basal lamina of various epithelia. At least in one instance they may have been seen in connection with the external lamina along Schwann cells in unmyelinated nerve fibres in human skin (Swanson and Helwig, 1968)†. Interesting also is the finding of anchoring fibrils in fibrotic but not normal lungs (Kawanami *et al.*, 1978) for this suggests that such fibrils can develop or increase in numbers given an appropriate stimulus. Such a thesis is also supported by the observation that when the skin of rat ears is subjected to repeated mild frictional stimulation there is an increase in the numbers of anchoring fibrils (Banks *et al.*, 1984).

In dermatitis herpetiformis, the anchoring fibrils in the skin have been shown to bind IgA (Yaoita and Katz, 1976). In the skin of patients with epidermolysis bullosa dystrophica-recessive, the anchoring fibrils are said to be absent (Briggaman and Wheeler, 1975b), and it has been postulated that the defect in the disease is associated with impaired formation of anchoring fibrils. In epidermolysis bullosa dystrophica-dominant, the anchoring fibrils are present but in significantly reduced numbers or they may be few and rudimentary (Hanna *et al.*, 1983; Tidman and Eady, 1985).

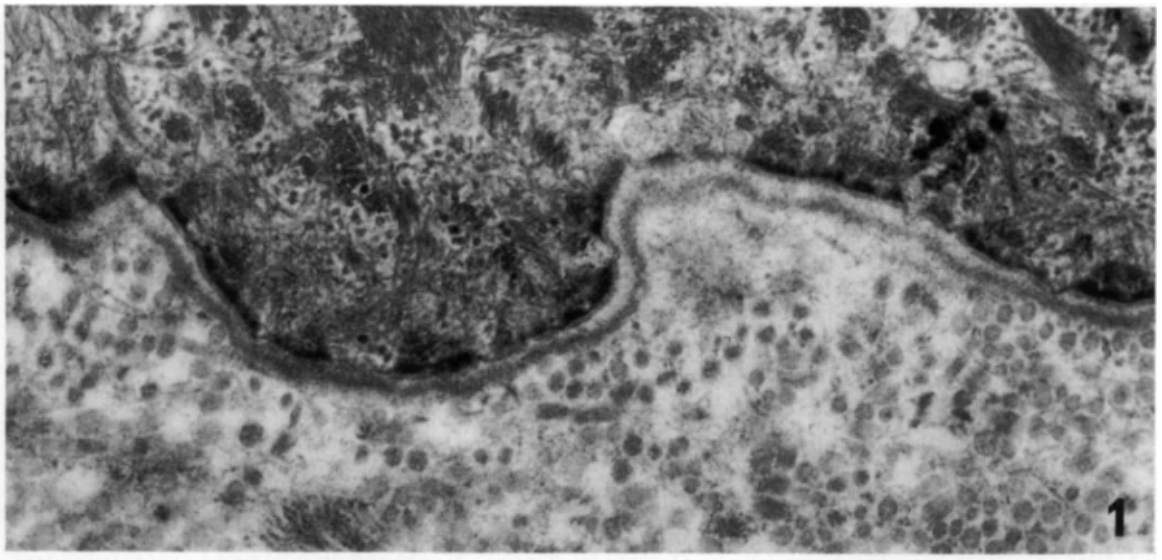
*This tumour arose as a nodule on the lower lip of a man aged 76 years. Histological diagnoses proffered by some 11 pathologists include: malignant fibrous histiocytoma, neurofibrosarcoma, malignant schwannoma, desmoplastic melanoma and spindle cell squamous carcinoma. The tumour infiltrated the mandible and extended to the pterygoid fossa. Ultrastructural examination of the mass in the pterygoid fossa showed groups of cells surrounded by interrupted basal lamina and segments of lamina densa or anchoring plaques in the matrix, to which were attached numerous anchoring fibrils. Various ultrastructural features suggest that the tumour is probably a spindle cell squamous carcinoma or a malignant schwannoma. Immunocytochemical studies carried out in several laboratories were inconclusive or conflicting. Thus for example the tumour was found to be negative for S100 protein in three different laboratories, and strongly positive in one laboratory. Immunoreactivity for cytokeratins was ambiguous or negative.

†I am unable to confirm this finding. Further, I know of no other report where anchoring fibrils have been seen in association with nerves be they in the skin or elsewhere. The illustration presented by Swanson and Helwig (1968) purporting to show anchoring fibrils associated with an unmyelinated nerve fibre is of poor quality and quite unconvincing. Further studies are needed to confirm or refute the idea that anchoring fibrils may occur in association with nerve fibres.

Plate 517

Fig. 1. Biopsy of grossly unaffected skin from a case of epidermolysis bullosa dystrophica. Note the absence of anchoring fibrils. In some areas of the specimen a few small slender rudimentary fibrils were present. × 50 000

Fig. 2. Malignant spindle cell tumour. Note the native collagen fibrils (arrows) and the numerous anchoring fibrils (arrowheads) attached to or radiating from structures acceptable as anchoring plaques or segments of basal lamina (B). Compare with *Plate 516, Fig. 1.* ×46 000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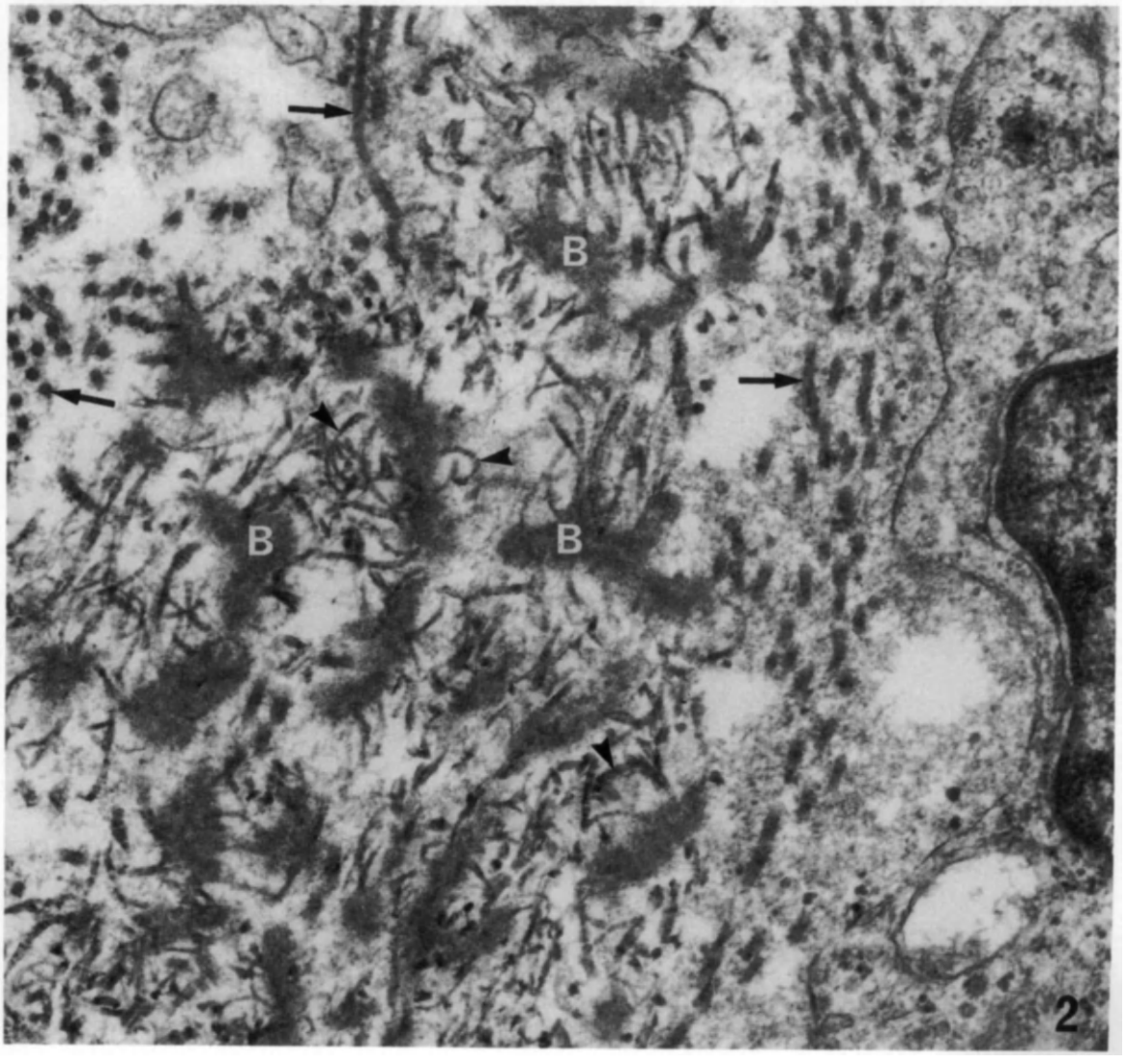
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Spiny collagen

The term 'spiny collagen' was coined by Banfield *et al.* (1973) to describe 'mature-type striated collagen fibrils with irregular granules of relatively dense material adhering to the band'. Zwillenberg *et al.* (1972) describe them as 'fibrils bearing cloud-like structures periodically attached to the fibrils' while Schafer *et al.* (1967) refer to them as fibrils showing 'beading at intervals of approximately 640 Å'. Although spiny collagen occurs in published illustrations it usually draws no comment from the authors (the list that follows includes such papers).

Spiny collagen (*Plate 518*) has been seen in: (1) cultures of fibroblasts grown in a medium containing 50 μg/ml of ascorbic acid (Schafer *et al.*, 1967); (2) wall of human varicose veins and bovine veins in culture (Zwillenberg *et al.*, 1971, 1972); (3) aorta of lathyritic chick embryo (Banfield *et al.*, 1973); (4) human heart* (Banfield *et al.*, 1973; Renteria *et al.*, 1976); (5) experimentally produced subcutaneous haematoma in the rabbit (Ghadially and Lalonde, unpublished observation) (*Plate 518*); (6) pulmonary adenomatosis of sheep (Perk *et al.*, 1971); (7) tumours produced in the rat by intramuscular injection of transformed tissue culture fibroblasts (Cornell, 1969); (8) mesenchymal renal tumour in an infant (Favara *et al.*, 1968); (9) human medullary thyroid carcinoma (Grimley *et al.*, 1969); and (10) Hodgkin's lymphoma (Stiller and Katenkamp, 1978); and (11) extrapulmonary silicotic lesions (Slavin *et al.*, 1985).

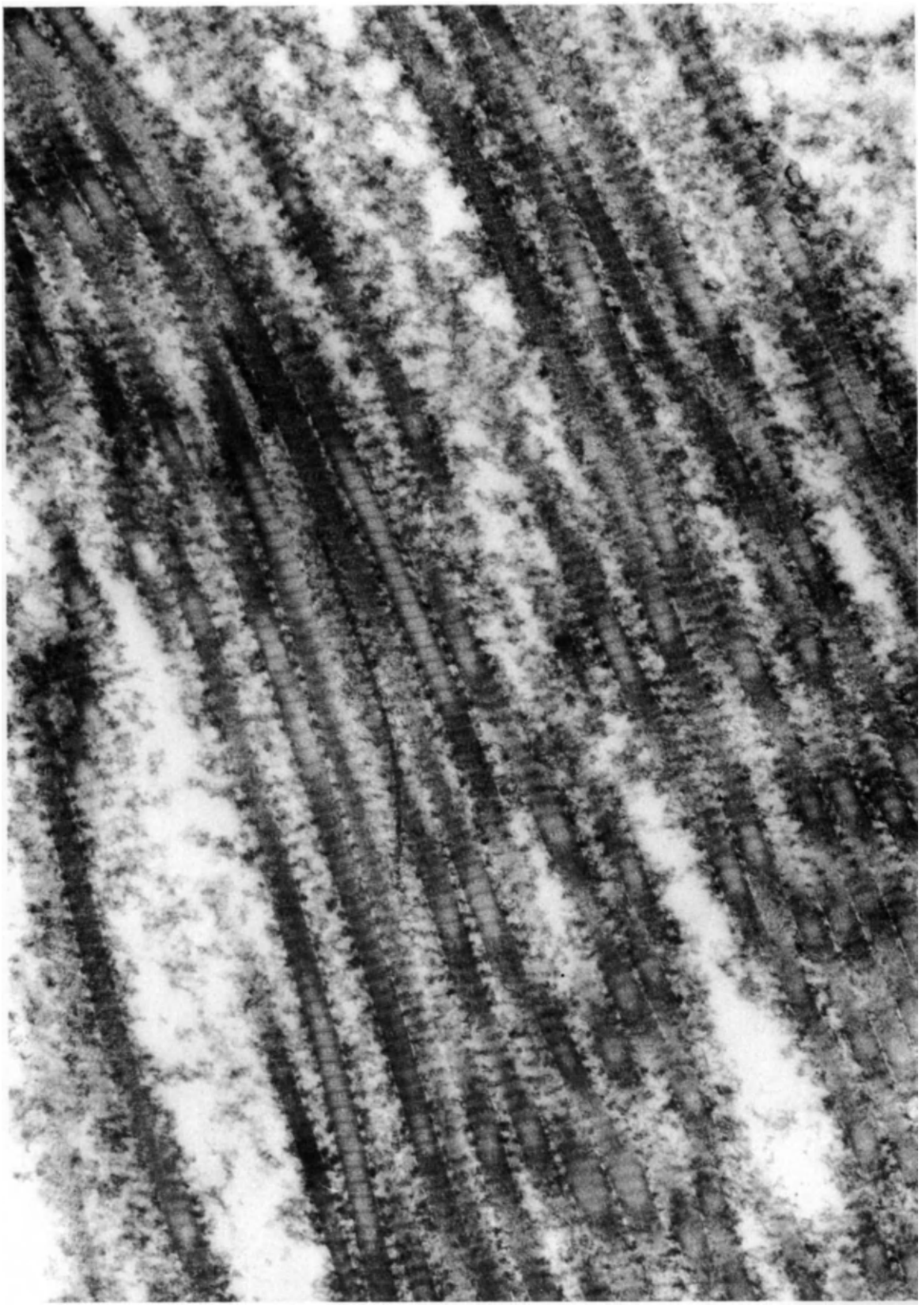
Little is known about the nature or composition of the electron-dense granules decorating spiny collagen. The significance of this change (i.e. formation of spiny collagen) is also obscure. It is not uncommon to find a few proteoglycan particles 'attached to' the collagen in the matrix of cartilage (*see* for example *Plate 538, Fig. 1*) or in the nucleus pulposus (Smith and Serafini-Fracassini, 1968). Arguing from such observations, Banfield *et al.* (1973) put forward the suggestion that 'spiny collagen is a manifestation of a proteoglycan–collagen complex'. This is a reasonable proposition but one cannot help but note that the morphology of the granules of spiny collagen is somewhat different from that of the proteoglycan particle seen in the matrix of most connective tissue (page 1272).

At higher magnifications the former has a woolly filamentous texture (suggesting a predominant protein component), while the proteoglycan particle is homogeneously electron dense.

*Found in biopsy of heart muscle in various pathological states. Said to occur more frequently in cases of acute rheumatic fever.

Plate 518

The spiny collagen shown in this illustration was found in the subcutaneous tissue of a rabbit, one hour after injection of autologous blood to produce a subcutaneous haematoma (*Lalonde and Ghadially, unpublished electron micrograph*). ×47 000



Fibrous long-spacing collagen

Terms such as 'fibrous long-spacing collagen', 'FLS collagen', 'FLS fibrils' and 'FLS fibres' have been employed to designate collagen fibrils where the periodicity is markedly greater than the 64 nm periodicity of the common or native collagen fibril. Such fibrils are also much wider (thicker) than the native collagen fibril.

Broadly speaking* two varieties of fibrous long-spacing collagen occur: (1) *in vitro* fibrous long-spacing collagen which usually exhibits a major banding periodicity of about 240 nm (range 200–280 nm) and bears several minor bands or striations. Here the tropocollagen molecules polymerize side by side in register, so that the length of the period is about the same as the length of the tropocollagen molecule; and (2) *in vivo* fibrous long-spacing collagen which usually has a major banding periodicity of about 120 nm (range 100–150 nm) and minor bands are as a rule absent or sparse†. Here the tropocollagen molecules polymerize side by side in a half-staggered arrangement, hence the length of the period is approximately half the length of the tropocollagen molecule. This type of fibrous long-spacing collagen fibril contains longitudinally oriented filaments which are as a rule clearly seen traversing the length of the fibril.

Fibrous long-spacing collagen was first produced *in vitro* by dialysing a solution containing tropocollagen (citrate extract of skin collagen) against water (Highberger *et al.*, 1950). Later it became apparent that adding serum acid glycoproteins to the solution before dialysis greatly increased the yield of fibrous long-spacing collagen (Highberger *et al.*, 1951) and that glycoproteins were incorporated into the fibrous long-spacing collagen fibrils (Gross, 1956).

The first report on the *in vivo* occurrence of fibrous long-spacing collagen was by Jakus (1956) who found banded structures in the Descemet's membrane of the cornea. Particularly fine examples of fibrous long-spacing collagen (called 'Luse bodies') occur in schwannomas (Luse, 1960; Luse *et al.*, 1963). Luse believed them to be peculiar to this type of tumour but this is not true any more, as will be apparent from the list that follows.

Fibrous long-spacing collagen has now been found in a variety of normal and pathological tissues and organs. These include: (1) normal Descemet's membrane (Jakus, 1956); (2) Descemet's membrane in cornea guttata and in Kayser-Fleischer rings (Jakus, 1962); (3) cornea injured by pronase (Mohos and Wagner, 1969); (4) trabecular meshwork of the eye in the

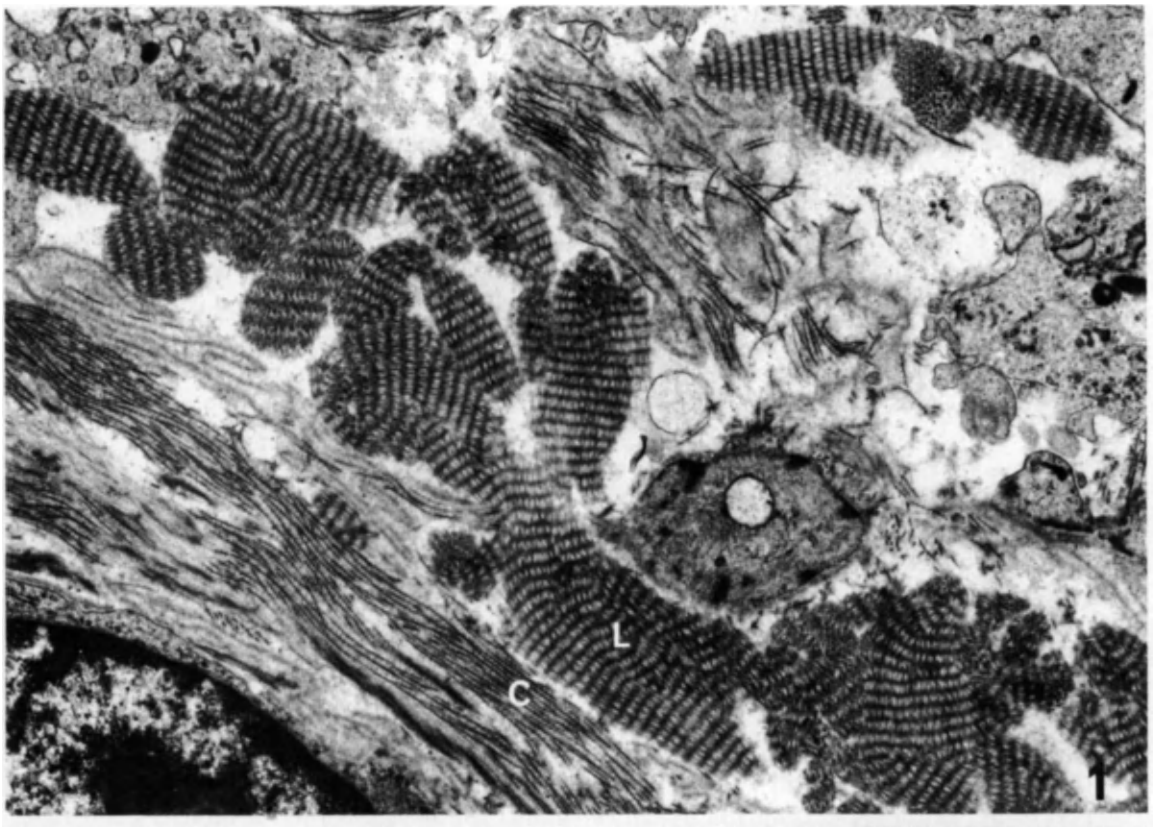
*The generalizations made in this paragraph are valid and useful, but there are exceptions. For example, four forms of *in vitro* fibrous long-spacing collagen (FLS) are known. The best known FLS I and II have a periodicity of about 230–260 nm, FLS III has a reported periodicity of about 100 nm (i.e. in this it resembles *in vivo* FLS) and FLS IV has a periodicity of about 170 nm (Chapman and Armitage, 1972).

†Three minor bands have been seen on a few occasions and Kimura *et al.* (1975) illustrate fibrous long-spacing collagen with nine minor bands, but this is exceptional.

Plate 519

Fig. 1. Schwannoma. Note the collagen (C), fibres, and Luse bodies (L). × 13 000 (*From Ghadially, 1980*)

Fig. 2. Higher-power view of Luse bodies. The axial periodicity is about 130 nm. × 46 000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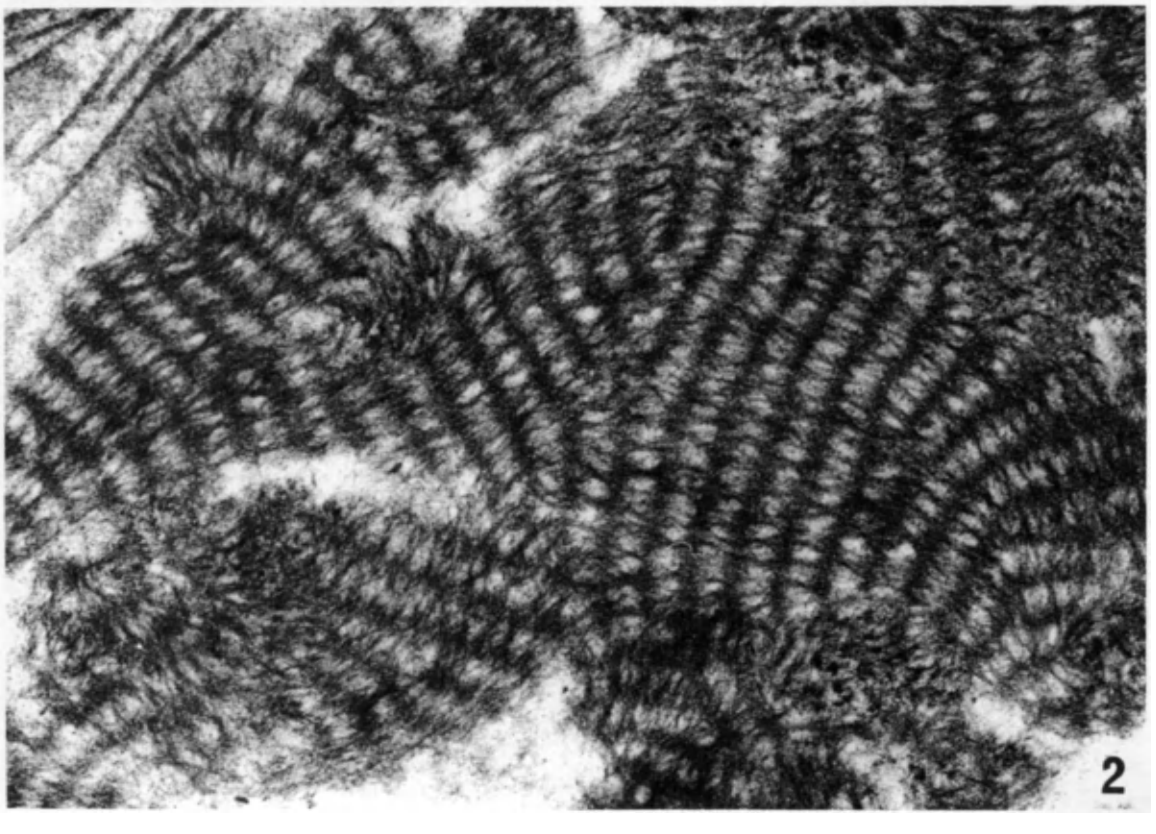
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normal state, in the exfoliative syndrome and in glaucoma (Garron *et al.*, 1958; Holmberg, 1965; Iwamoto *et al.*, 1971; Ringvold and Vegge, 1971; Vegge and Ringvold, 1971; Lütjen-Drecoll, 1972; Rohen and Witmer, 1972; Rohen *et al.*, 1973); (5) pectinate ligament of iris (Leeson and Speakman, 1961; Rohen, 1962; Spelsberg and Chapman, 1962); (6) macular region of the utricle (Friedmann *et al.*, 1965a); (7) human heart, normal and in rheumatic carditis (Banfield *et al.*, 1973); (8) cultures of heart fibroblasts (chick and mouse) (Goldberg and Green, 1964; Yardley, 1965); (9) fibromatosis hyalinica multiplex juvenilis (Woyke *et al.*, 1970); (10) angiofibromas in tuberous sclerosis (Bhawan and Edelstein, 1977); (11) cauda equina (Scholtz, 1975); (12) skin of patients with lepromatous leprosy (Edwards, 1975); (13) systemic hyalinosis (juvenile hyaline fibromatosis) (Ishikawa *et al.*, 1979); (14) experimentally constricted nerve (Pillai, 1964); (15) traumatic neuroma of the acoustic nerve (Hilding and House, 1965); (16) acoustic neurinomas (schwannomas) (Luse, 1960; Poirer and Escourolle, 1967; Cravioto and Lockwood, 1968; Friedmann, 1974; Ghadially, 1980) (*Plate 519*); (17) some peripheral nerve tumours in Von Recklinghausen's disease and other schwannomas (Friedmann *et al.*, 1965b; Ramsey, 1965; Fisher and Vuzevski, 1968; Gould and Battifora, 1976; Heine *et al.*, 1976); (18) blue naevus (Bhawan and Edelstein, 1976); (19) astrocytoma (Ramsey, 1965); (20) nucleus pulposus of the intervertebral disc in animals including humans (Smith and Serafini-Fracassini, 1968; Cornah *et al.*, 1970; Meachim and Cornah, 1970; Ghadially, 1978) (*Plate 520*); (21) articular cartilage of old mice (Silberberg and Hasler, 1970); (22) granuloma produced in lymph node by Freund's adjuvant (Bairati *et al.*, 1967); (23) lymph node in Hodgkin's disease and other lymphomas, lymphadenitis and thymomas (Mollo *et al.*, 1968; Mollo and Monga, 1971); (24) mouse haemangiosarcoma and canine mastocytoma (Banfield *et al.*, 1973); (25) murine osteosarcoma (Marquart *et al.*, 1976); (26) glomeruli in human amyloidosis and casein-induced amyloidosis in the rabbit (Kimura *et al.*, 1975); (27) kidney of patients with multiple myeloma (Schubert and Adam, 1974); (28) dermis in scleroderma (Kobayasi and Asboe-Hansen, 1972); (29) basal cell carcinoma, squamous cell carcinoma and melanoma (Hashimoto and Ohyama, 1974); (30) parathyroid carcinoma (Urbanski *et al.*, 1981*); (31) Ewing's sarcoma (Ghadially and Mierau, 1985) (*Plate 521*); and (32) extrapulmonary silicotic lesions (Slavin *et al.*, 1985).

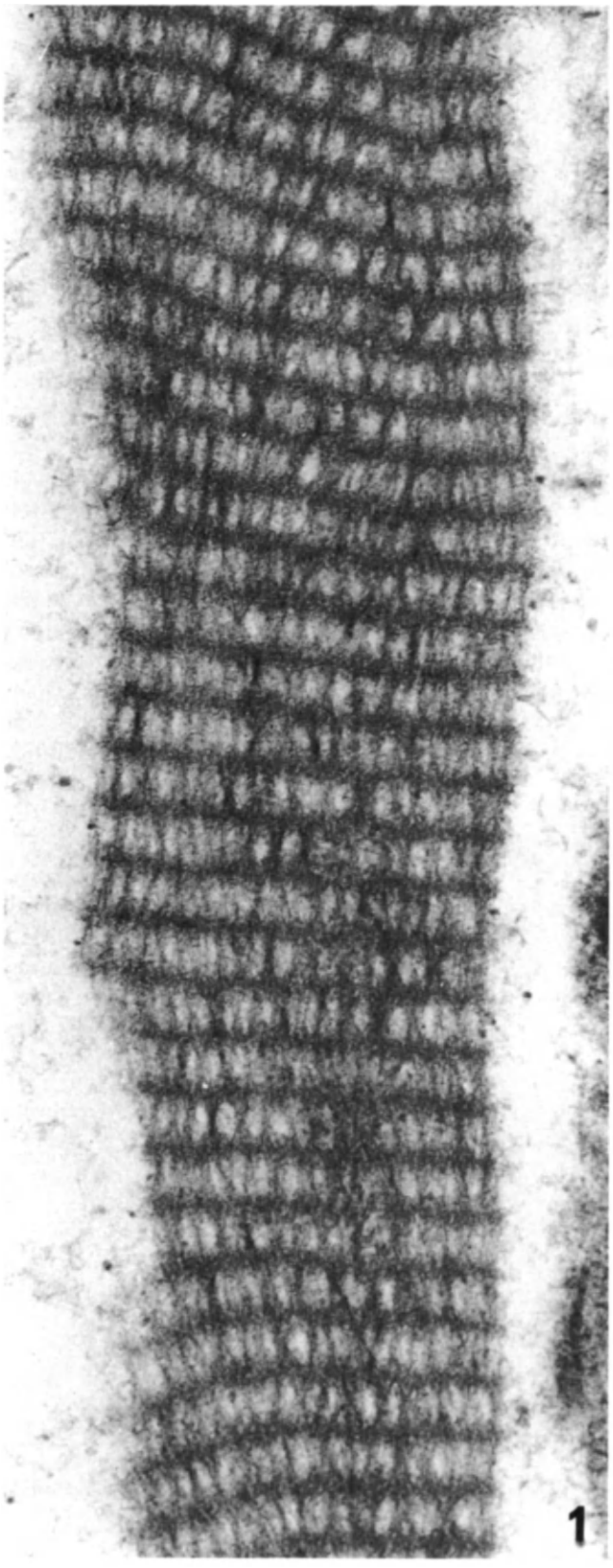
In the publications cited above the term 'fibrous long-spacing collagen' has often been employed to describe the banded structures seen in various tissues, but some workers doubt whether the material seen *in vivo* is the same as the fibrous long-spacing collagen produced *in vitro*. Such cautious students of the subject have adopted other terms like, 'banded structures', 'curly collagen', 'lattice collagen', 'sheath collagen', 'kollagenoid' and 'gitterkollagen'. Luse *et al.* (1963) state that 'By electron microscopy sectioned reconstituted collagen was indistinguishable from the fibrils present in Schwann cell tumours'. Unfortunately no

*They describe the structures they found as 'Luse bodies consisting of extracellular cross-banded structures with a periodicity of 735 Å'. Their description of these structures is difficult to comprehend. They seem to think that the width of the light band is a measure of the periodicity. My calculations of the periodicity of the structures illustrated in their *Fig. 4* show that the periodicity is a little over 100 nm and not 73.5 nm as they claim.

Plate 520

Fig. 1. Schwannoma (same case as in *Plate 519*) showing fibrous long-spacing collagen with a periodicity of approximately 125 nm. ×66 000

Fig. 2. Rabbit nucleus pulposus showing fibrous long-spacing collagen with a periodicity of approximately 100 nm. ×67 000 (*From Ghadially, 1978*)



illustrations are presented and there is no mention about the periodicity of banding. I am unable to support this statement because all the Luse bodies in the schwannomas that I have seen have shown the periodicity of banding (100–150 nm) and absence of minor bands one associates with *in vivo* fibrous long-spacing collagen and not the periodicity of banding (200–280 nm) and abundance of minor bands one associates with *in vitro* fibrous long-spacing collagen.

However, it is worth noting that not all fibrous long-spacing collagen produced *in vitro* is identical. While the classic variety has a major period of 240 nm and shows numerous minor bands, one can also produce fibrous long-spacing collagen *in vitro* which has a 100 nm periodicity and no minor bands (see for example illustrations in Ludowieg and Parker, 1973) and which is remarkably similar to the *in vivo* product. Conversely, we (Ghadially and Mierau, 1985) have seen banded structures in the stroma of an Ewing's sarcoma (*Plate 521*) acceptable as fibrous long-spacing collagen with a banding periodicity similar to that of *in vitro* fibrous long-spacing collagen*.

It has been shown that fibrous long-spacing collagen found in the nucleus pulposus contains sulphated glycosaminoglycans (Yardley and Brown, 1965; Smith and Serafini-Fracassini, 1968; Ludowieg and Parker, 1973). Furthermore, it has been shown that the extractable glycosaminoglycan is associated with a protein suggesting that it is a proteoglycan (Balazs, 1970).

Thus it would appear that both *in vitro* and *in vivo* fibrous long-spacing collagen comprises a combination of proteoglycans or glycoproteins and collagen, because, as mentioned at the beginning of this section, acid glycoproteins are needed in the collagen solution to produce fibrous long-spacing collagen. From this collective evidence one may speculate that one of the possible ways in which fibrous long-spacing collagen may form in a tissue is by depolymerization of existing native collagen fibrils and the repolymerization of the collagen molecules to form fibrous long-spacing collagen when this is favoured by a high concentration of glycosaminoglycans.

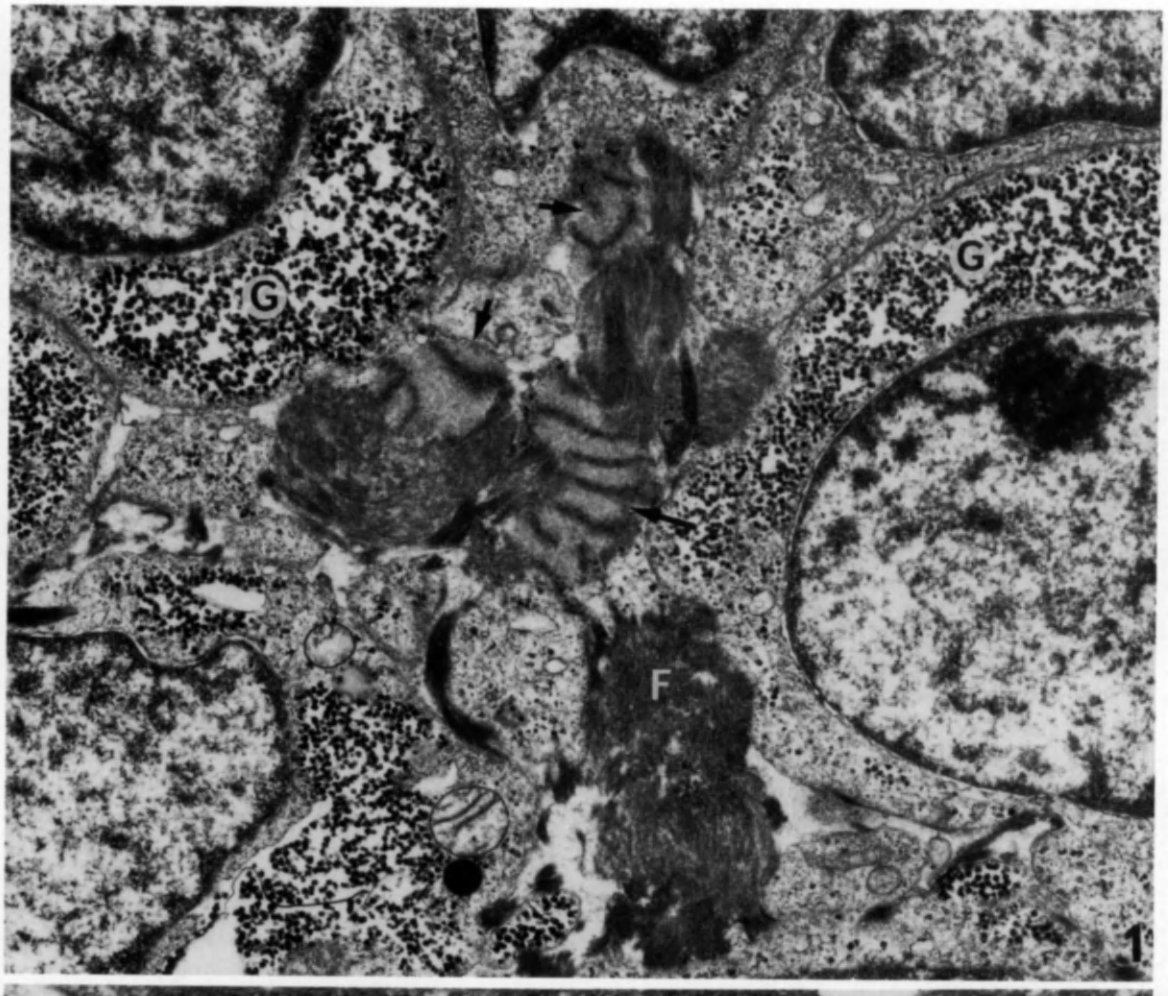
*In a largely haemorrhagic and necrotic specimen of Ewing's sarcoma, we found banded structures where the average periodicity of banding was 250 nm (range 200–276 nm). Morphological appearances suggested that these structures had evolved from the fibrin which was often closely associated with them. However, this idea was abandoned because the periodicity of banding of these structures is about ten times greater than the known periodicity of banding of fibrin (usually about 23 nm, range 19–35 nm). These structures have the same periodicity of banding as *in vitro* fibrous long-spacing collagen and about twice the periodicity of banding of *in vivo* fibrous long-spacing collagen. However, *in vivo* fibrous long-spacing collagen usually lacks the fine striations or minor bands of *in vitro* fibrous long-spacing collagen. Hence, it would appear that the banded structures in this tumour represent fibrous long-spacing collagen which shares the features of *in vitro* and *in vivo* types of fibrous long-spacing collagen.

Plate 521

Ewing's sarcoma (*From Ghadially and Mierau, 1985*)

Fig. 1. The tumour cells contain the characteristic focal deposits of glycogen (G). Fibrin (F) and banded structures (arrows) are seen between the tumour cells. The periodicity of banding of the fibril indicated by the long arrow is 252 nm. × 14 500

Fig. 2. High-power view of a banded fibril which has a banding periodicity of 272 nm. × 71 000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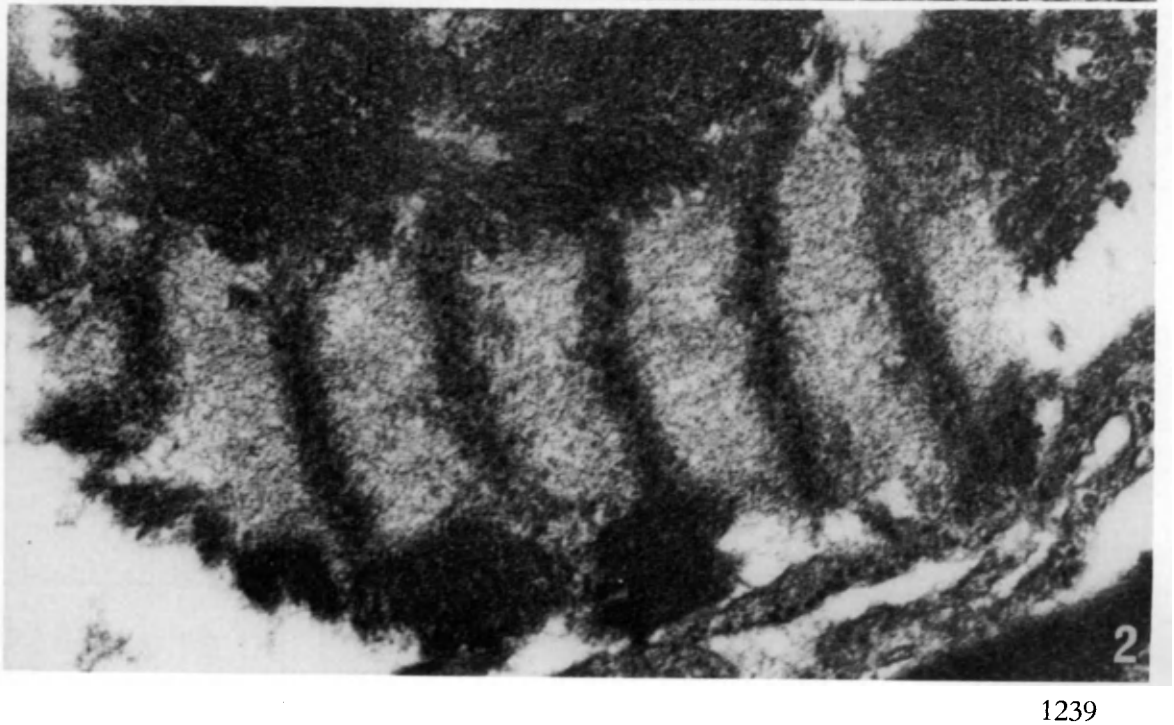
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Segment long-spacing collagen

Segment long-spacing collagen is a variety of long-spacing collagen which occurs as crystallites* or short segments, 240–280 nm long (i.e. about the same length as a tropocollagen molecule). This contrasts it from fibrous long-spacing collagen which occurs as quite long fibrils (Schmitt *et al.*, 1955; Bruns and Gross, 1973).

As mentioned before (page 1234) collagen of various morphologies can be produced *in vitro* from solutions of collagen. Native collagen is produced when a solution of tropocollagen is dialysed against 1 per cent NaCl; fibrous long-spacing collagen is produced when a solution of tropocollagen and acid glycoprotein of serum is dialysed against water; and segment long-spacing collagen is produced when ATP is added to an acidic solution of tropocollagen.

The length of the period in both forms of *in vitro* long-spacing collagen is usually about 300 nm in the wet state† and 240–280 nm in the dry state (i.e. length of a tropocollagen molecule) hence it is thought that they result from side to side in register polymerization of tropocollagen molecules‡ (Schmitt *et al.*, 1955; Bloom and Fawcett, 1975).

The first report on the occurrence of segment long-spacing collagen *in vivo* is by Pérez-Tamayo (1972); where *in vivo* segment long-spacing collagen crystallites found in carrageenen granulomas (produced in rat, rabbit and guinea-pig) were compared with *in vitro* segment long-spacing crystallites prepared from collagen extract of guinea-pig skin. The length of the *in vitro* crystallites prepared from guinea-pig skin was about 298 nm long, while the *in vivo* crystallites in sections from routinely processed Araldite-embedded carrageenen granuloma were about 282 nm long.

The only other report that I know of on *in vivo* segment long-spacing collagen is by Bockus *et al.* (1985) who illustrate an aggregate of these structures (*Plate 522*) in a spindle cell tumour of stomach. Measurements carried out on their published electron micrograph (their *Fig. 23*) indicate that the segments are about 375 nm long, which is much longer than the expected maximum of about 280 nm. In retrospect it is impossible to determine whether this figure (375 nm) is correct or stems from an error in assessing or recording magnification.§

*Because of the highly ordered substructure, not just segment long-spacing collagen but all forms of axially banded collagen including native collagen fibrils are rightly considered to be crystalline.

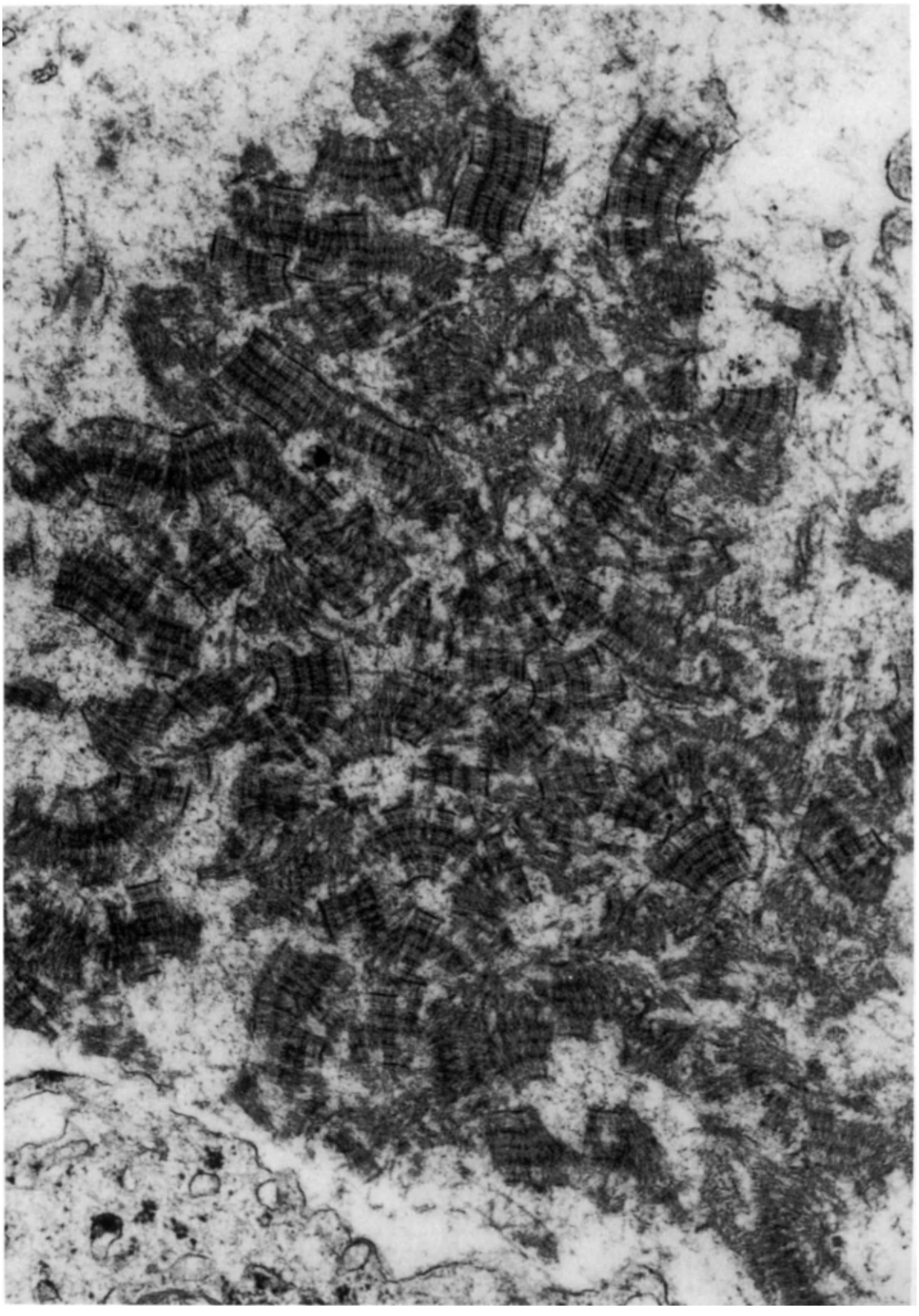
†The term 'wet state' refers to preparations derived by placing an aqueous suspension of collagen fibrils or crystallites on a coated grid. (Such preparations can be examined fixed or unfixed, stained (negatively or positively) or unstained). Needless to say, such specimens do suffer air drying and further desiccation when exposed to high vacuum in an electron microscope. Nevertheless, the shrinkage is consistently less than that seen in the 'dry state', i.e. in sections of routinely processed plastic-embedded material.

‡Segment long-spacing collagen can also be produced from acidic solutions of procollagen when ATP is added (Goldberg, 1974). These procollagen aggregates can be distinguished from tropocollagen aggregates because: (1) the former bear a bushy cap at one end (which represents the aminoterminal peptides) but the latter do not (because the aminoterminals have been excised by procollagen peptidase); and (2) the minor bands or striations are centrosymmetric. A sort of fibrous long-spacing collagen is also seen in such preparation, where between each period bushy masses of unexcised peptides are present. This bears little resemblance to the *in vitro* and *in vivo* fibrous long-spacing collagen described in the previous section of the text.

§It is virtually impossible to guarantee the accuracy of magnification of each and every one of the thousands of routine electron micrographs one takes over the years. This is so even in the best run laboratories with competent workers conscious of the many things that can go wrong. In view of such difficulties in the second edition of *The Cell* (Fawcett, 1981) magnifications are not given and he states 'Absence was considered preferable to inaccuracy in stated magnification'. This is an honest but rather drastic solution of the problem, which I feel reduces the value of the work. All magnifications stated in the 3rd edition of *Ultrastructural Pathology of the Cell and Matrix* have been calculated, recalculated and checked several times now. I am sure that the vast majority are accurate enough; but a rare one may be well off the mark. The reader should be cognizant of this fact.

Plate 522

Spindle cell tumour from wall of stomach showing an aggregate of segment long-spacing collagen. × 32 000 (*From Bockus, Remington, Friedman and Hammar, 1985*)



Giant collagen fibrils and amianthoid fibres

Normal or native collagen fibrils with characteristic periodic banding usually range in thickness from about 30*–120 nm, but fibrils up to about 160 nm have been found in rabbit semilunar cartilages (personal observation). However, much thicker collagen fibrils deserving to be called giant collagen fibrils have been seen in some species and in certain situations. Collections of giant collagen fibrils are found in a condition often referred to as amianthoid degeneration or amianthoid change†.

Amianthoid (asbestoid) change is one of several age-associated regressive changes seen in hyaline cartilage (Böhmig, 1929; Hass, 1943). It is characterized by the development of quite coarse fibres called 'amianthoid fibres', which in gross wet specimens of human costal cartilage present as silvery threads and plaques in the central regions of costal cartilage. Light microscopic studies show that amianthoid areas have less glycosaminoglycans in them than the surrounding cartilage and the lacunae in the vicinity of such areas are devoid of chondrocytes. Such findings are in keeping with the idea that amianthoid change is part of a regressive or degenerative change in cartilage.

This condition has also been called 'fibrillary transformation' and 'fibrous transformation' and light microscopic studies on the same or similar changes in: (1) laryngeal cartilage (Pascher, 1923); (2) tracheobronchial cartilage (Nevinny, 1927); (3) articular cartilage in degenerative joint disease (Grasset, 1960; Bennett *et al.*, 1942); (4) epiphyseal plates in acromegalic arthropathy (Urist, 1972); and (5) costal cartilage of small laboratory rodents (Dawson and Spark, 1928) have been published, but it is not too clear whether the structures described in all these instances are identical with amianthoid degeneration. Much doubt has been expressed by light microscopists about the nature of these fibres and in 1968 Bloom and Fawcett concluded that they 'have nothing in common with collagenous fibres'. However, ultrastructural studies (Hough *et al.*, 1973; Hukins *et al.*, 1976) on human costal cartilage, where amianthoid change occurs quite consistently between the second and the fourth decade, show that amianthoid fibres comprise aggregates of collagen fibrils of quite remarkable thickness up to about 1000 nm (i.e. 1 μm) in width or slightly greater. Such fibrils deserve to be called 'giant collagen fibrils'.

Situations in which collections of giant collagen fibrils (i.e. amianthoid fibres) have been seen with the electron microscope include: (1) human costal cartilage (Hough *et al.*, 1973; Hukins *et al.*, 1976); (2) lathyritic rat articular cartilage (Durroux *et al.*, 1972); (3) osteoarthrotic human

*Collagen fibrils less than about 30 nm thick usually show faint banding and few or no fine striations. Very fine unbanded collagen fibrils (i.e. about 20 nm or slightly less) no doubt exist, but without the characteristic banding it is difficult to confidently identify them.

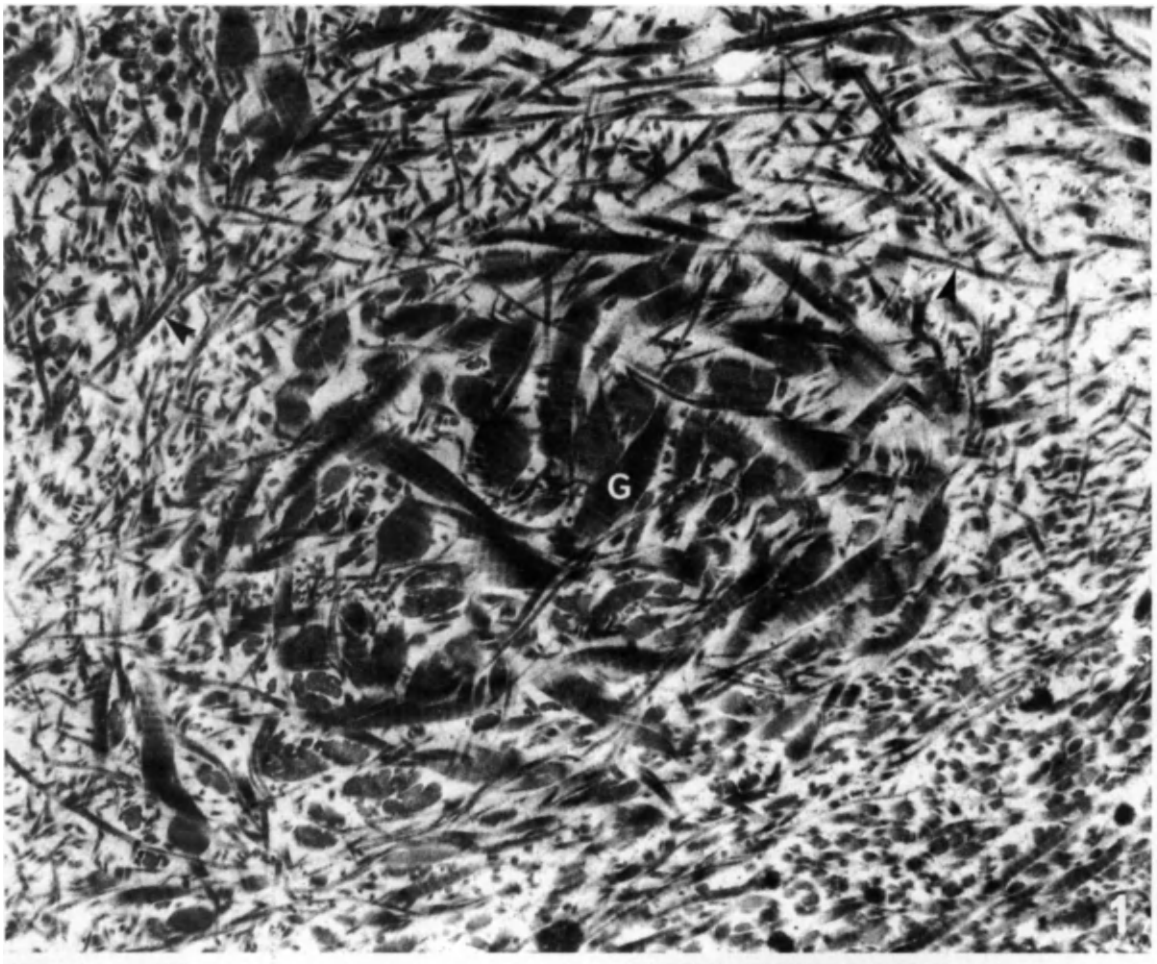
†The nomenclature used in this section of the text varies from that used in our previous papers (Ghadially *et al.*, 1979, 1980) on amianthoid fibres, where the term amianthoid fibre used by light microscopists was retained to describe the giant fibrils in these fibres, because of their large size (thickness) and because they probably represent a group of 'fused' collagen fibrils. The discovery of the giant collagen fibril in the gonopodium of the mosquito fish (Riehl, 1980) and the fact that not all unusually thick collagen fibrils are likely to be associated with degenerative changes, has led me to evolve the system of nomenclature used in this section, which is more in line with the classic concepts of fibrils and fibres presented on pages 1215–1221.

Plate 523

Giant collagen fibrils in osteoarthrotic cartilage (*From Ghadially, Lalonde and Yong, 1979*)

Fig. 1. A focal area of amianthoid change (microscar) in osteoarthrotic cartilage is seen here. Compare the thickness of the giant fibrils (G) with the native collagen fibrils (arrowheads). × 27 000

Fig. 2. Another focal area of amianthoid change showing transversely (T) and longitudinally (L) cut giant collagen fibrils. Major and minor bands are evident in the giant fibril (L) running horizontally across the picture but minor bands are not evident in the obliquely (O) cut fibril. × 66 000


G
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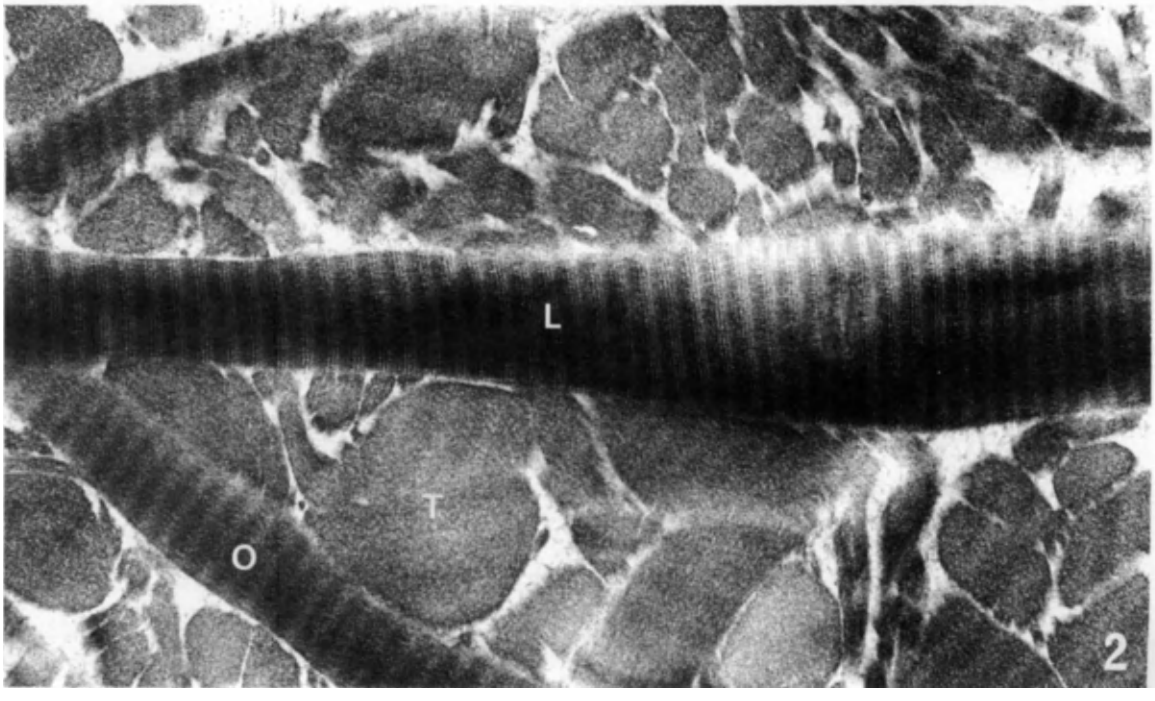
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2

articular cartilage* (Ghadially *et al.*, 1979) (*Plates 523* and *524*); (4) chondrosarcoma* (Ghadially *et al.*, 1980) (*Plate 525*); and (5) 'an unusual benign soft tissue tumour' which Connolly (1981) suggests should be called an 'amianthioma'†; and (6) synovial sarcoma and malignant schwannoma (Orenstein, 1983).

These ultrastructural studies have now clearly established that amianthoid fibres are collections of giant collagen fibrils some 6–10 times thicker (up to about 1 μm or slightly more) than the native collagen fibril (20–120 nm). Such a contention is now based not only on morphological appearances and periodicity determined by electron microscopy and high angle x-ray diffraction studies, but also on the fact that amianthoid fibres are susceptible to digestion by purified collagenase.

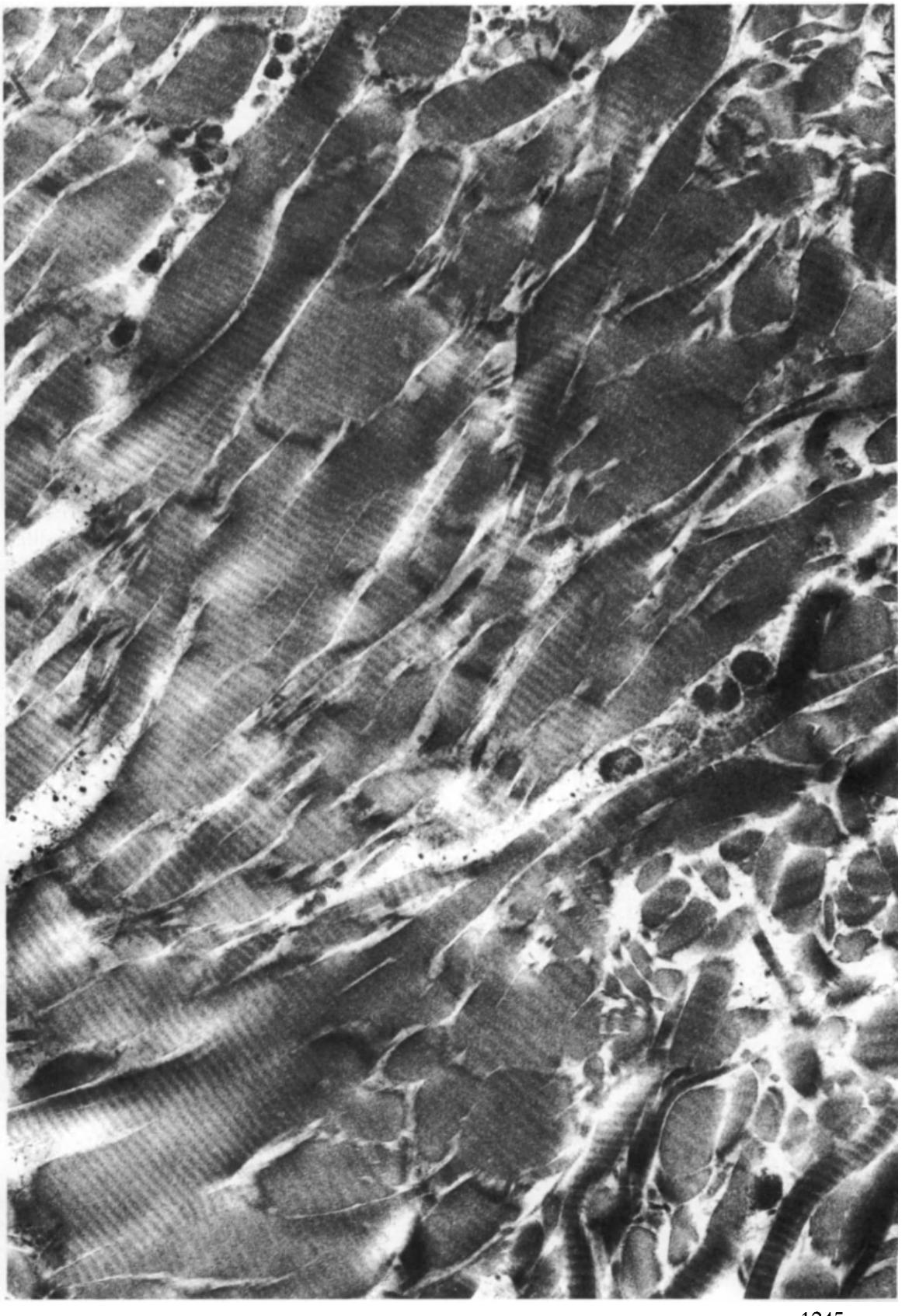
It is notoriously difficult to obtain accurate estimates about the periodicity of collagen fibrils from electron micrographs. Obliquity of sectioning, varying preparative procedures and the inevitable shrinkage due to dehydration are bound to affect the final figure. In ultrathin sections of giant collagen fibrils in amianthoid fibres in costal cartilage Hough *et al.* (1973) found that the period length varied from 56 nm to 62 nm. The figure (55 nm) obtained by us (Ghadially *et al.*, 1979, 1980) for giant fibrils in osteoarthrotic cartilage and chondrosarcoma was slightly smaller, but this is almost certainly due to different degrees of shrinkage engendered by different preparative procedures. In support of this one may point out that in wet preparations of costal cartilage x-ray diffraction studies (Hukins *et al.*, 1976) show that the periodicity of the giant fibrils in amianthoid fibres is 67 ± 1 nm, a value comparable to that obtained for native collagen fibrils in wet tendon (beef Achilles 67 nm, rat tail 67.1 nm and kangaroo tail 67.3 nm) (Tomlin and Worthington, 1956; Hukins *et al.*, 1976). Thus there is no need to evoke the idea that atypical or abnormal collagen occurs in amianthoid fibres.

The manner in which amianthoid fibres develop and the significance of this change is not too clear. The idea that amianthoid fibres represent newly synthesized collagen is not too attractive for Hough *et al.* (1973) found a paucity of chondrocytes in the neighbourhood of such fibres in costal cartilage and the same was true for osteoarthrotic cartilage (Ghadially *et al.*, 1979) and for the chondrosarcoma we (Ghadially *et al.*, 1980) studied where most of the cells in the blocks of

*In our material (chondrosarcoma and osteoarthrotic cartilage) the dark and light bands were easily seen in all giant fibrils but the minor bands or striations were found only in fibrils up to about 450 nm thick.
†Fortunately this quite unwarranted term has not been adopted in the literature.

Plate 524

Giant collagen fibrils in a microscar in osteoarthrotic cartilage (same case as *Plate 523*). The major dark and light bands are easily seen but the minor bands are not evident. The periodicity is 55 nm. × 32 000 (*From Ghadially, Lalonde and Yong, 1979*)



tissue examined were degenerate or necrotic. On the other hand the idea that the giant fibrils in amianthoid fibres result from a falling together of pre-existing normal-sized collagen fibrils with their alternating dark and light bands in register is more attractive for histochemical studies (Hough *et al.*, 1973) have shown a paucity of glycosaminoglycans in amianthoid areas of costal cartilage, while we have noted a paucity of proteoglycan particles in the osteoarthrotic cartilage. In this connection it is worth recalling that other age-associated changes that occur in cartilage are a decrease in water content (Meyer and Kaplan, 1959; Linn and Sokoloff, 1965) and a reversal of the chondroitin sulphate : keratan sulphate ratio (Mathews and Glagov, 1966; Kröz and Buddecke, 1967). Amprino (1938) has suggested that amianthoid fibres result from a 'pulling out' or 'unmasking' of collagen from the interfibrillary matrix, while anoxia has been considered to be a factor (Rahlf, 1972) because this change is seen in the central rather than the peripheral zone of costal cartilage.

It is now well known that with age collagen becomes more crystalline through cross linking and that the fibrils become thicker. For example, an age related increase in the width of the collagen fibril has been noted in rabbit articular cartilage (Davies *et al.*, 1962; Barnett *et al.*, 1963) and in the human iris (Okamura and Lütjen-Drecoll, 1973). Occasional collagen fibrils as wide as 240 nm have been demonstrated in osteoarthrotic cartilage by Weiss (1973). This author also illustrates a partially calcified collagen fibril 420 nm wide, but it is shown at such a low magnification that the banding is barely discernible, and the fact that it is encrusted with calcium salts makes it difficult to say what its true thickness is.

Be that as it may, there is little doubt that thicker collagen fibrils are seen in older articular cartilage whether it is normal or osteoarthrotic. One may therefore argue that the giant fibrils in amianthoid fibres are an exaggeration of the same process (i.e. an increase in width of collagen fibrils with age), but it is not clear whether spatial apposition through depletion of ground substance or cohesive forces (i.e. cross links) hold the component fibrils together.

However, ageing is hardly likely to be a factor in the production of amianthoid fibres in a malignant tumour like a chondrosarcoma, but the idea that this is a type of degeneration engendered perhaps by anoxia would be consistent with our findings.

Finally, it is worth noting that the occurrence of giant collagen fibrils may not always be an age-associated or a degenerative change for solitary giant collagen fibrils up to about 6 μm thick occur normally in the gonapodium of the mosquito fish (*Heterandria formosa*) (Riehl, 1980).

Plate 525

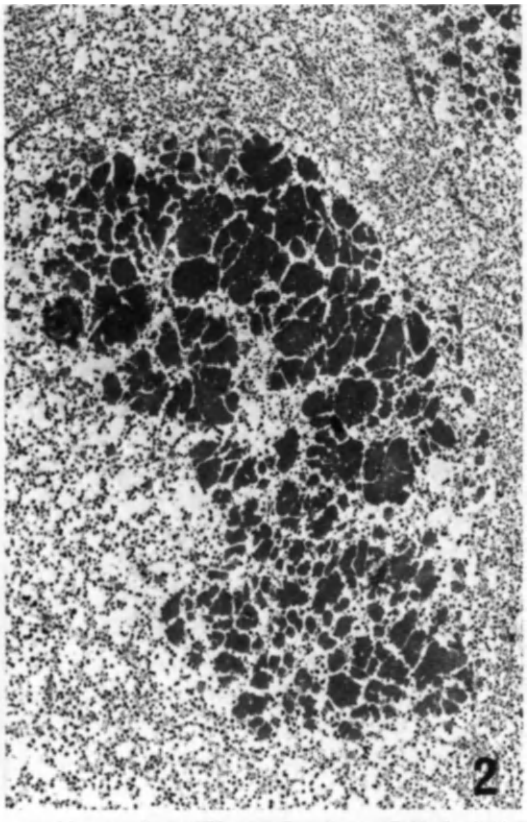
Amianthoid fibres in a chondrosarcoma.

Fig. 1. A collection of obliquely and longitudinally cut amianthoid fibres. × 5000

Fig. 2. A collection of cross cut amianthoid fibres. × 7000 (*From Ghadially, Lalonde and Yong, 1980*)

Fig. 3. In these giant fibrils major dark and light bands are clearly seen but not the minor bands. The length of the period (i.e. one light band + one dark band) is about 55 nm. The 'branched' appearance seen here supports the notion that the giant fibril derives from fusions of more slender fibrils. Note the continuity of the bands across the width (about 1 μm between arrowheads) of the giant fibril. × 44 000 (*From Ghadially, Lalonde and Yong, 1980*)


1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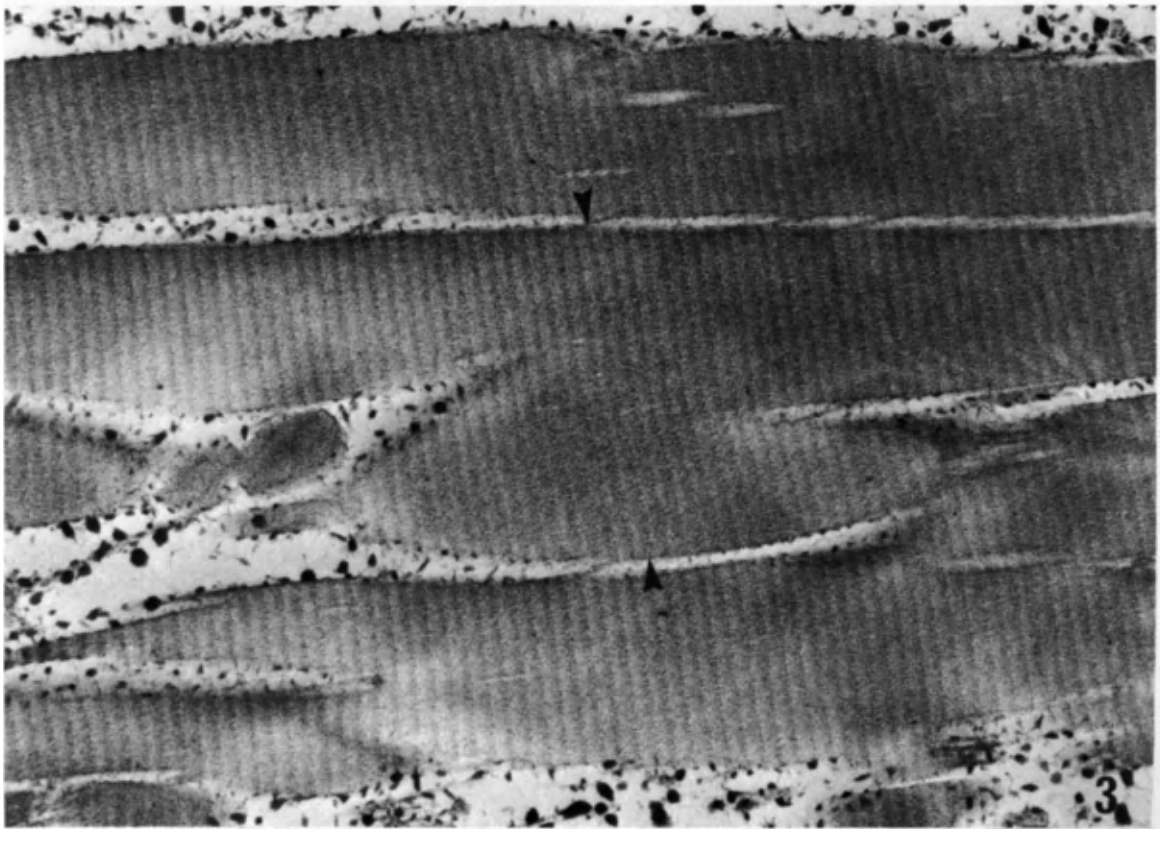

3

Spiralled collagen (poorly packed collagen and frayed collagen)

In the abnormality referred to as 'spiralled collagen' or 'helical collagen' the collagen fibril shows a spiralled or frayed appearance in longitudinal section. In cross-section it shows great variability in width and shape (which is no longer circular) and an increase in overall size. In some instances in cross-section it appears as a ragged mass beset by 'holes' or 'cavities' (moth-eaten appearance), while in others it has a 'flower-like appearance', 'stellate appearance' or a 'hieroglyphic appearance'. A review of the published electron micrographs suggests that while some of the smaller 'flower-like' or moth-eaten fibrils are altered or atypical solitary fibrils, the larger ones are probably derived by a falling together of two or more such fibrils. Spiralled collagen has now been seen not only in certain heritable connective tissue dysplasia where the skin is hyperextensible and fragile, but also in several other situations.

Spiralled collagen has been seen in: (1) the skin in some types of Ehlers–Danlos syndrome* (EDS-I and EDS-IV) (Steinmann *et al.*, 1975; Vogel *et al.*, 1979); (2) the skin of cattle and sheep with dermatoparaxis (O'Hara *et al.*, 1970; Simar and Betz, 1971; Fjolstad and Helle, 1974); (3) the skin of cats, dogs and mink with cutaneous asthenia (Patterson and Minor, 1977; Byers *et al.*, 1978); (4) epiphysis in a case of epiphysial dystosis (Ghadially and Wedge, unpublished observation) (*Plate 526, Figs 1 and 2*); (5) apparently normal human skin (on rare occasion a spiralled collagen fibril may be found) (Danielsen and Kobayasi, 1972); (6) dermal eruptions in pseudoxanthoma elasticum (Danielsen *et al.*, 1970); (7) shagreen patch (Kobayasi *et al.*, 1971); (8) elastosis perforans serpiginosa (Volpin *et al.*, 1978); (9) elastofibroma (Waisman and Smith, 1968); (10) lesions produced in human skin by Norway saltpetre (a mixture of calcium nitrate and ammonium nitrate) (Otkjaer-Nielsen *et al.*, 1978); (*Plate 526, Figs. 3 and 4*); (11) human emphysematous lungs (Belton *et al.*, 1977); (12) lungs of rats exposed to nitrogen dioxide (Stephens *et al.*, 1971); and (13) amyloid kidney (Ghadially *et al.*, 1981) (*Plate 527*); (14) atheromatous plaques in human carotid arteries (Laschi, 1985); (15) flexor digitorum longus tendons of mice treated with anabolic steroid (methandienone) (Michna, 1986); and (16) Ewing's sarcoma, giant cell tumour of bone, adenocarcinoma metastatic to bone (primary site unknown), leiomyoma, haemangioendothelioma, haemangiopericytoma, malignant lymphoma, neuroendocrine carcinoma of lung metastatic in skin, neuroendocrine carcinoma of skin (Hull and Warfel, 1986).

A priori one may argue that there are two ways in which the appearance we call 'spiralled collagen' may develop: (1) a defective aggregation (i.e. a packing defect†) of collagen filaments

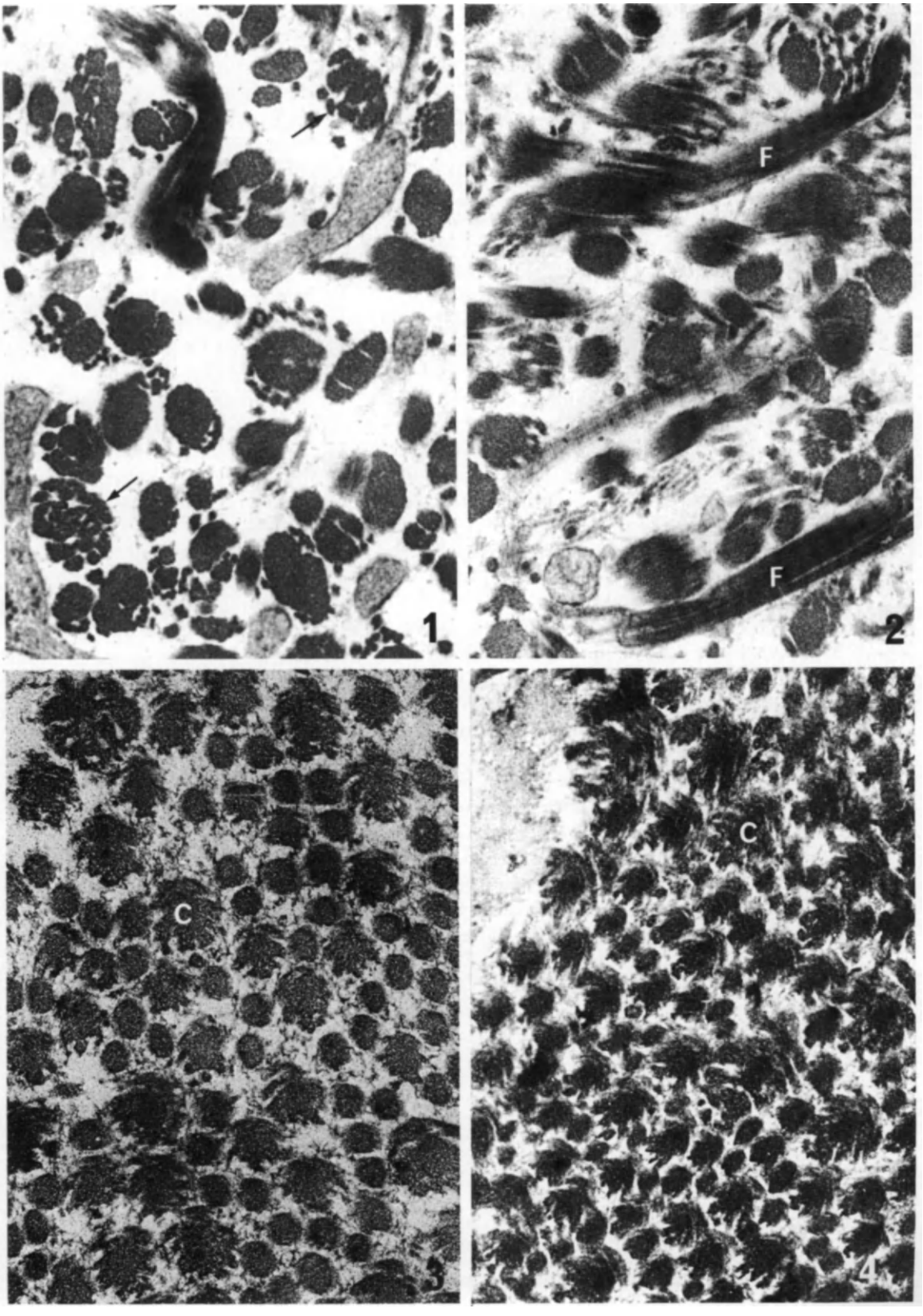
*Clinically, genetically and biochemically EDS is a heterogeneous group of disorders. In several of these disorders biochemical defects in collagen synthesis and metabolism have been identified. However, ultrastructural studies have been unremarkable except in cases of EDS-I and EDS-IV where spiralled collagen fibrils have been found. (For references *see* Vogel, 1979.)

†For biochemical and metabolic aspects of this (i.e. packing defect) and other disorders affecting collagen, the reader should consult the brief but excellent review by Minor (1980).

Plate 526

Figs. 1 and 2. Spiralled collagen from a case of epiphyseal dystosis. In transverse section (arrows) the fibrils have a ragged moth-eaten appearance. In longitudinal section the fibrils (F) appear spiralled or frayed. × 58000; × 56000

Figs. 3 and 4. The transversely sectioned spiralled collagen (C) in these electron micrographs has a flower-like appearance. It comes from skin injured by handling Norway saltpetre. × 47000; × 40000 (*From Otkjaer-Nielsen, Christensen, Hentzer, Johnson and Kobayasi, 1978*)


1
F
F
2
C
3
C
4

to form compact fibrils; and (2) dissociation, disaggregation or fraying of previously normal fibrils (i.e. an unpacking or unravelling phenomenon).

On surveying the list presented above one gets the impression that both mechanisms are valid and that either can produce the appearance we call 'spiralled collagen'. The idea of a genetic defect in collagen synthesis and polymerization, to form fibrils with suitable cross-links, is attractive in conditions such as the Ehlers–Danlos syndrome and dermatoparaxis in cattle and sheep, but obviously not in situations such as the lungs of rats exposed to nitrogen dioxide or amyloid kidney.

In the recessively inherited disorder, dermatoparaxis, in sheep and cattle there appears to be a deficiency of procollagen peptidase, which is the excising enzyme that removes the N-terminal extension of the procollagen molecule to form tropocollagen. This leads to an accumulation of procollagen molecules in the abnormal fibrils. It would appear that this extension impairs, but does not totally inhibit, fibril formation (Lapière *et al.*, 1971; Lenaers *et al.*, 1971; Lapière, 1973; Fjolstad and Helle, 1974). According to Bailey and Lapière (1973), the impaired mechanical properties of these fibrils (and hence the hyperextensibility and fragility of the skin) are probably related to 'the steric hindrances introduced by the uncleared polypeptide preventing the cross-linking reaction through the lysyl aldehyde near the amino-end of the α-chains'.

The situation regarding Ehlers–Danlos syndrome is quite complex; neither the biochemical abnormalities nor ultrastructural appearances of fibrils in every variety of EDS are known or fully documented. Thus for example in EDS-I to date no biochemical defect has been found, but spiralled collagen has been seen in the dermis (Vogel *et al.*, 1979). However, abnormalities of lysyl hydroxylation are found in EDS-VI and abnormalities of collagen fibril morphology are also evident (Holbrook and Byers, 1978).

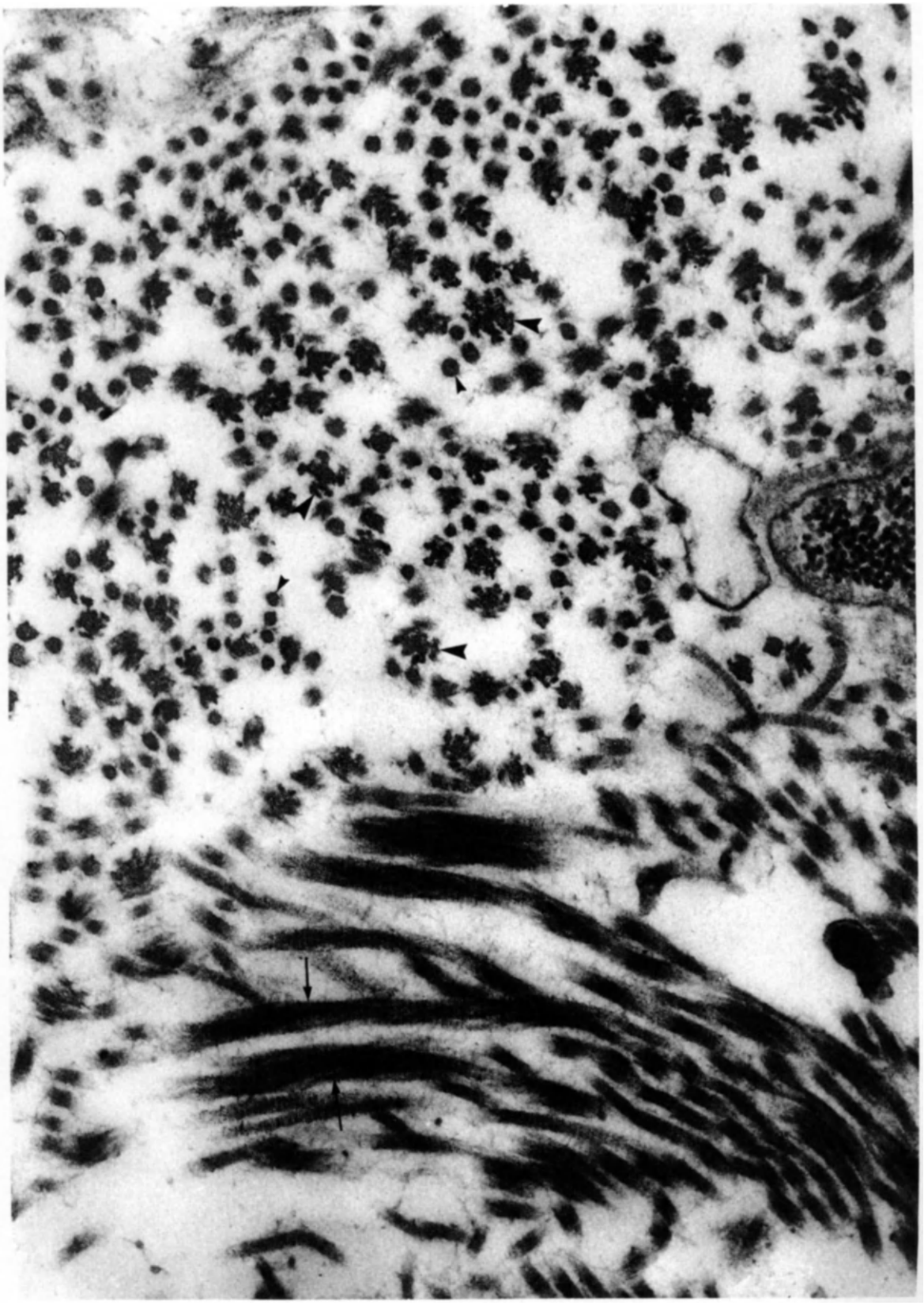
It is difficult to believe that a genetic defect is responsible for the formation of spiralled collagen in such diverse situations as skin exposed to saltpetre (*Plate 526*), amyloid kidney (*Plate 527*) and emphysematous lung. Here the alternative hypothesis that a disaggregation of fibrils (i.e. fraying) is involved seems more attractive*. As is to be expected such frayed fibrils would have a greater diameter than the normal fibril, but in some instances the frayed fibrils appear so large that one has to postulate that they are derived from two or three fibrils that run into each other as they expand in diameter.

It is worth recalling that the filaments in the native collagen fibril usually pursue a low pitched spiral course (in some but perhaps not all instances) (Ruggeri *et al.*, 1979), and so the spiralled appearance seen in the so-called 'spiralled collagen' described in this section of the book may be little more than a demonstration of this fact when fibrils dissociate or are not firmly packed during their genesis. The experiments of Kischer *et al.* (1980) underline this point by showing that when collagen fibrils dissociate *in vitro* after exposure to urea they also present an appearance somewhat similar to the spiralled collagen described in this section of the text.

*Yet another possibility would be that normal fibrils are destroyed and replaced by abnormal poorly packed fibrils. However, there is no evidence to support such a contention. This combined with the fact that the turnover rate of collagen in the adult human is so slow as to be barely detectable argues against this suggestion.

Plate 527

Longitudinally cut (arrows) and transversely cut (large arrowheads) spiralled collagen found in a biopsy specimen of amyloid kidney. Some normal (small arrowheads) transversely cut collagen fibrils showing circular profiles are also present. × 61 000



Elastic fibres, elaunin fibres and oxytalan fibres

These three 'types' of fibres constitute what some authors call the 'elastic system' or the 'elastic system fibres' (Gawlik, 1965; Cotta-Pereira *et al.*, 1976a). In many, but not in all, situations they represent developmental stages of elastic fibre formation; the order being oxytalan fibres, elaunin fibres and elastic fibres. It is, however, essential to study the better known elastic fibres first and the less well known oxytalan and elaunin fibres later.

Ultrastructural studies have shown (*Plate 528*) that elastic fibres* have two distinct morphological components, and it is now established that they represent two discrete proteins (Ross and Bornstein, 1969, 1970; Ross, 1971). In mature elastic fibres the bulk of the elastic fibre (about 90 per cent) comprises the well known protein elastin which has been known to light microscopists for over a 100 years because of its characteristic staining properties. In routine electron microscopic preparations this component presents an amorphous appearance; the electron density ranges from lucent to moderately electron dense†. The other component of the elastic fibre is an electron-dense filament about 11 nm in diameter. Filaments and aggregates of such filaments (i.e. fibrils) occur on the surface and within the amorphous elastin of elastic fibres. Such filaments and fibrils are also found lying free in the extracellular matrix. Here they are called oxytalan filaments or fibrils and as discussed later (page 1254), elastic fibres develop by deposition of elastin within such fibrils.

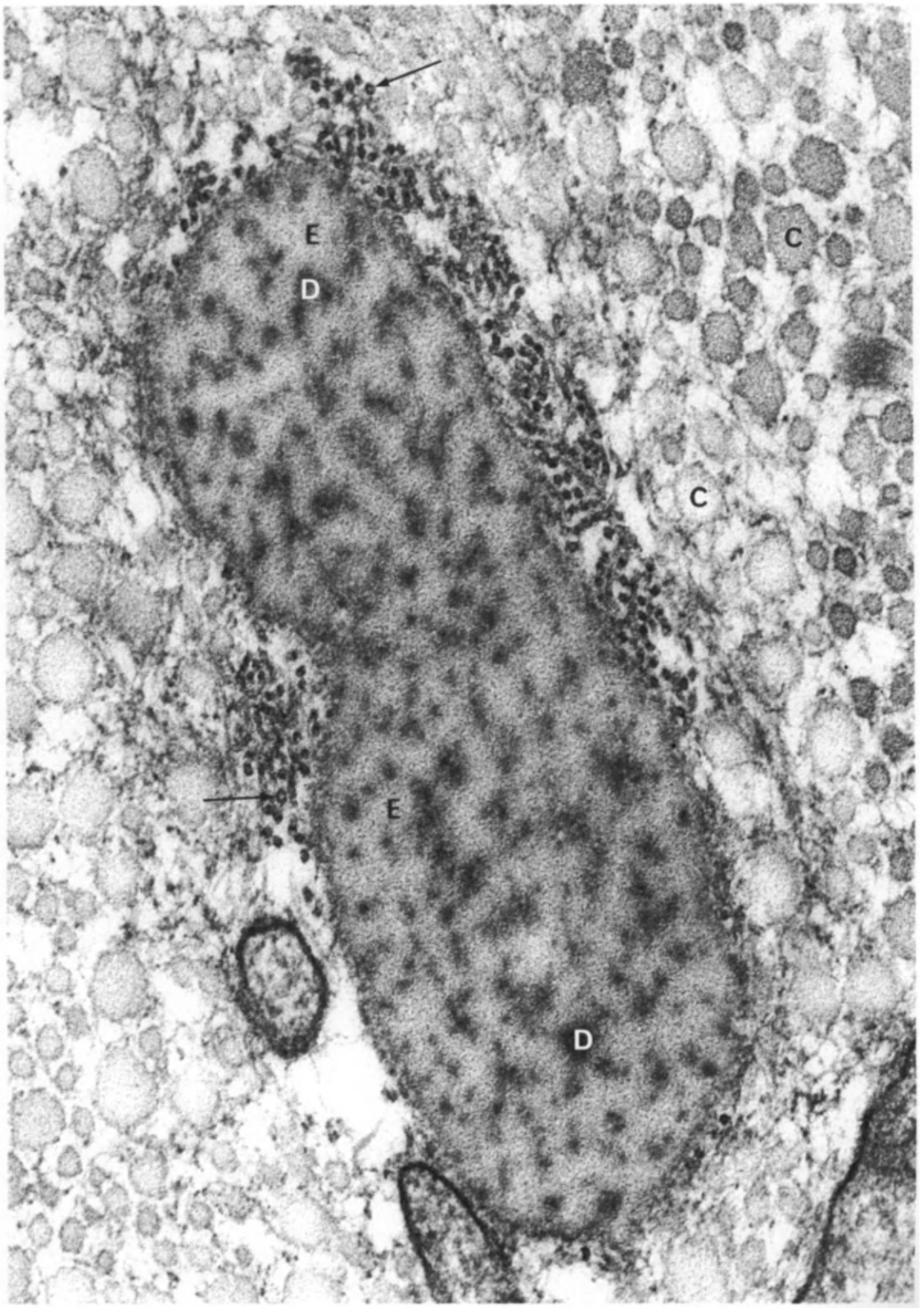
These electron-dense filaments (i.e. oxytalan filaments and electron-dense filaments associated with elastic fibres) have at times been called by various terms such as 'fibrotubules', 'tubulofilaments' and 'filamentous tubules'. Many workers believe that such terms are undesirable and inappropriate and that these designations spring from the fact that in ultrathin sections stained with lead alone (or when staining with uranium has more or less failed) the stain deposits on the surface of the filament but does not stain the bulk of the filament so that a spurious image suggesting a tubular structure is seen (Fahrenbach *et al.*, 1966). However, others (Inoué and Leblond, 1986) claim that these structures are truly 'hollow' or 'tubular' in nature, and some authors (e.g. Garner and Alexander, 1986) have called them 'electron-dense microtubules' (because their diameter is only about 10–13 nm and not over 30 nm as is the case with tubules. *See* footnote on page 938). My experience is that when this 'microtubular appearance' is seen, the staining of adjacent collagen fibrils is patchy or the fibrils present as 'hollow collagen'. Thus the capriciousness of staining seems to determine whether these structures present as filaments or microtubules (*Plate 528*).

*Besides elastic fibres there also occur elastic lamellae, such as the elastica interna and elastica externa of arteries.

†Variations in staining density may be found in sections from the same tissue block. Such variations may also be age dependent, the mature older fibres being more electron lucent. There may also be site dependent differences. Skin elastin seems to stain more heavily than aortic elastin (personal observation). Variations in preparative techniques also affect staining density (Bartman, 1968).

Plate 528

Elastic fibre from a human semilunar cartilage removed at surgery. Some of the elastic fibre filaments have a microtubular appearance (arrows) on cross section. Note also the paler staining elastin (E) and the dense (D) zones which probably represent filaments trapped within the elastin. The surrounding collagen fibrils (C) show much variation in staining density. This combined with the microtubular appearance presented by some of the elastic fibre filaments suggests a less than optimum staining of the section. × 105 000


E
D
C
C
E
D

For some time now the consensus of opinion has been that these electron-dense filaments are composed of or contain glycoproteins which are rich in polar amino acids (hydrophilic amino acids), contain much less glycin than in elastin and have no hydroxyproline, desmosine and isodesmosine (Ross and Bornstein, 1969; Cleary and Gibson, 1983). Later work (immunohistochemical studies) suggests that fibronectin (a glycoprotein) occurs as a coat on these filaments (Goldfischer *et al.*, 1985; Schwartz *et al.*, 1985) and that the filaments contain amyloid P component (a glycoprotein) (Breathnach *et al.*, 1981). Inoué and Leblond (1986) and Inoué *et al.* (1986) propose that these electron-dense filaments comprise a stack of pentagonal amyloid P components surrounded by a spiralling band with protruding spikes of unknown chemical composition.

Elastin, the amorphous component of the elastic fibre is resistant to digestion by trypsin, to boiling and to hydrolysis by weak acids and alkali. It is composed mainly of hydrophobic nonpolar amino acids. Unlike collagen it has a high content of valine, little hydroxyproline and no hydroxylysine. However, elastin bears some resemblance to collagen in that about one third of the residues of elastin are glycine and about 11 per cent proline. Elastin also contains two unusual amino acids desmosine and isodesmosine which act as covalent cross-links between the polypeptide chains.

In routine light microscopic or routine electron microscopic preparations elastin does not show a fibrillar or filamentous substructure. However, elastin is said to show a fibrillar substructure with the scanning electron microscope and negatively contrasted preparations of elastin show the presence of 'filaments' or fibrils about 2.5 nm in diameter (Gotte and Serafini-Fracassini, 1963; Gotte *et al.*, 1965, 1972; Kewley *et al.*, 1977; Serafini-Fracassini *et al.*, 1978).

The manner in which elastic fibres develop (elastogenesis) (*Plates 529* and *530*) has been studied in a variety of situations such as: (1) flexor digital tendon of fetal rat (Greenlee *et al.*, 1966); (2) fetal and adult bovine ligamentum nuchae (Fahrenbach *et al.*, 1966; Greenlee *et al.*, 1966; Kewley *et al.*, 1978); (3) fetal chick and rat aorta (Takagi and Kawase, 1967; Kadar *et al.*, 1971; Ross and Klebanoff, 1971; Albert, 1972); (4) chick lung (Jones and Barson, 1971); (5) tendon and ligament from the horse (Parry *et al.*, 1978); (6) healing wound (Williams, 1970a,b); and (7) in tissue culture (Ross, 1971; Narayanan *et al.*, 1976).

In all the above mentioned studies it was noted that during elastogenesis the electron-dense elastic fibre fibrils are laid down first and later the elastin is laid down within the fibrils which serve as a scaffolding or template for its deposition. For example, in the digital flexor tendon the mature elastic fibres are cylindrical in shape and the aggregates of fibrils that form during early stages of elastogenesis have the same shape (Greenlee and Ross, 1967) and similarly, in the aorta the elastic laminae are preceded by sheet-like aggregates of electron-dense filaments (Ross *et al.*,

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Plate 529

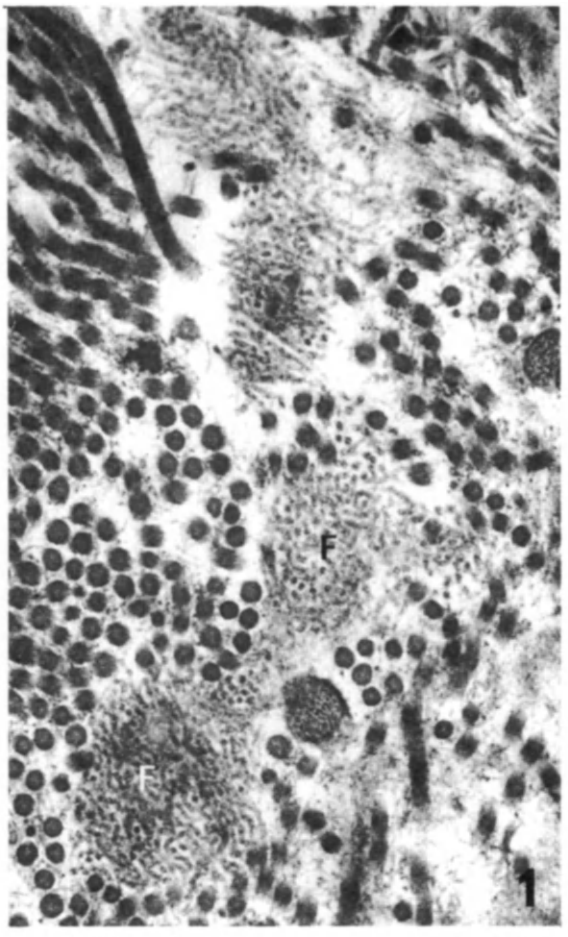
From biopsies of human skin.

Fig. 1 Collections of electron-dense filaments (F) which are the equivalent of the oxytalan fibres of the light microscopist. × 54 000

Fig. 2. Amongst the electron-dense filaments one may discern homogeneous material which is probably elastin. This structure is acceptable as the elaunin fibre of the light microscopist. × 52 000

Fig. 3. Another fibre acceptable as an elaunin fibre. Elastin deposition is more clear-cut than in *Fig. 2.* × 53 000

Fig. 4. A mature elastic fibre. Electron-dense filaments are few and evident clearly only at the periphery of the fibre. The major part of the fibre comprises elastin with focal densities derived from included electron-dense filaments. × 36 000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F
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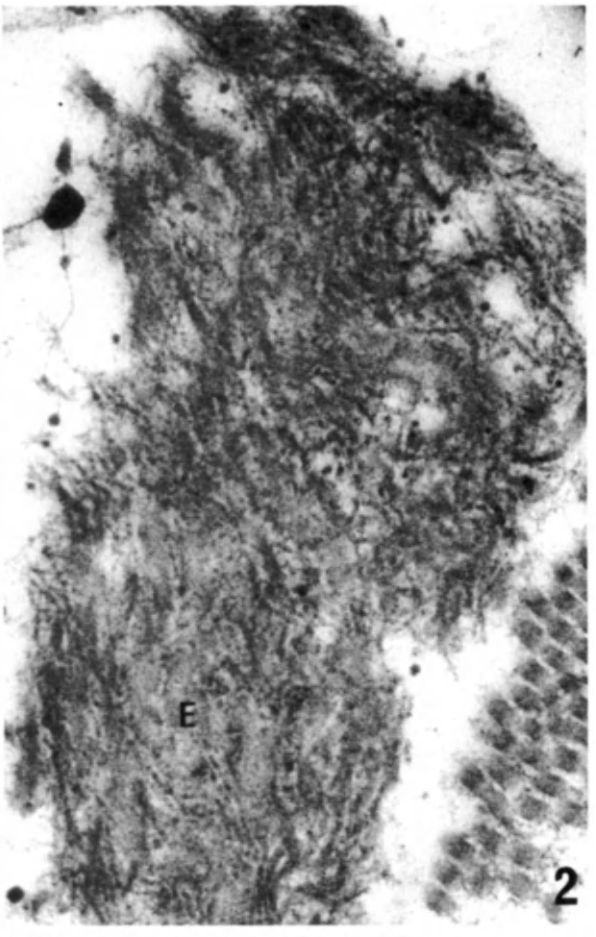
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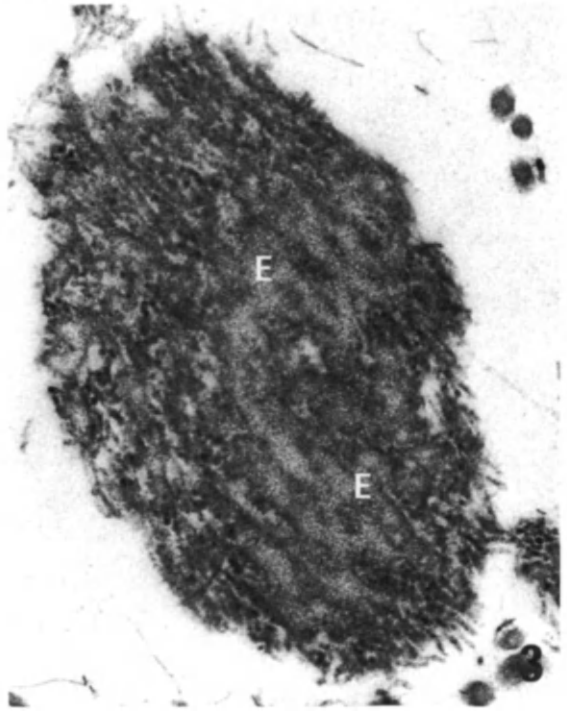
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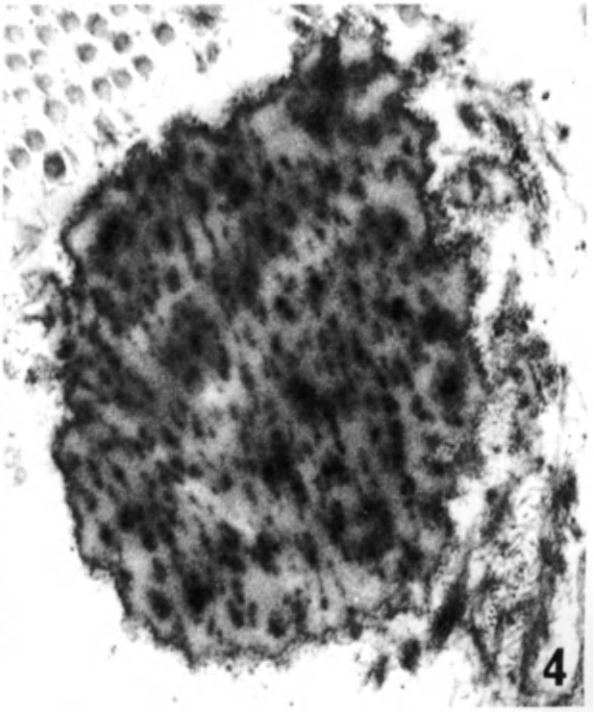
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1977). Another point that emerges from the above-mentioned studies is that it is only in the mature or old elastic fibre that the elastin does not accept uranium and lead stains and hence tends to be more or less electron lucent. Newly laid down elastin shows a variable but greater intensity of staining.

Elaunin fibres were first described by Gawlick (1965) and later by others (Pearse, 1968; Cotta-Pereira *et al.*, 1976b; Böck, 1979). Its claim to being a different type of connective tissue fibre rests on the fact that unlike elastic fibres, it is not stained by Verhoeff's iron haematoxylin or orcinol-new fuschin but, like elastic fibres, it is stained by aldehyde fuchsin, orcein or resorcin fuchsin. However, published electron micrographs of elaunin fibres (e.g. *Fig. 4* in Cotta-Pereira *et al.*, 1976c) are interpretable as immature elastic fibres where the fibrillary component is quite prominent but the elastic component is not as abundant and compact as in the mature elastic fibre.

Oxytalan fibres were first described by Fullmer and Lillie (1958) as a new type of connective tissue fibre on the basis of their tinctorial properties. They are characterized by: (1) failure to stain with stains commonly used for collagen, elastin and reticulum; (2) stainability with aldehyde fuchsin, resorcin fuchsin or acid orcein after previous oxidation; (3) failure to stain with these dyes after digestion with testicular hyaluronidase β-glucuronidase or lysozyme between oxidation and staining; and (4) resistance to digestion with elastase applied prior to oxidation (Fullmer, 1958; Fullmer and Lillie, 1958; Rannie, 1963).

Oxytalan fibres have been described in: (1) the periodontal membrane of several mammals (Fullmer, 1958, 1960a; Rannie, 1963; Simpson, 1967; Carmichael, 1968); (2) dental granulomas and radicular cysts (Fullmer, 1960b); (3) tendons and ligaments (Fullmer and Lillie, 1958; Rodrigo *et al.*, 1975; Edmunds *et al.*, 1979); (4) dermoepidermal junction (Hasegawa, 1960; Cotta-Pereira *et al.*, 1976b); (5) human eccrine sweat glands (Cotta-Pereira *et al.*, 1975); and (6) cartilage of external ear of rat (Bradamante *et al.*, 1975; Bradamante and Svajger, 1977). It has been postulated that oxytalan fibres occur in areas where connective tissue is subjected to mechanical stress.

Electron microscopic studies show that the filaments comprising oxytalan fibres have about the same width as the filaments in elastic fibre fibrils; and histochemical studies after digestion with various enzymes show little difference either. Based on this evidence and on connective tissue fibres, which anatomically resemble elastic fibres but show no affinity for elastin dyes (found in various animals particularly invertebrates), Bradamante and Svajger (1977) conclude that 'bundles of structural glycoprotein microfibrils (microfibrillar fibres, oxytalan or pre-elastic fibres) are not only the ontogenetic but also the phylogenetic precursors of elastic fibres in vertebrates'. Based on histochemical and ultrastructural studies on human skin, Cotta-Pereira *et al.* (1976b) suggest that 'oxytalan and elaunin fibres may represent interruptions in successive phases in the development of elastic fibres'. Similar sentiments have

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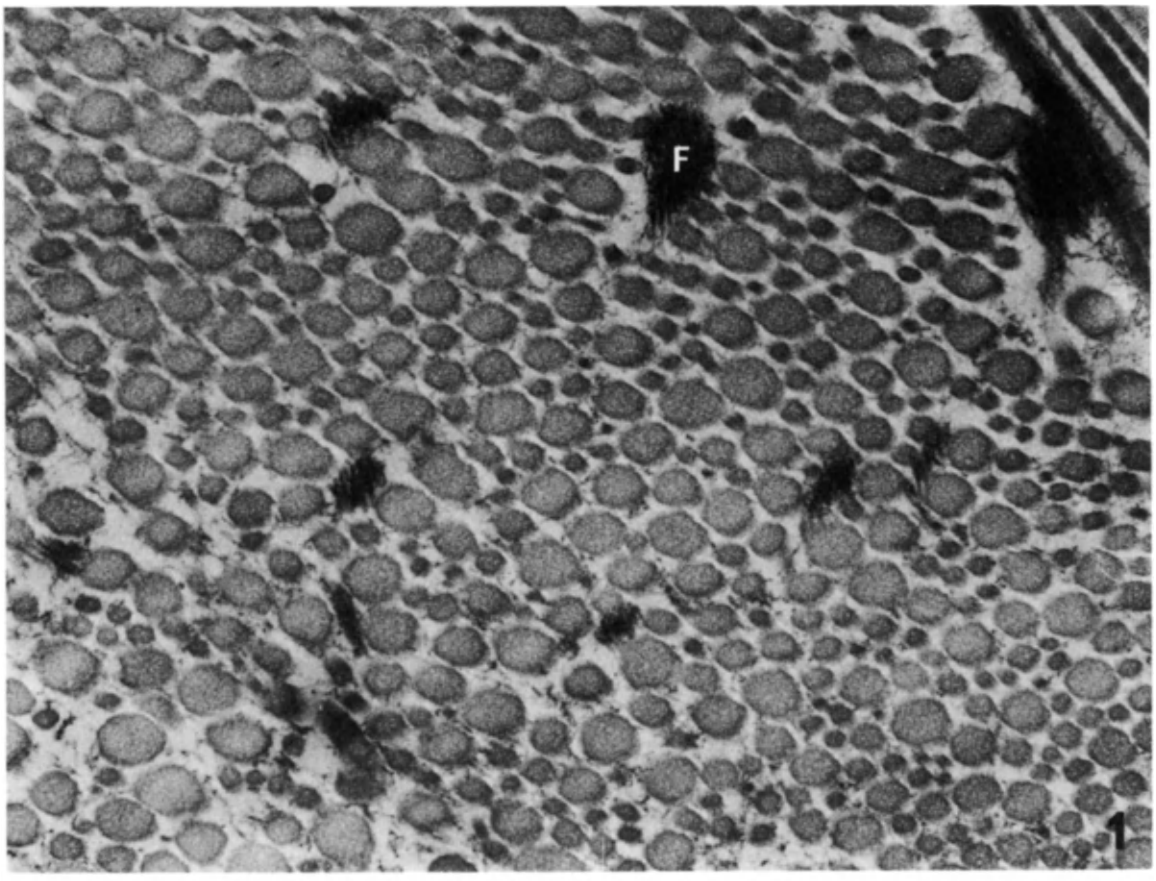
Plate 530

From normal rabbit semilunar cartilages.

Fig. 1. Fibrils composed of electron-dense filaments (F) acceptable as pre-elastic or oxytalan filaments are seen between transversely cut collagen fibrils of widely varying diameters. × 52000

Fig. 2. A fibril (between arrowheads) composed of electron-dense filaments acceptable as oxytalan or pre-elastic filaments is seen lying parallel to the collagen fibrils. However, the possibility that this is a section through the periphery of an elastic fibre cannot be completely ruled out. × 82000 (*From Ghadially, Thomas, Yong and Lalonde, 1978*)

Fig. 3. An obliquely cut elaunin fibre with a lucent core and a peripheral zone of electron-dense filaments. × 86000 (*From Ghadially, Thomas, Yong and Lalonde, 1978*)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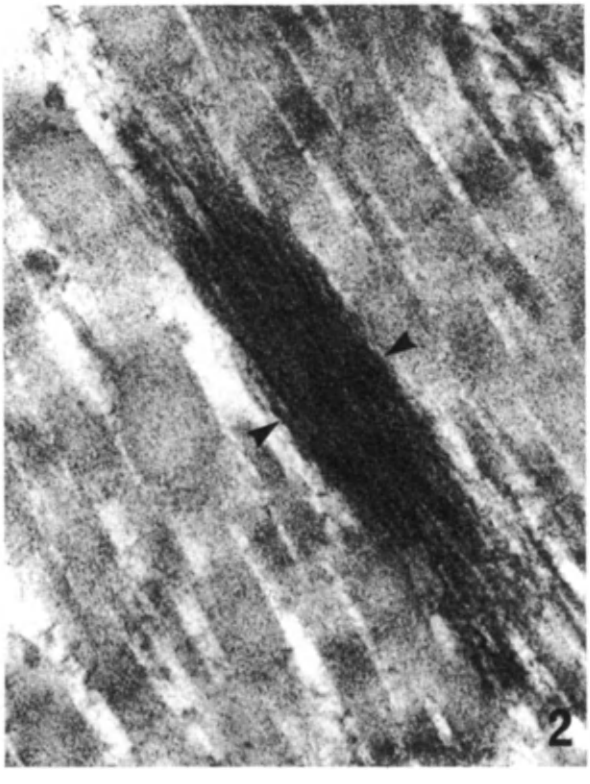
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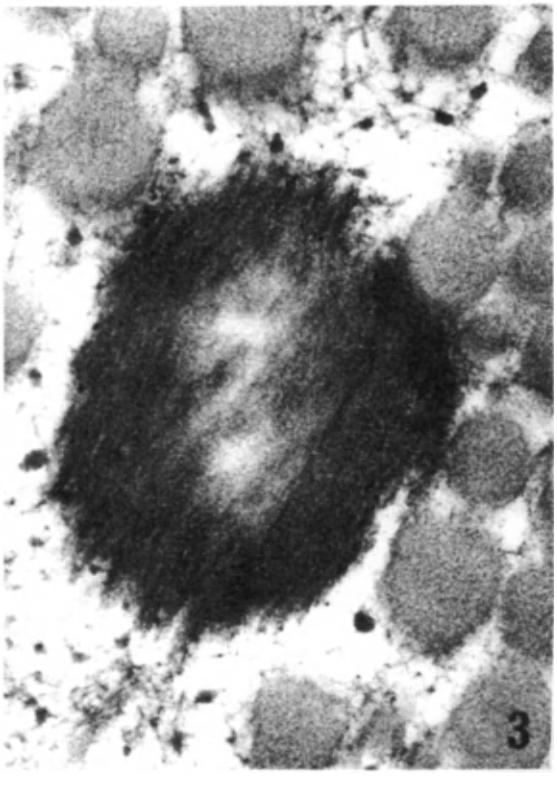

3

been expressed by various workers who have referred to them (oxytalan fibres) as 'related to elastic fibres', 'a type of incompletely or specially modified elastic fibres' (Fullmer, 1960a) or as a 'variant of elastic tissue that does not mature' (Carmichael, 1968). The idea that oxytalan fibres are often 'pre-elastic' fibres is attested also by the fact that they precede elastic fibres in post-inflammatory granulomas (Feher *et al.*, 1971). However, fibrils composed of 10–13 nm electron-dense filaments occur in tissues which even in the mature state do not contain elastic fibres. At least here perhaps one could regard them as true oxytalan fibres (Serafini-Fracassini and Smith, 1974).

In the fibrocartilagenous menisci a situation similar to that found in the skin prevails. Collections of electron-dense filaments acceptable as pre-elastic filaments or oxytalan filaments were noted by Silva (1969) in the intra-articular disc of the temporomandibular joint of the guinea-pig, but mature or maturing elastic fibres with the characteristic lucent core indicating the deposition of elastin were not found. In the rabbit semilunar cartilages (Ghadially *et al.*, 1978) (*Plate 530*) innumerable small fibrils composed of filaments acceptable as pre-elastic filaments or oxytalan filaments were present as also a few larger fibrils composed of such filaments. However, fully mature elastic fibres were virtually absent but occasional young elastic fibres with a small lucent core were present. These could be considered the equivalent of elaunin fibres in the skin. In the human semilunar cartilages the situation is slightly different in that one does find quite a few mature elastic fibres (i.e. fibres containing more elastin than elastic fibre filaments) (Ghadially *et al.*, 1983; Ghadially, 1983) (*Plate 528*), but here also oxytalan fibrils seem to persist. It is likely that in the semilunar cartilages (as in the skin), not all electron-dense fibrils are destined to serve as scaffolding for elastin deposition and conversion into elastic fibres.

The more or less electron-lucent appearance of the amorphous component (i.e. elastin) of elastic fibres often seen in routinely stained sections has some disadvantages. For example, one can easily miss the presence of small elastic fibres and thus underestimate the amount of elastic material present in a given tissue. Therefore, various methods have been tried to enhance the contrast of elastin. Alcoholic and aqueous solutions of phosphotungstic acid have been tried but they impart a variable density to elastin in the same block and at times in the same section (Albert and Fleischer, 1970). Futaesaku *et al.* (1972) found that tannic acid added to the fixative enhanced the staining of various connective tissue components including elastic fibres. According to Cotta-Pereira *et al.* (1976c) a regular and reproducible visualization of the elastic fibre is achieved if a fixative containing 0.25 per cent tannic acid in 3 per cent glutaraldehyde is used, followed by the usual method of tissue preparation and staining.

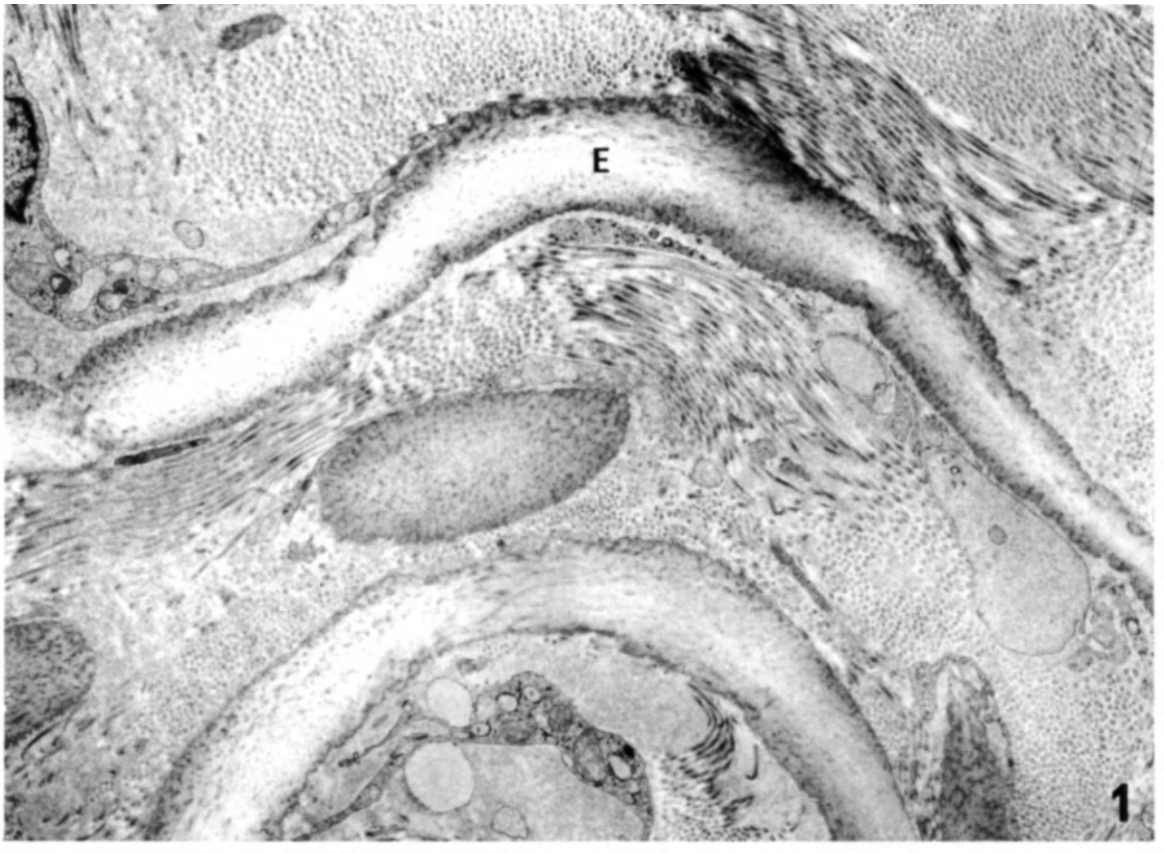
Tetraphenylporphine sulphonate has a specific affinity for elastic fibres (Winkelman and Spicer, 1962). It gives a bright red fluorescence to elastic fibres after injection into animals but it does not make the fibres electron dense. However, silver tetraphenylporphine sulphonate does impart a quite selective electron density to elastin after *in vitro* staining and seems to be a good stain for demonstrating elastic fibres and lamellae in ultrathin sections (*Plate 531*) produced from routinely processed blocks of tissue (Albert and Fleischer, 1970; Albert, 1973). Other tissue components show a paler non-specific background staining, which is usually quite adequate for electron microscopy. However, counterstaining with uranium and lead can be used if a further increase in density of elastin as well as other tissue components is desired.

Plate 531

From a block of rabbit aorta fixed in glutaraldehyde and post-fixed in osmium (*Ghadially and Yong, unpublished electron micrographs*).

Fig. 1. In this section stained with uranium and lead the electron-density of the elastic laminae varies from low to medium. × 8500

Fig. 2. In this section stained with silver tetraphenylporphine sulphonate followed by uranium and lead, the elastic laminae appear electron dense. × 13000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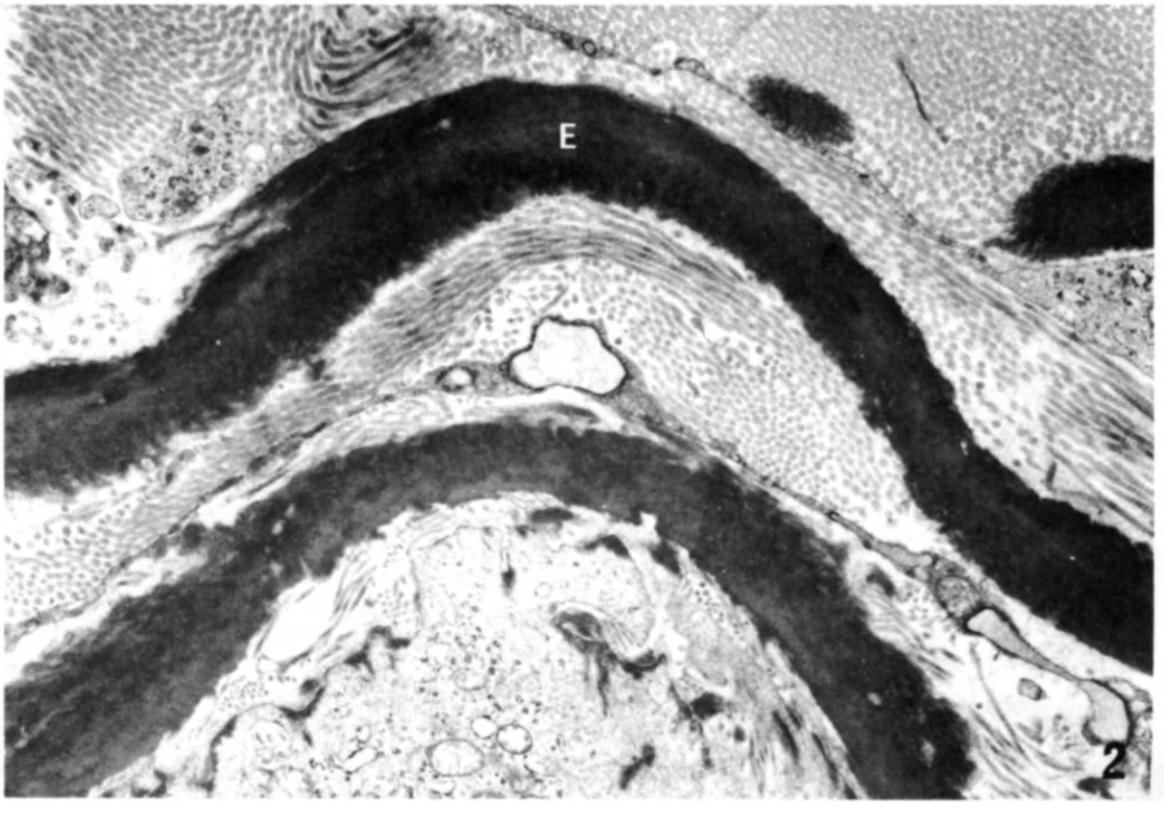
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Calcified elastic fibres

In certain pathological states calcium salts are known to be deposited in elastic fibres; one such condition is pseudoxanthoma elasticum (*Plate 532*). This disease is a genetic disorder in which abnormal mineralization of the dermis and vascular system occurs. Ultrastructural studies (Ross *et al.*, 1978) have shown that the principal alterations detectable in the skin involve the elastin of the elastic fibre. The elastic fibre filaments are apparently not affected. As we have seen (page 1252) normal elastin is amorphous and has a variable but usually feeble affinity for lead and uranium stains. In contrast to this much of the elastin in the affected areas of the skin in pseudoxanthoma elasticum has a granular appearance and shows an enhanced affinity for stains. A later change is calcification which presents as dense deposits within the elastin or around the elastic fibre (Otkjaer-Nielsen *et al.*, 1977). Electron-dense particles and apatite crystals are seen in such deposits. Electron-diffraction studies confirm that these are calcium apatite crystals (Otkjaer-Nielsen *et al.*, 1977) and electron-probe x-ray analysis has demonstrated calcium and phosphorus in these deposits (Blümcke *et al.*, 1974; Blumenkrantz *et al.*, 1974).

The reason for the increased affinity of elastic fibres for calcium in pseudoxanthoma elasticum is not clear but the histochemical studies of Martinez-Hernandez and Huffer (1974) have demonstrated polyanions (glycosaminoglycans, acidic glycoproteins or polyphosphates) and they suggest that 'this phenomenon may play a role in increased calcium binding by dermis and in mineral deposition within elastic fibres in affected dermis'. A similar conclusion was also reached by Tereau *et al.* (1979) who state: 'in this hereditary disease there is an initial modification of glycoprotein synthesis by fibroblasts and that calcification is only secondary to these alterations'.

Elastic fibres containing calcium deposits have been considered characteristic of pseudoxanthoma elasticum (Huang *et al.*, 1967; Danielsen *et al.*, 1970) but Otkjaer-Nielsen *et al.* (1978) have shown that identical deposits (as judged by ultrastructure and electron diffraction analysis) occur in saltpetre-induced cutaneous lesions and they also point out that 14 cases have been reported in the literature where cutaneous calcification had occurred following contact with calcium chloride. Finally, it is worth recalling that elastic fibre calcification is seen in some examples of metastatic calcification and in atherosclerosis (Blumenthal *et al.*, 1944; Yu and Blumenthal, 1963; Jacotot *et al.*, 1973; Urry, 1975) so this phenomenon on its own is neither specific nor diagnosic of pseudoxanthoma elasticum.

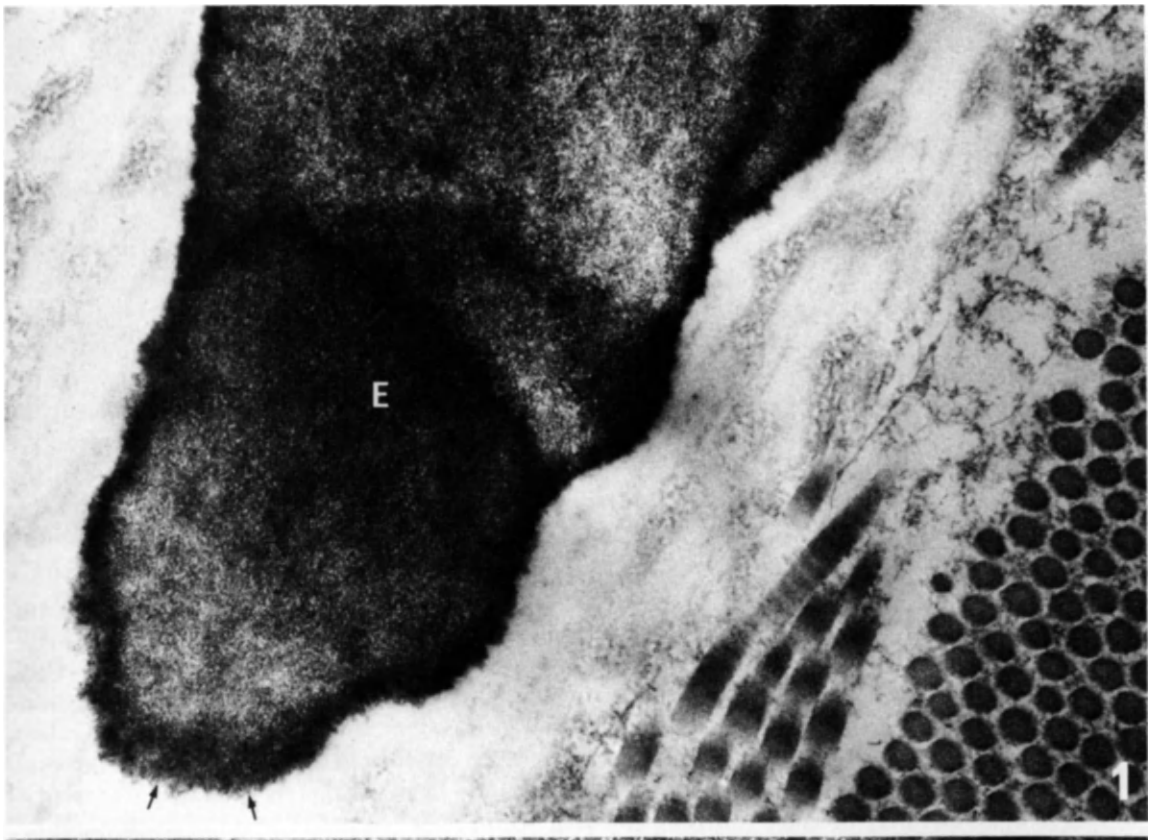
*Another alteration reported is the occurrence of spiralled collagen (see page 1248) but this does not appear to be a constant feature for few authors report on its occurrence in pseudoxanthoma elasticum.

Plate 532

A calcified elastic fibre from the skin of a case of pseudoxanthoma elasticum (*From Otkjaer-Nielsen, Johnson, Hentzer, Danielsen and Carlsen, 1977*).

Fig. 1. Bright-field electron micrograph (i.e. routine electron micrograph) from a stained section showing electron-dense calcium deposits in a dermal elastic fibre (E). These calcium deposits present as particles and needle-like structures (arrows). × 30000

Fig. 2. Dark-field electron micrograph of the same fibre. The calcium deposits present as 'bright particles' (i.e. particles which scatter electrons strongly) in the elastic fibre (E). In passing one may note that it would not be correct to call these particles 'electron lucent'. Electron-lucent areas in bright field are where electrons are not scattered; in dark-field these (i.e. bright areas) are the areas where the electrons are scattered. × 30000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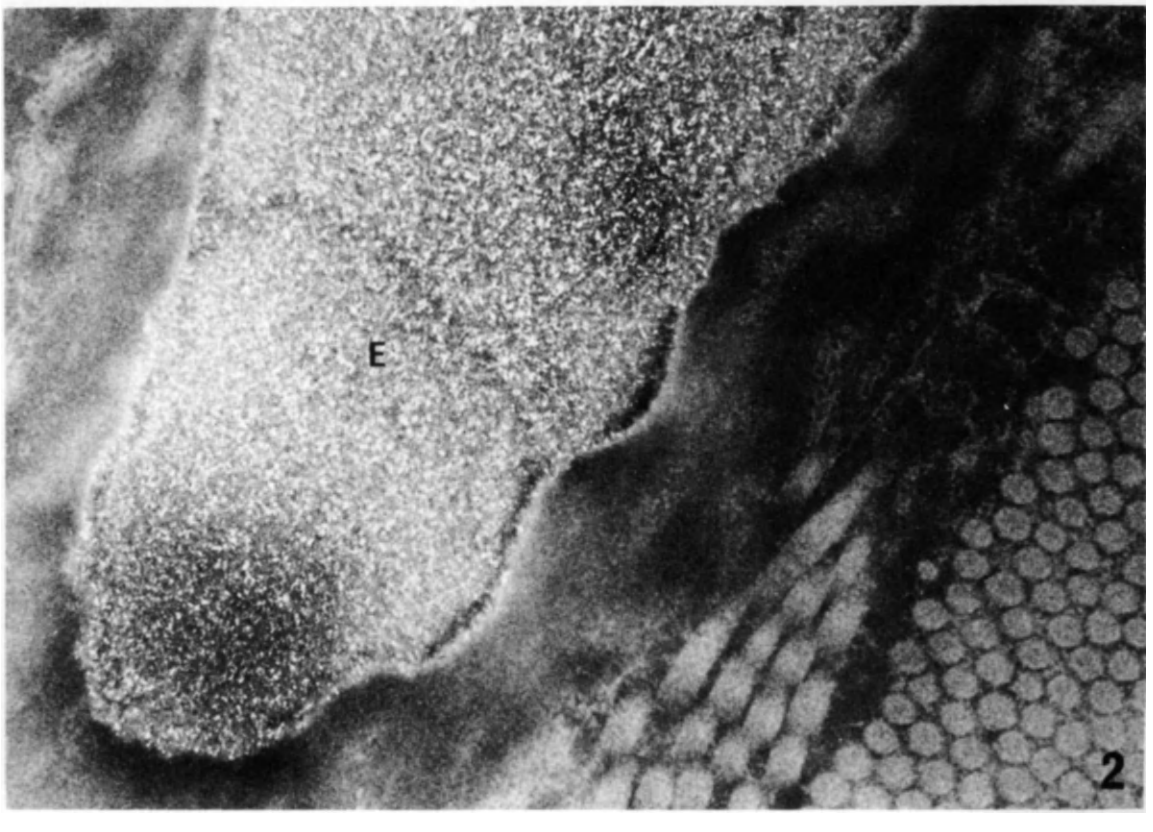
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Amyloid

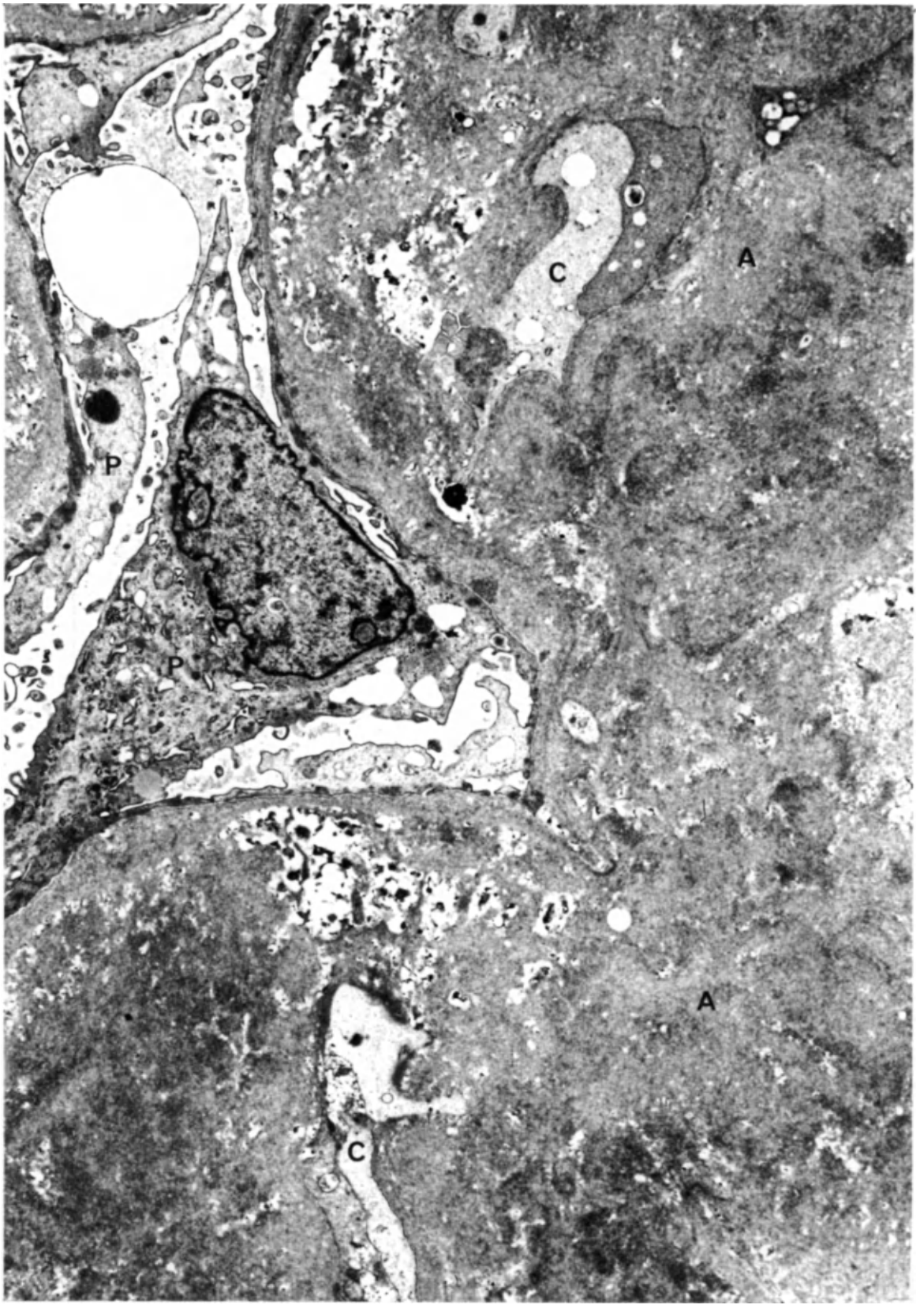
The term 'amyloid' (starch-like) was coined by Virchow (1852) to describe certain deposits in tissues, which he found developed a blue tint when treated with iodine followed by sulphuric acid. Meckel (1856) was unable to obtain this blue coloration in amyloid deposits and later workers concluded that amyloid was a pathological proteinaceous substance (Friedreich and Kekule, 1859) which at light microscopy presents as a hyaline, eosinophilic extracellular deposit. Amyloid gives various tinctorial reactions such as: (1) metachromatic staining with crystal violet; (2) yellow fluorescence after staining with toluidine blue; and (3) green birefringence after staining with congo red (Divry and Florkin, 1928).

The congo red birefringence test is highly selective and quite sensitive (Glenner and Page, 1976), but some care is needed in its execution (Carson and Kingsley, 1980). However, the congo red test does not delineate a substance with a specific chemical composition, or define amyloid as such, for proteins that possess conformations similar to that of amyloid also give this reaction (Gueft, 1972). X-ray diffraction and infrared analyses of amyloid show that the polypeptide chains are arranged in a β-pleated sheet conformation which is largely responsible for the congo red birefringence (Glenner and Page, 1976). Thus the notion that amyloid is a material of specific chemical composition has changed to a class of materials that share a common physical characteristic (i.e. β-pleated sheet conformation).

Electron microscopy of amyloid deposits from a variety of diseases in man and animals, and amyloid produced by experimental manoeuvres shows the same or a similar filamentous and fibrillar structure (*Plates 533–535*). The filamentous nature of amyloid was first demonstrated by Cohen and Calkins (1959) and Spiro (1959). Subsequent studies (Shirahama and Cohen, 1967; Cohen and Shirahama, 1973; Westermark, 1977) on isolated amyloid show that the basic structure here is the 7–10 nm filament (called 'fibril' by some workers). Lateral aggregation of these filaments may produce fibrils up to about 40 nm thick. Negative staining, ruthenium red staining and dark-field electron microscopy (of isolated amyloid) show that the 10 nm filament is a ribbon-like structure composed of two or more 3–4 nm thick protofilaments (referred to by some as 'protofibrils' or filaments). The 3–4 nm protofilaments are unlikely to be visualized in sections of plastic embedded tissues although poorly focused electron micrographs taken at high magnifications have been published which may create such an impression.

Plate 533

Amyloid kidney.
Glomerular amyloid deposits, in all clinical types of amyloidosis, are initially laid down in the mesangium. Progressive deposition in the mesangium and capillary loops leads to occlusion of capillary lumen and finally glomerular obliteration. This electron micrograph depicts a fairly late stage of amyloid (A) deposition where capillary remnants (C) and some surviving podocytes (P) are seen. × 6500


C
A
P
P
A
C

In routine ultrathin sections amyloid presents as a collection of unbranched, usually straight or slightly bent, rigid-looking, thread-like structures which are usually about 10 nm in diameter (filaments) but they can measure up to about 40 nm in diameter (fibrils). Although the filaments and fibrils are often randomly oriented and hence present as a meshwork, sometimes a parallel alignment is also evident, particularly near the cell borders where the filaments appear juxtaposed parallel to or at right angles to the cell membrane (Heefner and Sorenson, 1962).

A vexatious question that has generated much debate is whether or not amyloid filaments occur in an intracellular location. Confusion can arise from two sources: (1) vagaries of sectioning geometry producing appearances suggesting an intracellular location of amyloid filaments lying close to the cell; and (2) the ubiquitous 10 nm intracytoplasmic filaments (i.e. intermediate filaments), may be mistakenly imagined to be amyloid filaments. The morphological similarity of amyloid filaments with intermediate filaments in plasma cells, monocytoid cells, endothelial cells, macrophages and others have led some authors to suggest that such cells synthesize amyloid filaments in their cytoplasm and then discharge them into the extracellular matrix in various ways (Ben-Ishay and Zlotnick, 1968; Ranløv and Wanstrup, 1968; Chai, 1976). One of the suggestions is that death and disintegration of such cells leads to a release of 'amyloid filaments' into the matrix.

It will be recalled (*see* page 892) that 10 nm intracytoplasmic filaments (intermediate filaments) are of common occurrence and quite large accumulations of such filaments are seen in many pathological states. It is therefore not surprising that cells surrounded by amyloid at times develop massive collections of intermediate filaments in their cytoplasm. Such intermediate filaments are morphologically similar, but not identical in appearance to amyloid filaments (Zucker-Franklin and Franklin, 1970; Beltran and Stuckey, 1972) and it has been shown that antisera specific for amyloid filaments (Franklin and Zucker-Franklin, 1972) do not localize on intracytoplasmic filaments (Zucker-Franklin, 1974).

Regarding the synthesis of amyloid*, it has also been suggested that various cells such as Kupffer cells (Battaglia, 1961, 1962); endothelial cells (Cohen and Calkins, 1960); glial cells (Terry, 1963; Terry *et al.*, 1964) and mesangial cells in the renal glomerulus (Cohen and Calkins, 1960) may take up amyloid precursor proteins from the blood and convert them into amyloid filaments or what is more likely into an amyloid precursor (preamyloid) (Glenner, 1972) and that this preamyloid is then secreted into the extracellular matrix where it polymerizes into amyloid filaments adjacent to the cell membrane (Gueft and Ghidoni, 1963; Heefner and Sorenson, 1962; Jao and Pirani, 1972; Glenner, 1972).

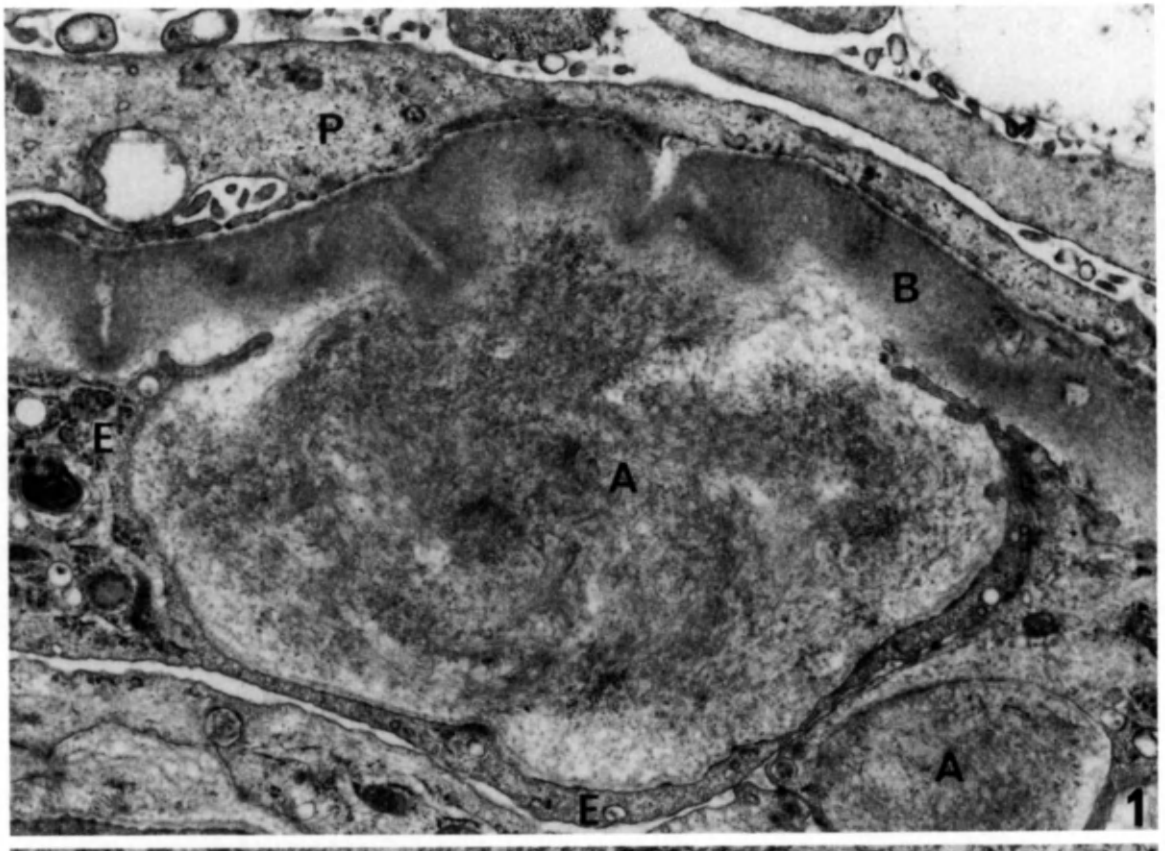
*The macrophage (histiocyte) has been implicated as the cell which makes amyloid more frequently than any other cell (*see* discussion following paper presented by Franklin *et al.*, 1974).

Plate 534

Amyloid kidney

Fig. 1. Seen here are amyloid deposits (A) in a renal glomerulus. The larger deposit lies between an endothelial cell (E) and the basal lamina (B). Note also the podocyte (P). ×12 500

Fig. 2. Seen here are amyloid filaments and fibrils at a higher magnification. The cross-cut filament indicated by an arrowhead measures about 9 nm in diameter, while the fibril (arrow) measures about 25 nm in diameter. ×56 000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B
E
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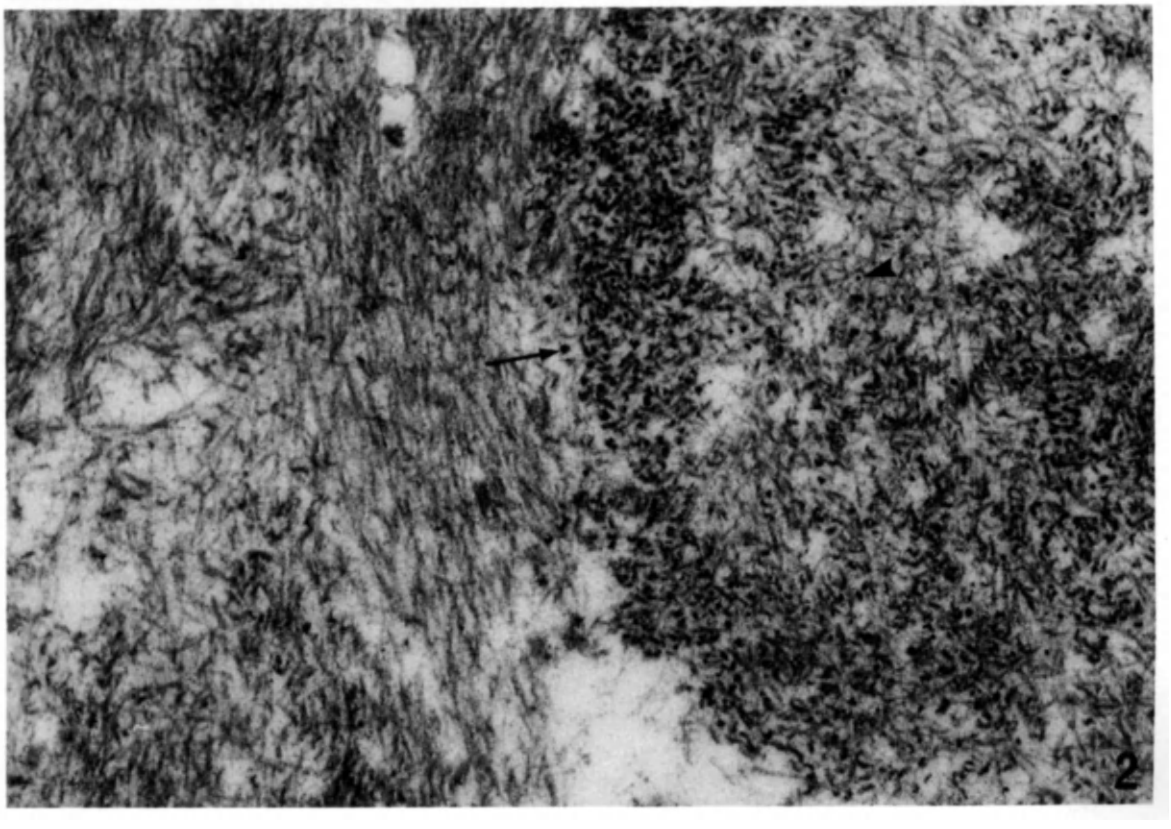
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Chemical analysis shows that amyloid is composed mainly of polymers of low, molecular weight proteins* (Cohen, 1967; Glenner, 1980; Glenner *et al.*, 1980). In cases of plasma cell dyscrasias (including myeloma) and primary idiopathic systemic amyloidosis, the amyloid proteins are derived from variable segments of immunoglobulin light chains (called 'AL proteins') (Glenner *et al.*, 1970a,b; Glenner *et al.*, 1971), but the amyloid of secondary systemic amyloidosis (called 'amyloid A', 'non-immunoglobulin protein' or 'A A protein') has quite a different amino-acid sequence and is of unknown origin (probably of local tissue origin) (Benditt *et al.*, 1971). Amyloid exhibiting all the well known histological characteristics of amyloid has also been found in endocrine organs and some neuroendocrinomas (also called 'APUDomas')†. This amyloid is produced by the neuroendocrine cells and not the mesenchymal cells. It would appear that the major proteins that occur in APUD amyloid are related to hormones or prohormones. Thus for example the amino acid sequence in the APUD amyloid of medullary carcinoma of the thyroid and in calcitonin are similar and insulin and glucagon easily form fibrils *in vitro* which have the β-pleated sheet conformation like amyloid fibrils. (For references and details *see* Westermark, 1977 and Westermark *et al.*, 1979.)

Several other moieties such as lipids, fibrinogen, complement and a structure known as the 'rod', 'doughnut', 'plasma component', 'pentagonal component', 'P component' or 'AP component' have been noted in partially purified preparations of amyloid (Glenner and Page, 1976) but they are not an integral part of amyloid. The P component first described by Bladen *et al.* (1966) represents normal serum α_1-glycoprotein (Binette *et al.*, 1974). It presents in electron micrographs as a pentagonal, doughnut-like structure measuring 8 nm in diameter. It has not been seen in sections from amyloid-containing tissues (probably because it is easily extracted), it is found only in partially purified preparations of amyloid.

*It is neither possible nor necessary to review the vast literature on the diverse amyloid fibril proteins which have now been identified by chemical, immunochemical and immunohistological techniques because whatever the type of amyloid the ultrastructural morphology is roughly the same. Thus, amyloid of all types is characterized light microscopically by Congo red positivity (and green coloration in polarized light) and electron microscopically by rigid looking 10 nm filaments in the extracellular compartment. When results of the Congo red test are equivocal, electron microscopy often settles the issue by demonstrating the characteristic filaments and fibrils which are virtually impossible to confuse with anything else.

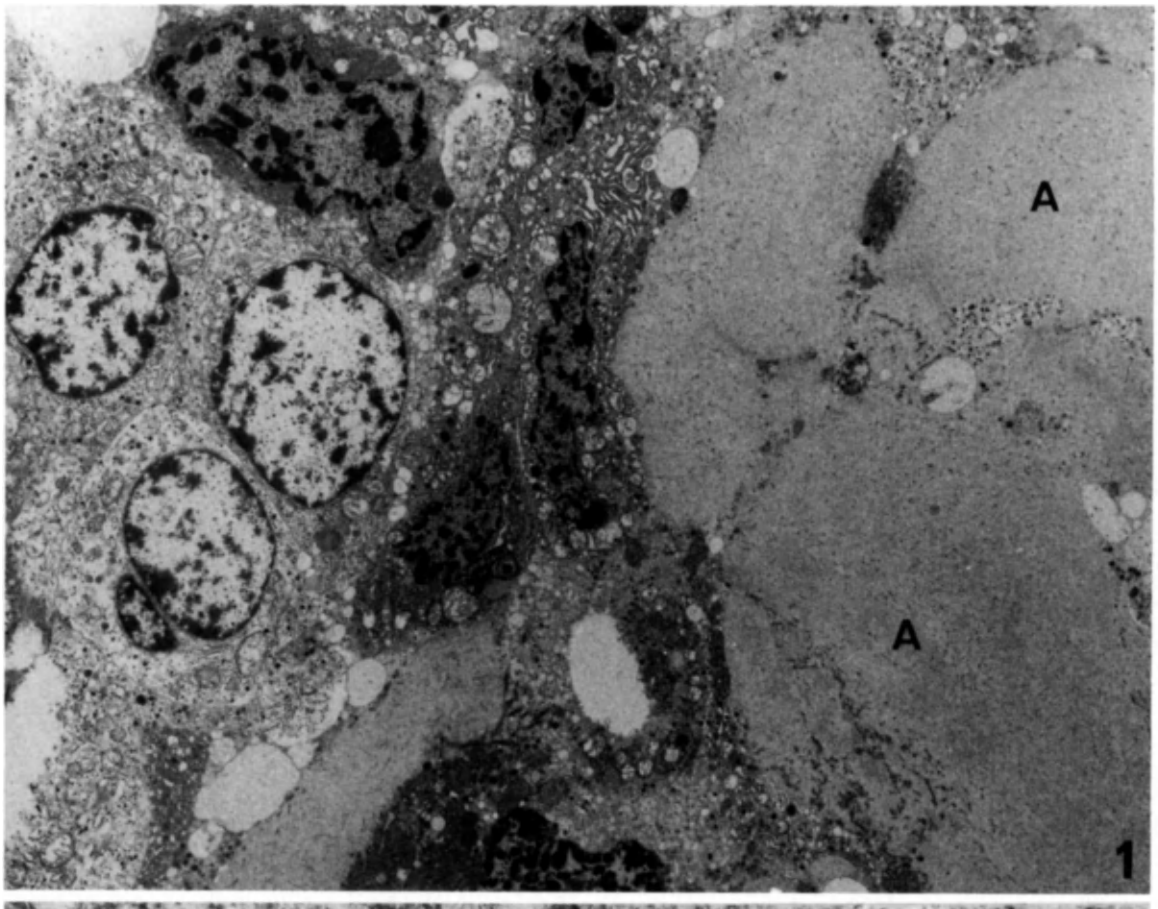
†Amyloid (often called 'APUDamyloid' to distinguish it from 'immunamyloid') is found in the stroma of some but not all neuroendocrinomas. The incidence of amyloid in medullary carcinoma of the thyroid is virtually 100 per cent. This has led some to imagine that if amyloid is present in the stroma of a neuroendocrinoma this is diagnostic of medullary carcinoma of the thyroid. This is incorrect, for amyloid has been documented (for references *see* Ghadially, 1985) to occur (albeit less frequently) in some phaeochromocytomas, carotid body tumours, bronchial carcinoids, islet cell tumours, gastrinomas and pituitary adenomas.

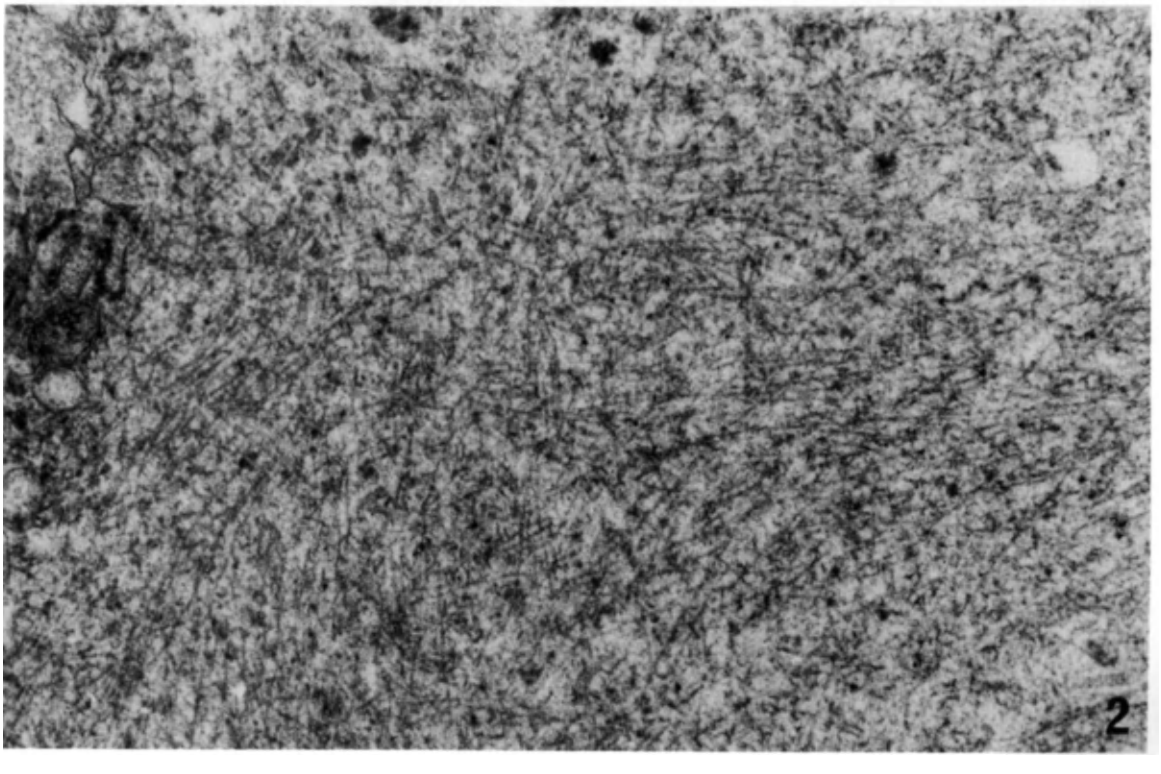
Plate 535

Medullary carcinoma of thyroid.

Fig. 1. Large amyloid deposits (A) are seen in the tumour stroma. × 3600

Fig. 2. A higher-power view showing APUD amyloid filaments, and fibrils about 8–14 nm wide. ×45000


A
A
1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Fibrin

Fibrin is not a normal tissue component but it has been found in the cytoplasmic matrix and extracellular matrix in some pathological states. The rare circumstances in which intracytoplasmic fibrin occurs have already been dealt with (pages 986–989) as also some aspects of the ultrastructural morphology of fibrin. Here we deal with the much more common extracellular fibrin deposits and the molecular morphology of fibrin.

A detailed list of all the situations and diseases where deposits of fibrin have been found is not warranted, because such information can be obtained from standard pathology texts. Suffice it to recall that in any pathological state where fibrinogen escapes from the blood vessels (increased permeability or rupture) into tissues clotting mechanisms are likely to convert fibrinogen into fibrin.

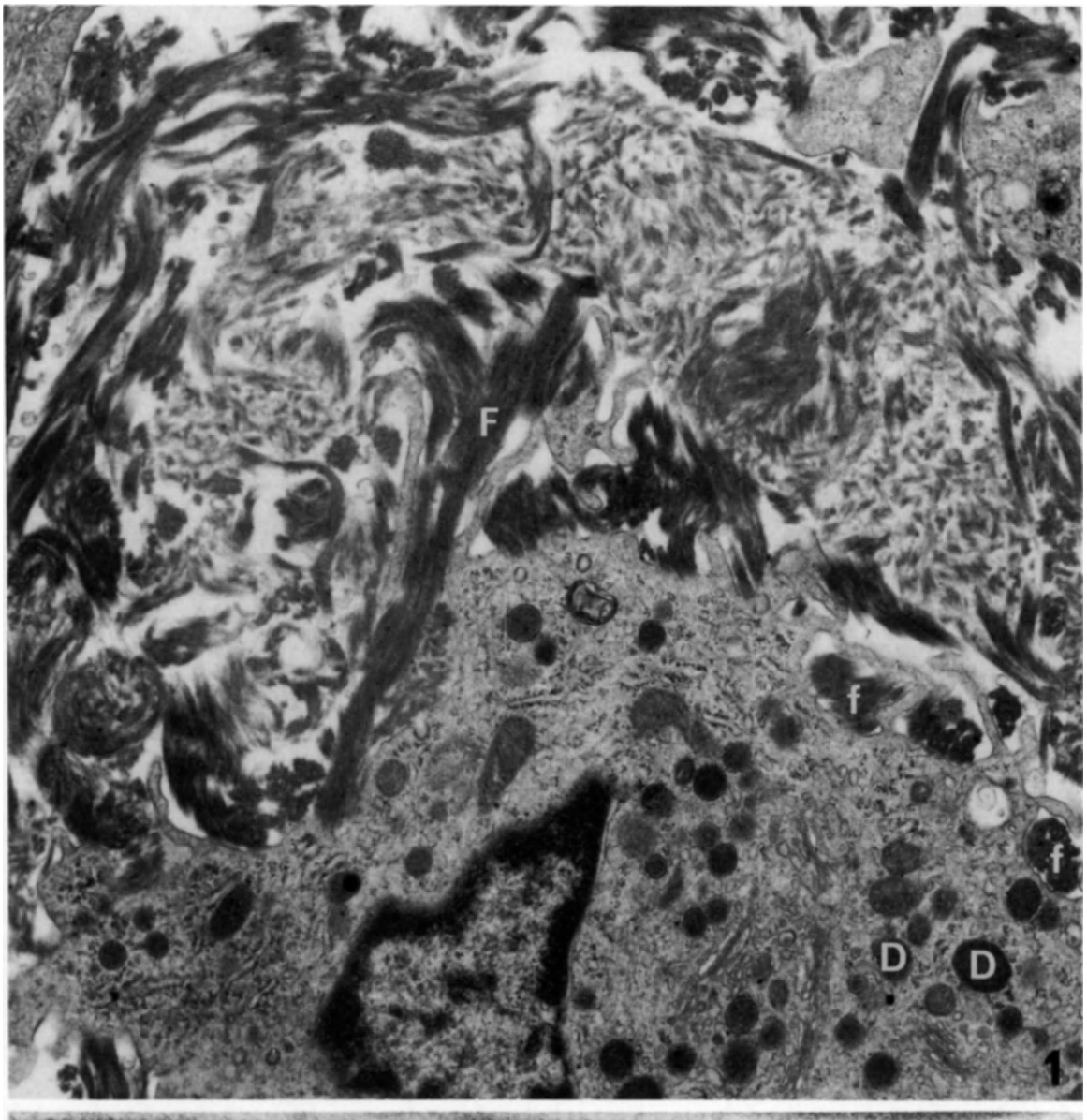
In haematoxylin- and eosin-stained sections, fibrin deposits present as deeply eosinophilic amorphous or fibrous masses. In routine ultrastructural preparation they present as markedly electron-dense masses and/or elongated fibrils or fibres. Because of their high electron density their filamentous substructure is at times difficult to discern. The electron-dense fibrous deposits of fibrin are characteristic enough and unlikely to be confused with any other extracellular matrix component. However, unequivocal identification of fibrin rests on the fact that fibrin shows a striated or banded pattern with a periodicity of about 23 nm (range 19–35 nm). Unfortunately, not all fibrin in a given specimen is banded and there are times when despite much searching characteristically banded fibrin is not found (*see* page 986 for reasons).

Plate 536

Rheumatoid synovial membrane. Fibrin was present in the joint space and also on and between synovial intimal cells.

Fig. 1. Fibrin in the form of fibrils and fibres (F) is seen lying on or adjacent to the surface of a synovial intimal cell. Cell processes wrapping around fibrin (f) suggest that fibrin is being endocytosed. Also seen are several dense bodies (D) acceptable as heterosomes and heterolysosomes derived from endocytosed fibrin. × 19500

Fig. 2. High-power view of fibrin showing banding or striations with a periodicity of about 20.7 nm. × 94000


F
f
f
D
D
1


2

Fibrinogen, the thrombin coagulable glycoprotein circulating in the blood, and its polymer fibrin, the essence of all thrombi, have been extensively studied by many techniques including electron microscopy of negatively stained and metal-shadowed molecules. Although several models have been proposed for the fibrinogen molecule, the one that is now widely accepted is the Hall and Slayter model (Hall and Slayter, 1959; Slayter, 1983) which depicts (based on ultrastructural observations) the fibrinogen molecule as a trinodular rod. (The outer nodules are called D nodules or D domains and the central nodule as the E nodule or E domain.) Thin 'strings' are at times seen to unwind from the D nodules and it is thought that these may be related to the alpha-chain extension. Chemical studies (reviewed by Doolittle, 1983, 1984) indicate that fibrinopeptides are associated with the E domain but this is not visualized by electron microscopy*.

Numerous chemical and ultrastructural studies (for details *see* Hantgan *et al.*, 1983) have led to the postulation of three principal steps in fibrin formation, namely activation, polymerization and lateral association (*Plate 537*). Proteolytic cleavage of fibrinopeptides by thrombin is believed to be the initial activation event. This excision of fibrinopeptides converts fibrinogen to fibrin molecules or fibrin monomers. The next step (which can be appreciated by electron microscopy) is polymerization of trinodular monomers to form a two-stranded (each strand may be regarded as a protofilament) filament (called 'protofibril' by some). In this two-stranded filament or polymer, the fibrin molecules are deployed in a half-staggered arrangement and this is what gives fibrin its 23 nm (approximately) periodicity (i.e. about half the length of the fibrin molecule). Linear growth of filaments dominate until a length of about 600 nm is reached. This heralds the third step whereby lateral aggregation of filaments leads to structures which may be regarded as fibrils or fibres (depending upon thickness). Lateral aggregation leads to the formation of fibres which are not only thick but also highly interconnected.

*In fact, ultrastructurally there is no constant discernible difference between the fibrinogen and fibrin molecule, because the thread-like structures associated with the D and E nodules are difficult or impossible to visualize. Their existence is largely surmised from chemical studies.

Plate 537

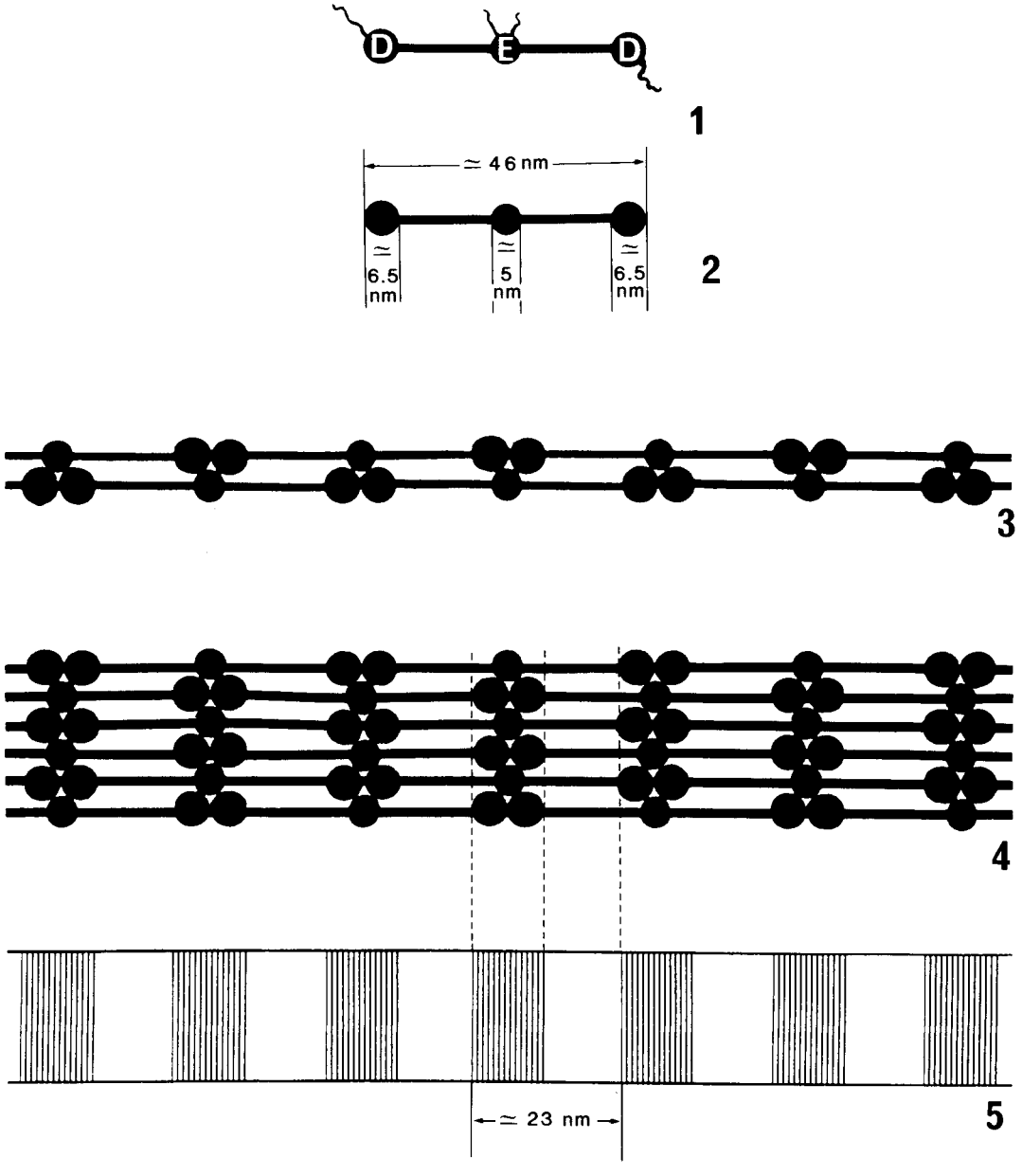
Fig. 1. Schematic diagram of fibrinogen molecule (based on chemical and ultrastructural studies) showing the trinodular rod, alpha chain extensions and fibrinopeptides which are depicted here as thread-like structures arising from the D and E nodules, respectively.

Fig. 2. Diagram shows the fibrin or fibrinogen molecule as usually visualized by electron microscopy.

Fig. 3. Fibrin filament composed of two protofilaments. Note the half-staggered arrangement of the fibrin molecules.

Fig. 4. Lateral aggregation of filaments produces slender fibrils and thicker fibres.

Fig. 5. A positively stained fibril or fibre showing alternating dark and light bands. The dark bands are produced by the aggregates of stained nodules. In negatively stained preparations, far more stain would penetrate the spaces (called 'holes') between the 'threads' connecting the nodules than between the nodules themselves. Hence, the density of the bands would be reversed, i.e. the band which appears dark in the positively stained preparation would become a light band in the negatively stained preparation and *vice versa*.


D
E
D
1
≃ 46 nm
≃ 6.5 nm
≃ 5 nm
≃ 6.5 nm
2
3
4
≃ 23 nm
5

Proteoglycan particles (matrix granules)

In the matrix of hyaline cartilage and various connective tissues one can identify two main macromolecular components by biochemical methods. These are: the fibrous protein collagen and the proteoglycans (once called 'mucoproteins'* or 'protein-polysaccharides'). Ultrastructurally one can also demonstrate both these components in connective tissues. Besides the numerous studies on collagen most reports (i.e. electron-microscopic studies) deal with the proteoglycans of hyaline cartilage which usually present as round, oval, triangular or stellate particles (non-membrane-bound) associated with fine filaments about 5 nm thick (*Plates 538–540*).

Particles of a similar morphology have also been demonstrated in the intra-articular discs and menisci (Silva, 1969, 1970; Ghadially *et al.*, 1978), and in the nucleus pulposus (Ghadially, 1978). Indeed, proteoglycan particles may be found in any connective tissue (*Plate 539, Fig. 1*), but as a rule such particles are small and sparse (because they are largely extracted) and hence do not attract attention in most routine preparations. Such particles are quite prominent in chondrosarcoma (*Plate 539, Fig. 2*), but smaller, fewer particles are seen also in various other tumours such as fibroadenoma, fibrous histiocytoma, schwannoma, myxoma and osteosarcoma (Ghadially, unpublished observations). Particles somewhat similar in appearance to proteoglycan particles have also been seen in the mucous and serous secretions produced by various glands (*see*, for example, illustrations in Simson *et al.*, 1978), but these probably represent glycoproteins rather than proteoglycans.

Various terms such as 'protein-polysaccharide particles', 'proteoglycan particles' and 'matrix granules' have been coined to describe these particles, which are quite abundant in the matrix (particularly the territorial matrix) of hyaline articular cartilage. None of these terms is entirely satisfactory. The first two terms can be criticized on the grounds that besides proteoglycans, some collagenous or non-collagenous protein (glycoprotein) may be present as evidenced by the associated filaments†. On the other hand, the term 'matrix granules' is confusing for electron-dense granular material of a lipidic nature (page 1278) occurring in the cartilage matrix has also at times been referred to as 'matrix granules'. Further, terms such as 'matrix granules' or 'intramatrical dense granules' have long been used to describe the electron-dense granules in mitochondria (*see* page 192).

On the whole, the term 'proteoglycan particles' seems more appropriate than others. The term 'particle' rather than 'granule' appears desirable because these structures in normal cartilage matrix measure only about 25–60 nm in long diameter. The occasional larger particle found in such preparations probably results from the aggregation of two or more particles.

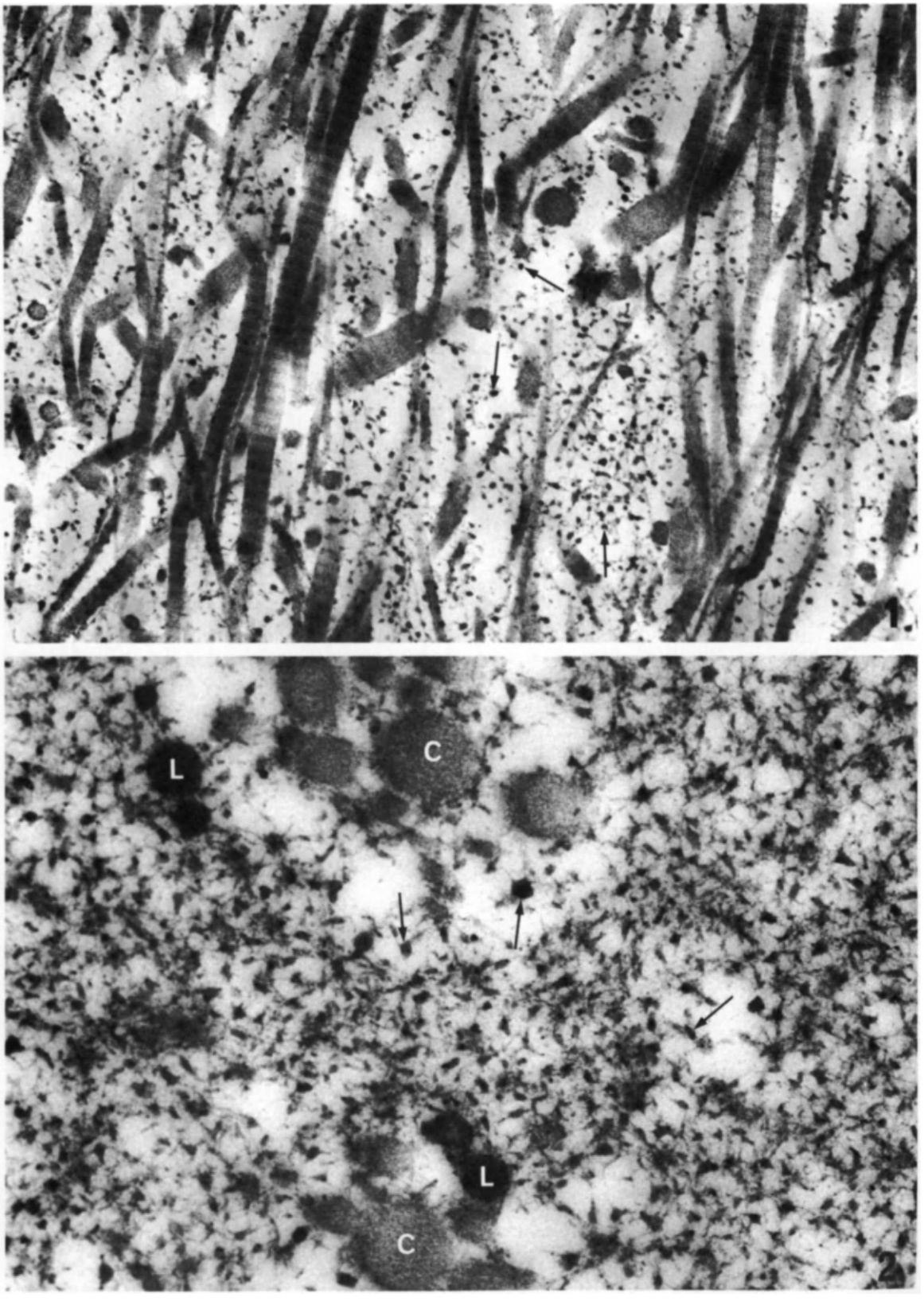
*For some time now a concerted effort has been made to eliminate the term 'mucopolysaccharides' and indeed all words with the prefix 'muco'. This has met with a considerable measure of success but it is doubtful whether all words with the prefix 'muco' will ever be totally eliminated. In any case, some of the mucopolysaccharides are now reclassified as glycoproteins while for others the term 'glycosaminoglycans' has been created.

†On the other hand, the filament could be the hyaluronic acid filament which forms the 'backbone' of the proteoglycan aggregate as seen in spread preparations (*see* page 1276 for more details).

Plate 538

Fig. 1. Zone III of rabbit articular cartilage. Numerous proteoglycan particles (arrows) are seen amongst the collagen fibrils. × 45 000

Fig. 2. Rabbit semilunar cartilage. Note the numerous proteoglycan particles (arrows), cross-cut collagen fibrils (C) and intramatrical granular lipidic debris (L). × 86 000


L
C
L
C

The manner in which cartilage is fixed and processed* has a bearing on the size, electron density and frequency with which proteoglycan particles are encountered. It would appear that it is the protein core of the proteoglycan which is fixed by aldehyde fixatives, the carbohydrate moiety is precipitated as a consequence of being covalently bound to the protein (Leppi, 1971). Usually a substantial loss of carbohydrate occurs during routine fixation, but this can be minimized to a greater or lesser extent by the addition of various substances such as ruthenium red, barium nitrate and cetylpyridinium chloride to the fixative.

Although proteoglycan particles were visualized by early workers in routine preparations of cartilage† their nature and significance was not at first appreciated. By combining ruthenium red and osmium tetroxide in a single reagent Luft (1965) demonstrated spherical electron-dense particles 30 nm in diameter (and associated filaments) and surmised that they were 'protein-polysaccharide macromolecules of cartilage preserved and stained by the ruthenium red-osmium tetroxide reaction'.

Many ultrastructural studies using ruthenium red and other agents to demonstrate proteoglycan particles have since been published. These include studies utilizing: (1) alcian blue (Thyberg *et al.*, 1973); (2) bismuth nitrate (Smith and Serafini-Fracassini, 1968); (3) colloidal iron (Matukas *et al.*, 1967; Hoshino and Yamada, 1972); (4) colloidal thorium (Revel, 1964); (5) lanthanum nitrate (Khan and Overton, 1970); and (6) ruthenium red (Eisenstein *et al.*, 1971; Luft, 1971a,b; Merker and Struwe, 1971; Thyberg *et al.*, 1973; Silbermann and Frommer, 1974).

Proteoglycan particles more or less completely disappear after digestion with hyaluronidase, chondroitinase, papain or trypsin (Matukas *et al.*, 1967; Khan and Overton, 1970; Thyberg *et al.*, 1973; Thyberg, 1977). Extraction with a 4 M guanidine-HCl or 1.9 M $CaCl_2$ removes almost all proteoglycan particles (about 85 per cent of proteoglycans measured as hexosamine are extracted) (Anderson and Sajdera, 1971; Campo and Phillips, 1973).

It has generally been held that these particles represent proteoglycans collapsed and condensed by preparative procedures. Hascall (1980)‡ has compared the size of the proteoglycan particle (as seen in sectioned material) with that of the large, whole proteoglycan aggregate (spread out with the Kleinschmidt *et al.* (1959) technique) (*see below*) and she has

*It is beyond the scope of this brief essay to deal with the large and complex literature on this subject. (For references *see* Szirmai, 1963 and Leppi, 1971.)

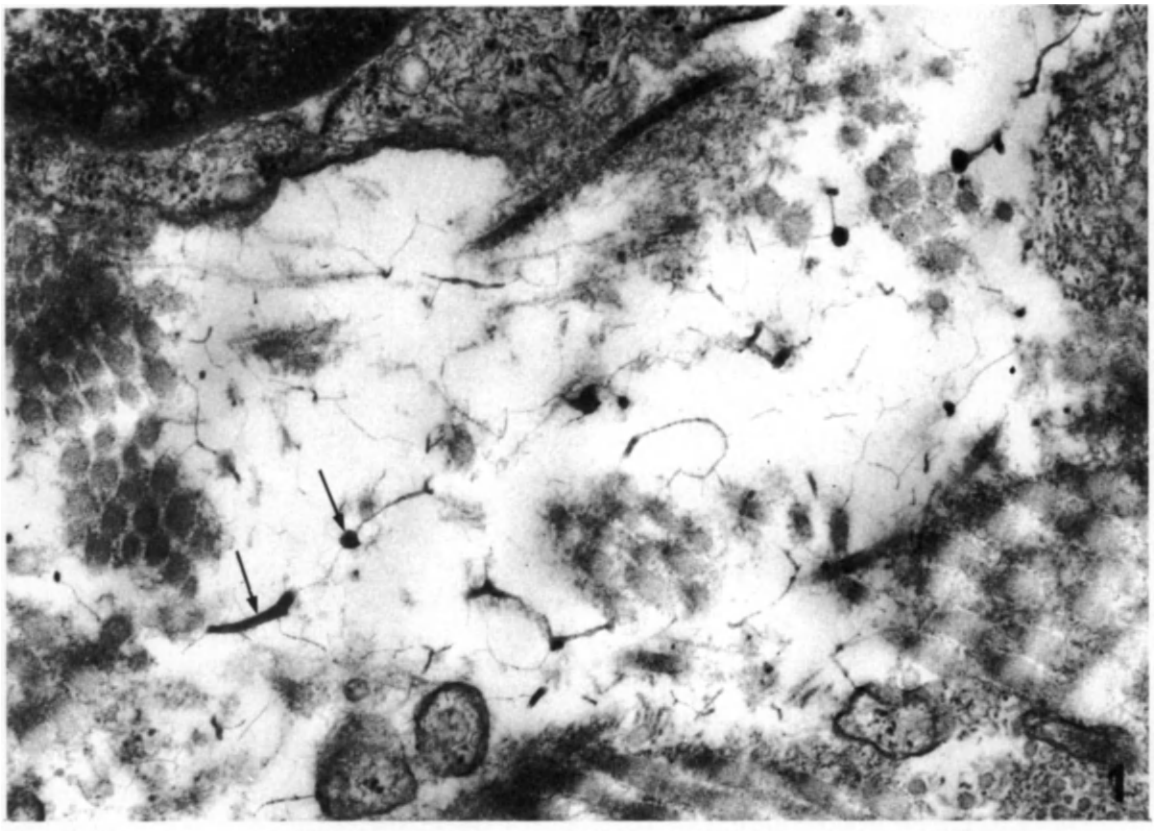
†The illustrations presented in *Plates 538–540* were obtained from material fixed in glutaraldehyde and post-fixed in osmium. The sections were stained with uranium and lead.

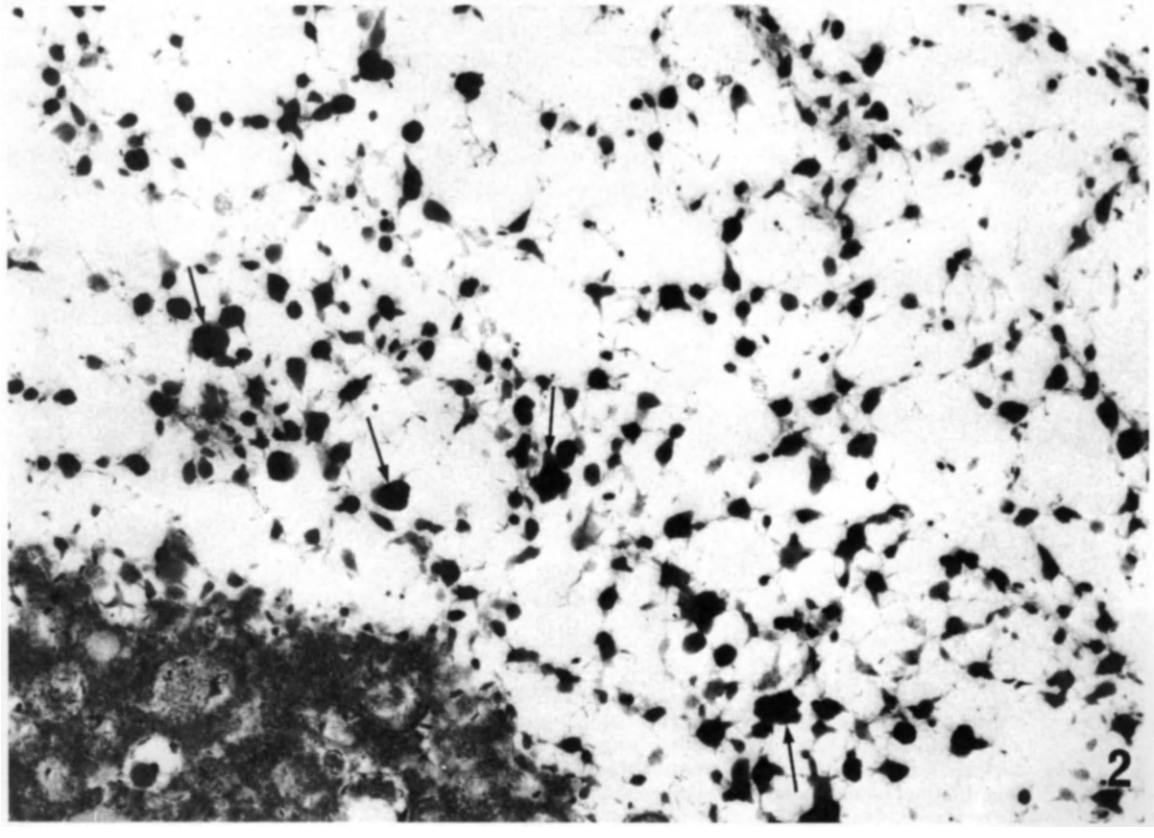
‡This study was carried out on the proteoglycans in Swarm rat chondrosarcoma. A medium-sized proteoglycan particle in sectioned material measured 160 nm × 25 nm. A medium-sized spread proteoglycan aggregate measured 2000 nm × 500 nm.

Plate 539

Fig. 1. Skin biopsy from a case of rheumatoid arthritis treated with gold. Proteoglycan particles (arrows) and associated filaments are seen in the dermis. × 46 000

Fig. 2. Chondrosarcoma. Quite large proteoglycan particles (arrows) are seen adjacent to a necrotic chondrocyte. Some of the larger particles (arrows) in this electron micrograph measure about 110–140 nm in long diameter. Compare these particles with those seen in normal cartilage (*Plate 538*). × 44 000





concluded that the proteoglycan particle is too small to be the whole aggregate and that it is more likely to represent one or several monomer portions of the aggregate*. A dissenting opinion regarding the nature of proteoglycan particles has also been expressed (Goel and Jacob, 1976). These authors report that if fresh cartilage is treated with collagenase both the particles and collagen fibrils in the matrix disappear, but if the cartilage is fixed in glutaraldehyde prior to exposure to collagenase neither the fibrils nor the particles disappear. From this they conclude that: (1) 'collagen is a major constituent of the granules'; and (2) 'the granules are fixation artefacts'. However, such conclusions do not flow logically or inevitably from the data presented. One may contend that anything which seriously disrupts the collagen meshwork in which large proteoglycan aggregates are trapped will inevitably lead to a release of proteoglycans from the matrix into the incubating medium.

In cartilage ground substance, most of the proteoglycans exist in the form of aggregates of quite high molecular weight. Entanglement between proteoglycan and collagen fibrils after the manner of the 'stag caught in the thicket' is thought to occur (Stockwell, 1974). The molecular architecture of proteoglycan aggregates has been visualized by electron microscopy (Rosenberg, 1975; Rosenberg *et al.*, 1975; Lohmander and Thyberg, 1975; Thyberg *et al.*, 1975; Thyberg, 1977). When isolated and spread by the Kleinschmidt *et al.* (1959) technique they present as large bottle-brush-like structures. The proteoglycan aggregate consists of numerous proteoglycan molecules (i.e. chains of chondroitin sulphate and keratan sulphate attached to core protein) of varying lengths attached to a hyaluronic acid filament. According to Rosenberg (1975), the filamentous backbone in individual aggregates of bovine articular cartilage (i.e. proteoglycans subunits) varies in length from 40 000–420 000 nm. The proteoglycan molecules vary in length from 10 000–40 000 nm.

Little is known about any morphological alterations that might occur in the proteoglycan particles in diseases or experimental situations. However, a few instances where the number and/or size of proteoglycan particles was reduced have been recorded. Such instances include: (1) cultured limb buds of mouse embryo incubated with 6-diazo-5-oxonorleucine (a substance known to inhibit synthesis of glycosaminoglycans) (Kochhar *et al.*, 1976); (2) lathyritic mouse embryo cartilage (Matukas *et al.*, 1967); and (3) cartilage of homozygous brachymorphic mice (condition resembles human achondroplasia) (Orkin *et al.*, 1976, 1977).

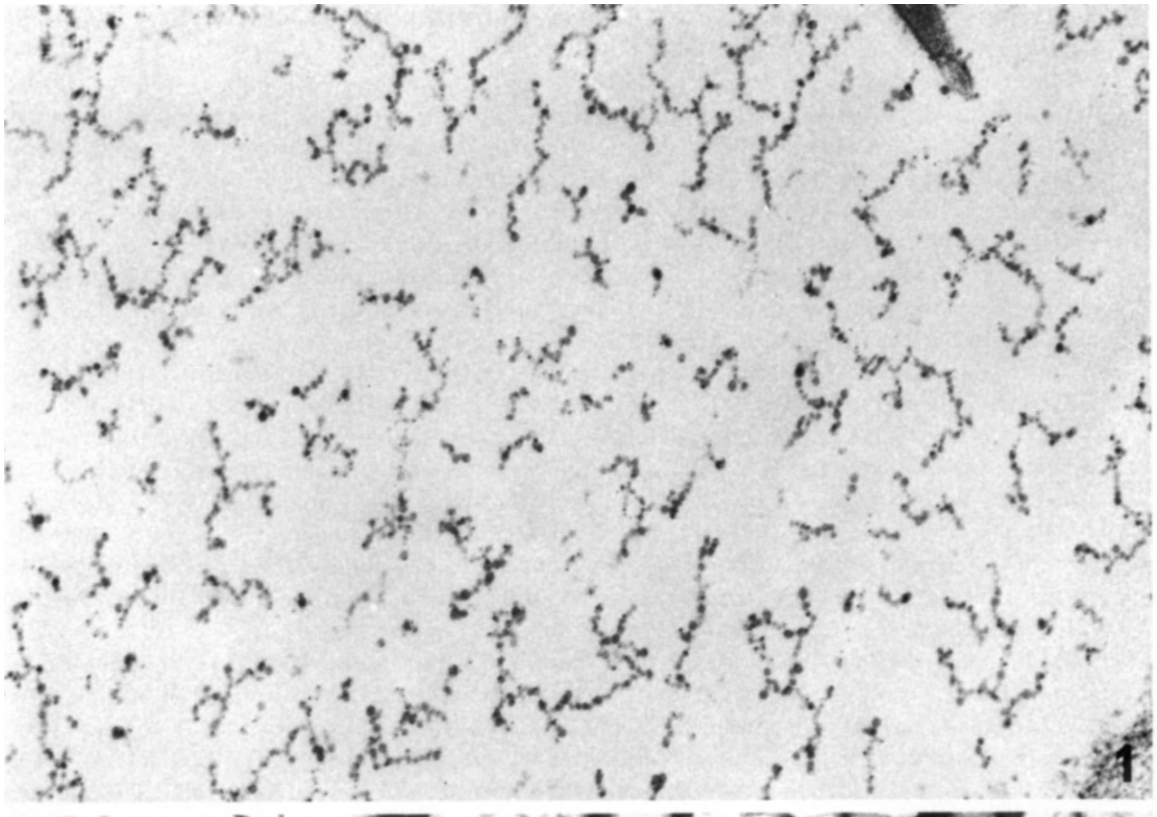
Proteoglycan particles show many morphological variations, as will be evident from *Plates 538–540*. Such differences could be meaningful and reflect site or disease dependent differences in composition or they may be largely if not entirely artefacts reflecting little more than various kinds and degrees of extraction and aggregation engendered by preparative procedures. Data permitting one to choose between such alternatives are lacking, but one may speculate that such dramatic variations in morphology probably reflect differences in chemical composition and size and complexity of proteoglycan aggregates.

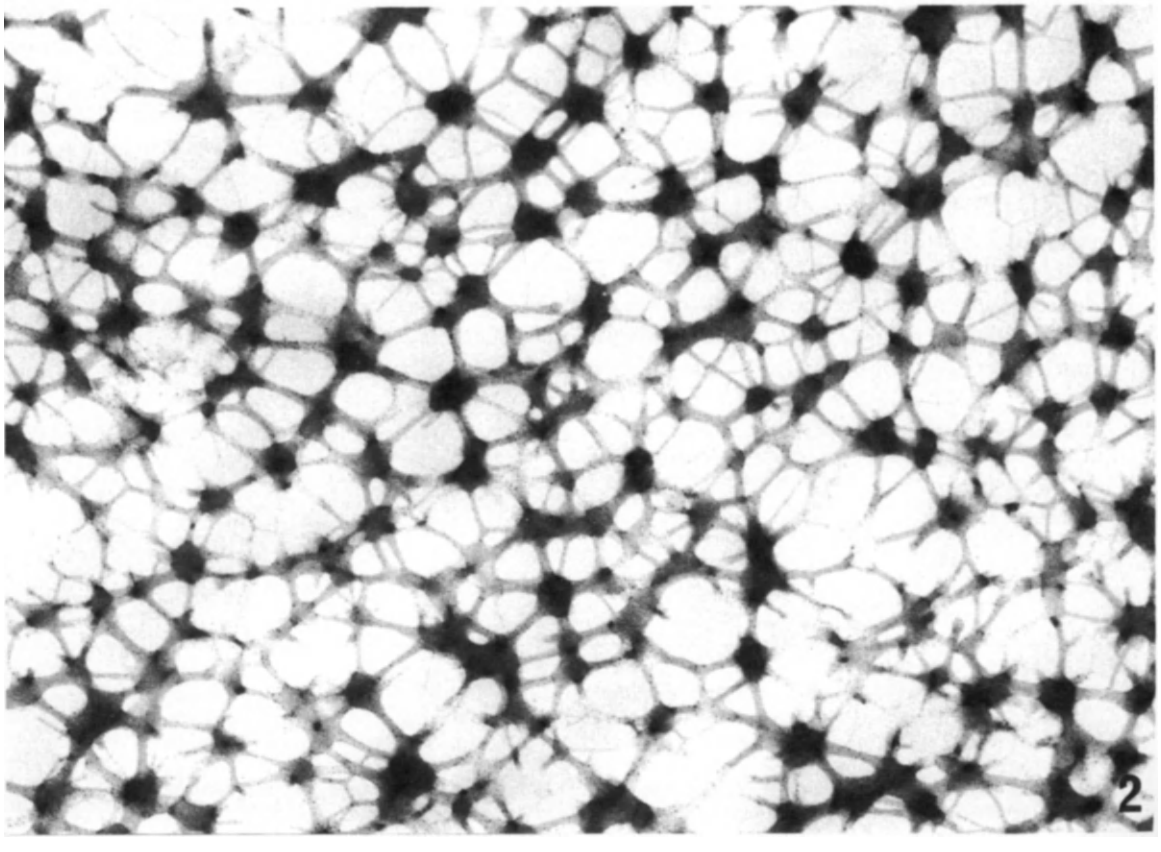
*Variously referred to as proteoglycan monomers, proteoglycan subunits or proteoglycan molecules. Several of these attached to a hyaluronic acid filament ('backbone') comprise a proteoglycan aggregate.

Plate 540

Fig. 1. Rabbit annulus fibrosus. The territorial matrix contains fine proteoglycan particles arranged like beads on angulated threads. I have seen this pattern of particles in osteoarthrotic cartilage. Meachim (1972) has found it in human nucleus pulposus and Stanescu (1975) has illustrated it in polyepiphyseal dysplasia. × 54 000

Fig. 2. Biopsy from pinna of the ear from a case of polychondritis. These proteoglycan particles formed a meshwork in the territorial matrix between the cell processes of a chondrocyte. × 44 000





Calcification (matrical lipidic debris, matrix vesicles, calcifying collagen and calcifying secretions)

Ultrastructural studies have added considerably to our knowledge of normal and pathological calcification. We now know that calcification can commence or occur in intracellular or extracellular sites. Instances of the former include intramitochondrial calcification seen in examples of metastatic calcification (*see* page 236) and intralysosomal calcification seen in malakoplakia (*see* page 680). Here we deal with calcification in the extracellular matrix which can occur in or around necrotic cell fragments or fragments cast off by viable cells (usually referred to as matrical lipidic debris or matrix vesicles) and collagen fibrils. Elastic fibres can also calcify but this has already been dealt with (*see* page 1260).

Terms such as 'matrical lipid', 'matrical lipidic debris'*, 'membranous debris', 'cytoplasmic fragments', 'osmiophilic bodies', 'corona vesicles', 'extracellular lipid', 'matrix granules' and 'matrix vesicles' have been used to describe granular and membranous (presenting as vesicles, myelinoid membranes and rarely as myelin figures) material found in the matrix of cartilage of various types and from various sites (Ghadially and Roy, 1969; Stockwell, 1979; Ghadially, 1983). The occurrence of such extracellular lipidic material was not too well recognized in the light microscopic era, but Montagna (1949) did speak of 'clouds of very fine sudanophilic particles visible only at high magnifications' and of 'a faint sudanophilic band in the new matrix just under the perichondrium' in adult tracheal cartilage.

In 1963, Barnett *et al.* described 'myelin bodies' together with 'electron-dense granules' in rabbit articular cartilage and suggested that this material was derived from chondrocytes that had suffered *in situ* necrosis. Similar material was found by us (Ghadially *et al.*, 1965) in human and rabbit articular cartilage and we concluded by saying 'Though some free lipid undoubtedly originates from disintegrating cartilage cells, most of the extracellular lipid lies in the neighbourhood of intact cells and the theory is advanced that it derives from their extruded cytoplasmic processes'. Hence the term 'matrical lipidic debris' was coined (Ghadially and Roy, 1969) to describe this material (*Plates 541* and *542*).

This lipidic debris in the matrix of cartilage increases with advancing age (Barnett *et al.*, 1963) and in experimental situations where cartilage is injured and a greater than normal number of chondrocytes suffer *in situ* necrosis (Ghadially *et al.*, 1971; Fuller and Ghadially, 1972). The fact that a 'cloud' of such particles is at times seen just under the articular surface with the light microscope and with the electron microscope one can often see particulate electron-dense material on the articular surface led me to suggest (Ghadially, 1978, 1980, 1983) that 'the lipidic debris finds its way to the surface and is discharged into the synovial fluid'.

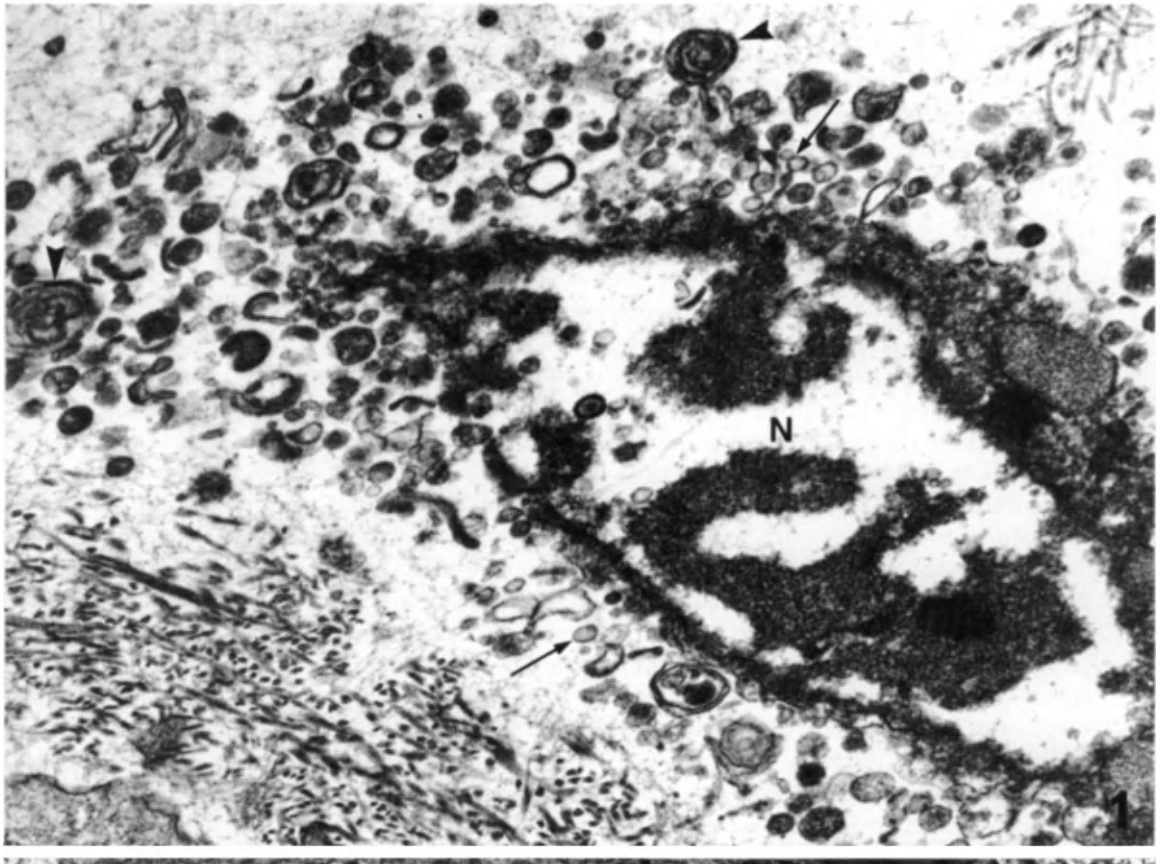
In epithelia, necrotic cells are shed from the surface; in some tissues they are removed by macrophages. Such mechanisms for the disposal of necrotic cells and debris derived from them

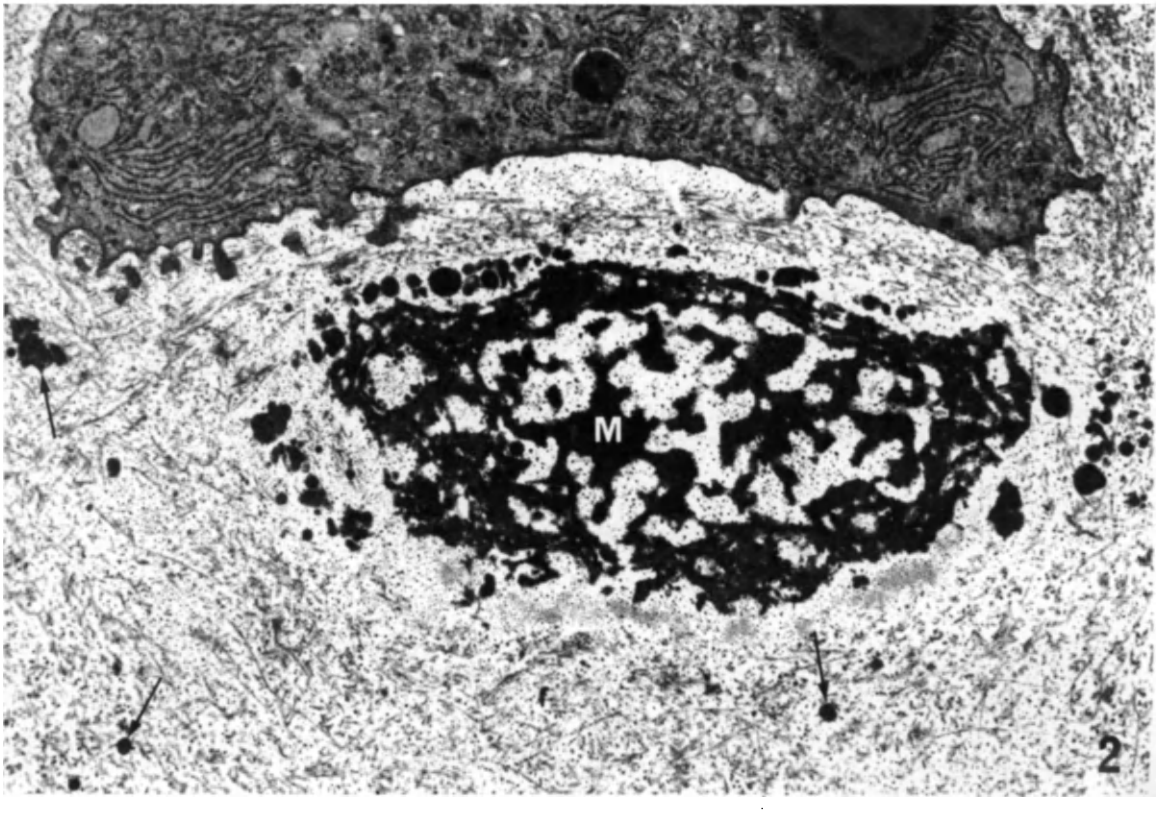
*This section on matrical lipidic debris should be read in conjunction with the section on spherical microparticles. For in the final analysis all these structures are essentially similar in that both represent material 'shed' from the cell into the matrix and both may calcify given a suitable milieu (*see* pages 1080–1083).

Plate 541

Fig. 1. *In situ* necrosis and disintegration of a chondrocyte from rabbit articular cartilage. Note the necrotic nucleus (N) and the membranous debris derived from the breakdown of cellular components. Some of these membranous formations present as small vesicles (arrows) others as larger vacuolar and whorled membranous structures (arrowheads). × 27 000

Fig. 2. *In situ* necrosis and disintegration of one member of a chondrocyte pair from rabbit articular cartilage. The resultant necrotic debris presents as granules and masses of electron-dense material (M). Some of it (arrows) appears to have drifted into the surrounding matrix. × 16 000 (*From Ghadially, 1978*)


N
1


M
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can hardly operate in an avascular tissue like articular cartilage where the sparse cells are locked in an abundant matrix. Hence, the formation and discharge of lipidic debris as described above is a satisfying hypothesis which explains the manner in which the products of cell necrosis and hence indirectly necrotic cells are disposed of from this tissue.

Similar membranous and granular material was noted in calcifying cartilage and it was at first referred to as 'membrane-bound cytoplasmic fragments' by Anderson (1967)* and as 'osmiophilic bodies' by Bonucci (1967). Since crystals of calcium apatite are found in and around some of these structures, the idea was born that they served as initial sites for calcium deposition. Anderson (1968) renamed these structures 'matrix vesicles' (a trilaminar membrane can be seen around some of them but most of them have a 'solid' rather than vesicular appearance) and Bonucci (1970a) called them 'calcifying globules'. Subsequent workers have proposed various other terms such as 'roundish osmiophilic bodies'; 'calcifying vesicles', 'osteoblastic buds', 'osmiophilic globules', 'membrane-bound vesicles', 'matrix saccules', 'crystal bodies', 'membrane-bound granules' and 'dense particles' to describe these structures in various sites and situations, but the quite inappropriate term 'matrix vesicles' is the one most commonly employed. Several workers (e.g. Holtrop, 1972) have regarded 'matrix vesicles' as the debris of chondrocytes, while Bonucci (1970a,b) has presented electron micrographs suggesting that matrix vesicles of epiphyseal cartilage arise by a blebbing of cell processes of chondrocytes. Thus the manner in which the so-called 'matrix vesicles' arise is analogous to the manner in which lipidic debris arises in articular cartilage (Ghadially *et al.*, 1965).

'Matrix vesicles' or calcifying bodies have now been seen in: (1) ossifying cartilage induced by the injection of amniotic cells in the thigh of cortisone-treated rats (Anderson, 1967); (2) epiphyseal cartilage and rib cartilage of humans, mouse, normal and scorbutic guinea-pig and normal and rachitic rat (Bonucci, 1967, 1970a,b, 1978; Bonucci and Dearden, 1976; Anderson, 1968, 1969, 1976; Cecil, 1972; Simon *et al.*, 1972; Ali, 1976; Ali *et al.*, 1978); (3) Zone IV of human articular cartilage (Dmitrovsky *et al.*, 1978); (4) antlers of deer (Sayegh *et al.*, 1973, 1974; Newbrey and Banks, 1973, 1975); (5) osteoarthrotic† cartilage (Ali, 1978); (6) torn semilunar cartilages (Ghadially and Lalonde, 1981) (*Plate 543*); (7) calcifying tendinitis (Sarkar and Uhthoff, 1978); (8) chondrosarcoma (Schajowicz *et al.*, 1974); (9) membranous bone of mouse (Bernard and Pease, 1969); fracture callus of dog (Schenk *et al.*, 1970); (10) healing alveolar bone sockets (Sela *et al.*, 1978); (11) cranium and long bone of rat, duck and pigeon (Ascenzi and Bonucci, 1971); (12) tooth of dogfish (*Squalus acanthias* L) (Garant, 1970); (13) predentin and dentin of humans, rabbit, mouse and rat (Croissant, 1971; Bernard, 1972; Eisenman and Glick, 1972; Ozawa and Najima, 1972; Sisca and Provenza, 1972; Slavkin *et al.*, 1972); (14) osteogenic sarcoma (Lee, 1974; Lee *et al.*, 1975; Muhlrad *et al.*, 1978; Sela and Bab, 1979); (15) atherosclerotic aorta and aortic valve (Paegle, 1969; Kim and Huang, 1972).

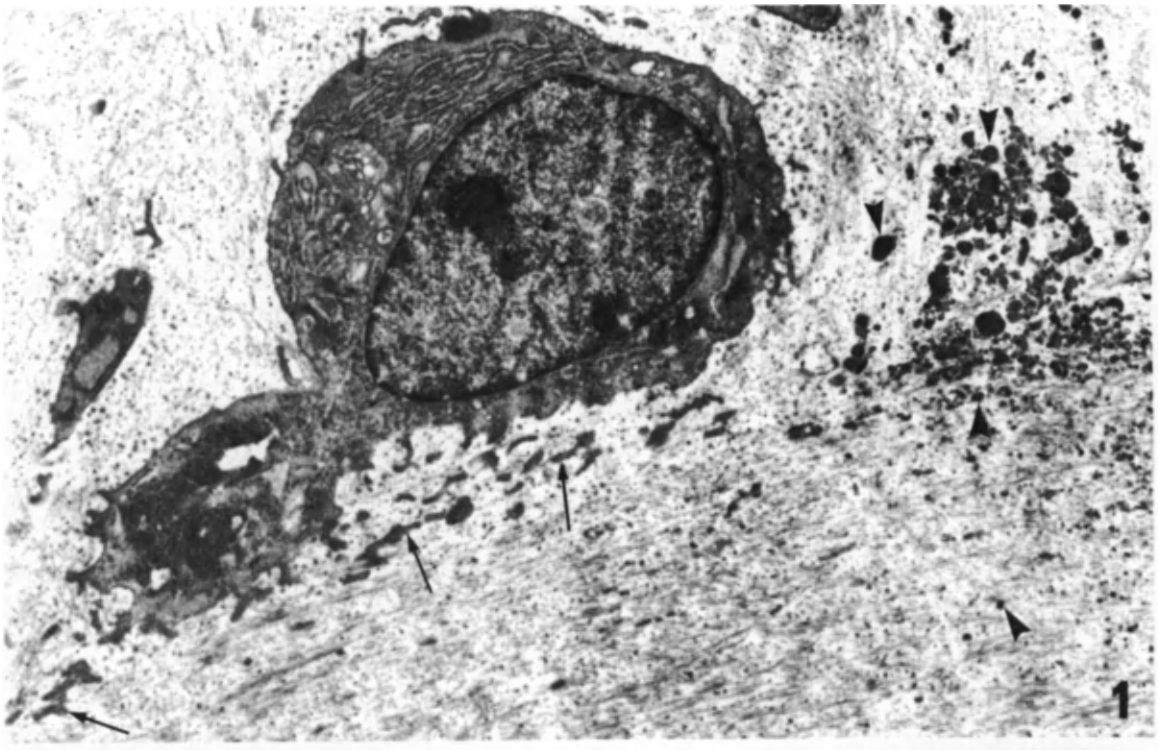
*Anderson's report (1967) deals with calcification in cartilage experimentally induced by injection of amniotic cells in the thigh of cortisone-treated rats. Bonucci's report (1967) deals with calcification in epiphyseal cartilage of rats and guinea pigs.

†The term 'osteoarthrosis' is preferable to 'osteoarthritis' because basically this is a degenerative rather than an inflammatory disease. However, both terms are in current usage.

Plate 542

Fig. 1. A chondrocyte from human osteoarthrotic cartilage. The matrix adjacent to the chondrocyte is littered with granular electron-dense debris (arrowheads). Numerous structures probably representing detached cell processes (arrows) are seen adjacent to the chondrocyte. × 9500

Fig. 2. A chondrocyte from human semilunar cartilage showing cell processes (arrows) and intramatrical lipidic debris (arrowheads). Appearances seen here are consistent with the idea that the lipidic debris seen here is derived from detached cell processes. × 13000 (*From Ghadially and Lalonde, 1981*)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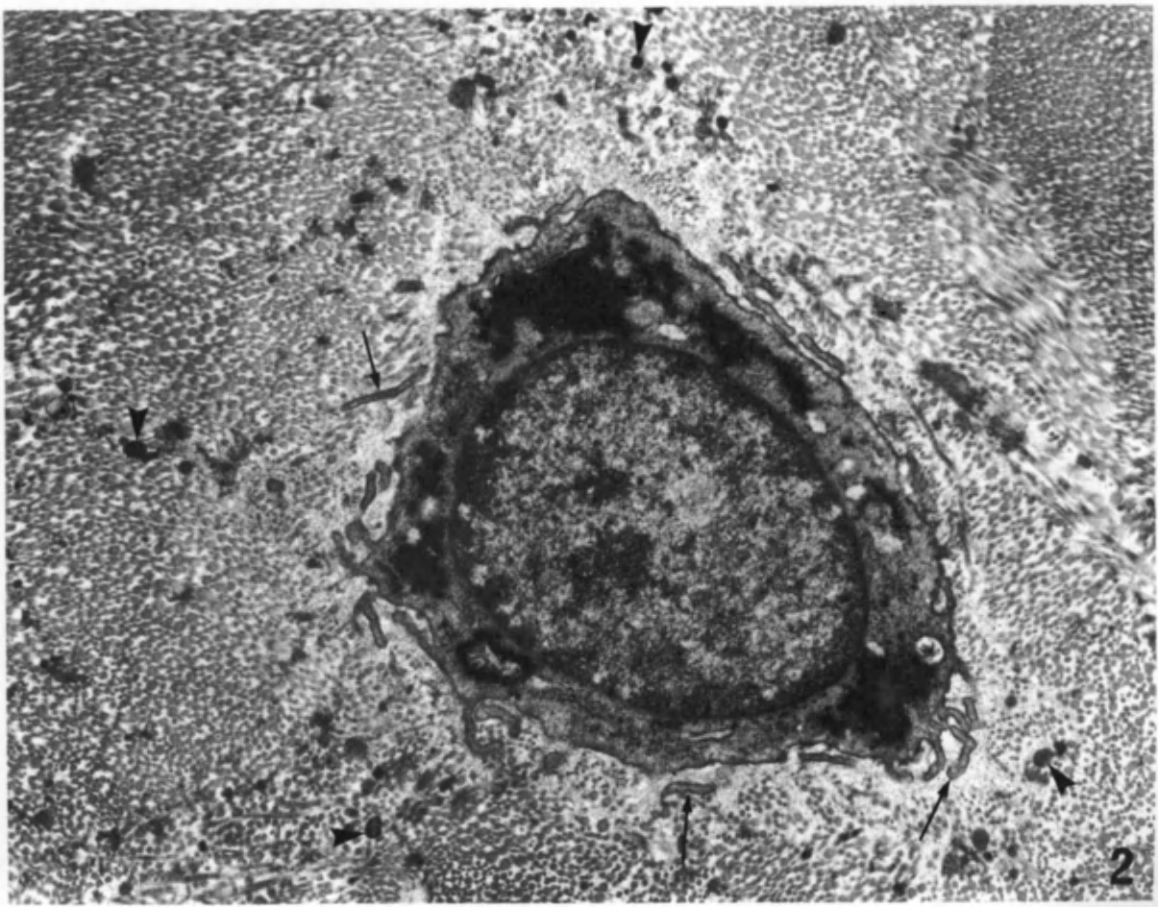
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Cytochemical studies at the ultrastructural level (Matsuzawa and Anderson, 1971; Anderson and Reynolds, 1973) and biochemical studies on isolated 'matrix vesicles'* from epiphyseal cartilage (Ali *et al.*, 1970; Ali and Evans, 1973) have shown that they contain alkaline phosphatase, adenosine triphosphatase and inorganic pyrophosphatases. Each of these phosphatases has been implicated one time or another in the mechanisms of calcification (Robinson, 1923; Cartier and Picard, 1955; Fleisch and Bisaz, 1962; Anderson, 1972). Isolated 'vesicles' show an enrichment of total lipids, cholesterol, phospholipid and glycolipid (Peress *et al.*, 1971, 1974; Wuthier, 1973). The most prominent phospholipids are sphingomyelin (which are also a prime component of plasma membrane) and phosphatidyl serine which has a strong affinity for calcium and might hence help to concentrate or sequestrate calcium in the 'vesicles'.

Pathology students have long been taught (e.g. Boyd, 1938) that it is common for calcium to be laid down in 'dead or dying tissues' and that this is due to 'calcium and phosphorus being adsorbed by the degenerated tissue'. Therefore, one is not surprised to hear that necrotic cellular debris (or 'matrix vesicles') can concentrate or sequester calcium, if this is available in the surrounding milieu, but one wonders about the role of various enzymes alleged to be present in 'matrix vesicles'. Thus for example Ali and Evans (1973) have postulated that 'matrix vesicles' might initiate calcification 'by generating energy by the hydrolysis of ATP to provide an active transport of ions inside the lumen of the vesicle for the formation of hydroxyapatite' but Felix and Fleisch (1976) found that 'isolated vesicles were unable to transport calcium actively' and they suggest that 'vesicles induce calcification by nucleating calcium phosphate precipitation and through the local destruction of pyrophosphate, a crystallization inhibitor'.

Although one may argue about the precise mechanisms of calcification one has to concede that this (i.e. deposition of calcium salts in or around matrical lipidic debris or so-called 'matrix vesicles') is at least one of the ways in which calcium is deposited in the growth plate. That this is not the only mechanism involved is attested by the fact that there is also a marked increase in the size of the intramitochondrial dense granules (*see* footnote on page 236) in calcifying cartilage. Studies utilizing potassium pyroantimonate (Brighton and Hunt, 1976, 1978) show that it is the mitochondria which first accumulate calcium and that they lose calcium (in the

*Sections through so-called isolated 'matrix vesicles' do not inspire confidence. The material looks like cell debris; either the variety pre-existing in the matrix and/or generated from intact cells during preparative procedures.

Plate 543

Calcifying bodies from torn human semilunar cartilages. Calcium has been demonstrated (*Ghadially and Lalonde, 1981*) in such bodies by electron-probe x-ray analysis.

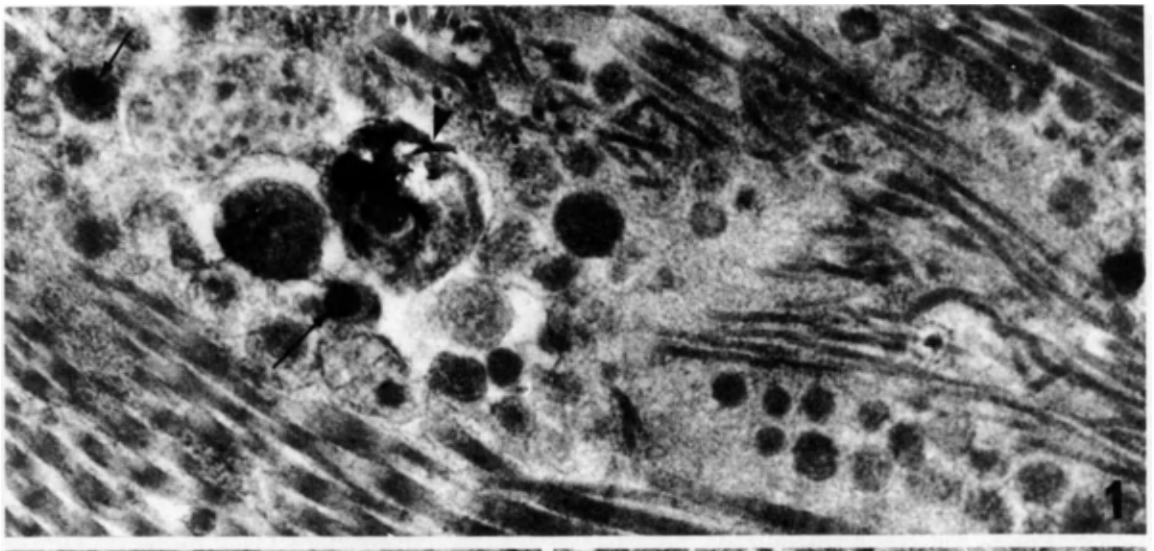
Fig. 1. A large globular or vacuolar body with calcium crystals (arrowhead). Note also the much smaller granular or vesicular bodies containing rounded electron-dense calcium deposits (arrows). Several other membranous and granular structures acceptable as matrical debris or 'matrix vesicles' are also present. × 48 000 (*From Ghadially and Lalonde, 1981*)

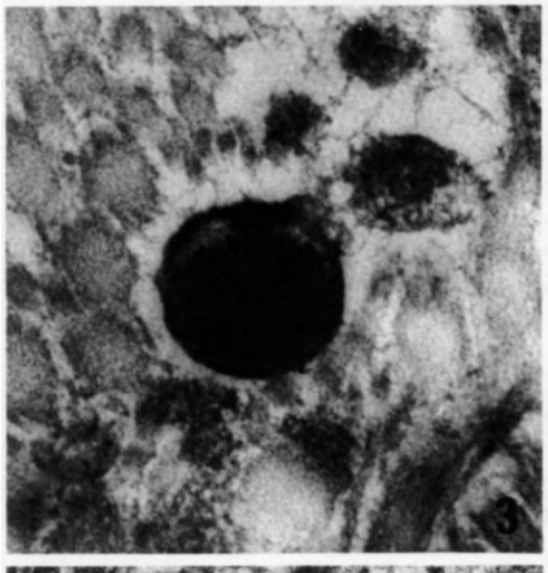
Fig. 2. Another large vacuolar body (arrow) with a corona of needle-shaped crystals. Note also the matrical debris. ×46 000 (*From Ghadially and Lalonde, 1981*)

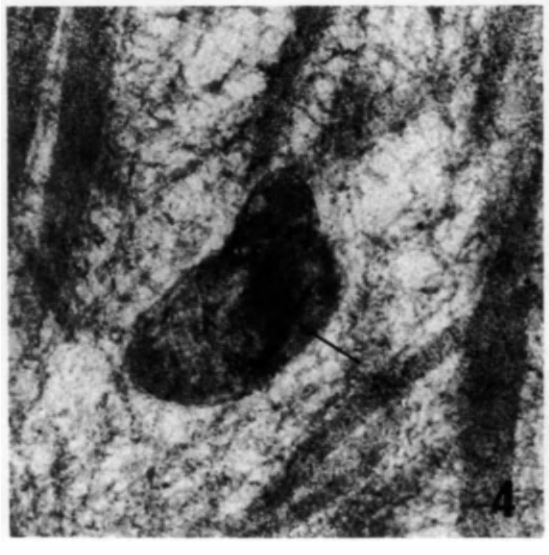
Fig. 3. A calcifying body with granular contents. × 100 000

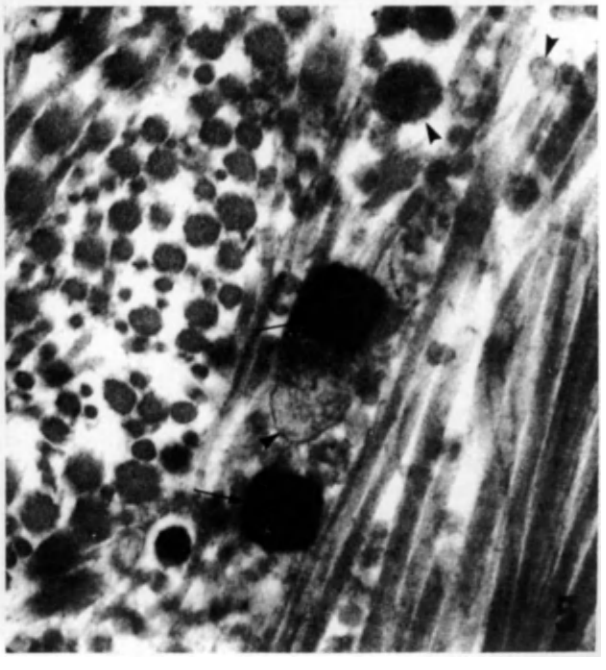
Fig. 4. A membrane-bound calcifying body with a needle-like crystal (arrow) in its interior. × 100 000

Fig. 5. Cuboidal calcified bodies (arrows) and matrical lipidic debris. One of these seems to be associated with a membranous structure (arrowhead). × 50 000 (*From Ghadially and Lalonde, 1981*)









degenerating zone of epiphyseal cartilage) which is then deposited in the calcifying bodies. A similar situation also probably prevails in Zone IV of articular cartilage for spherical and 'hedgehog-like' masses of apatite crystals are seen adjacent to Zone IV chondrocytes (*Plate 544*) (Ghadially and Roy, 1969). Yet here again this cannot be the only mechanism, for fine particles and needles of calcium salts are found between the collagen fibrils also.

Except in rare pathological conditions, Zones I to III of articular cartilage do not calcify, yet matrical lipidic debris which is morphologically indistinguishable from the so-called 'matrix vesicles' is of constant occurrence in these regions. It is also disconcerting to note that 'matrix vesicles' containing calcium deposits have been found in the elastic cartilage of the ear and semilunar cartilages, yet these are not calcifying cartilages.

The crucial question which emerges is: whether calcifying bodies in various cartilages and other sites derive by deposition of calcium salts in or on the membranous (including vesicular) and granular components of matrical lipidic debris or whether calcification occurs in or on a special vesicular structure (containing a battery of specific enzymes) of relatively constant size (about 100 nm) and form deserving to be called a 'matrix vesicle' or a 'special organelle of calcification' which is 'secreted' by the chondrocyte.

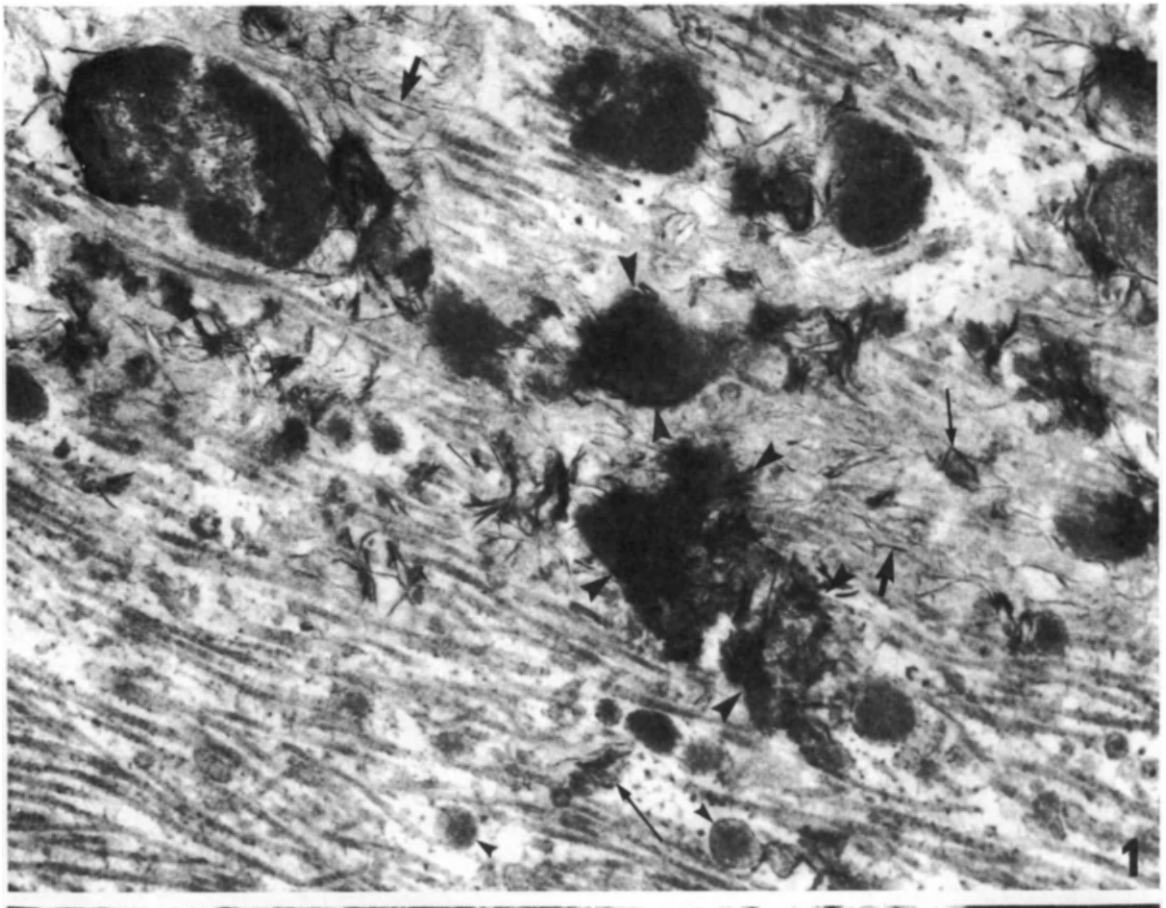
Besides the arguments already presented above, the observations made by us on human semilunar cartilages (Ghadially and Lalonde, 1981) do not support the thesis that such a distinct and different class of 'matrix vesicles' or 'organelles' exists because: (1) calcified bodies occurred, almost invariably, in company with pleomorphic lipidic debris; irrespective of whether it was granular or vesicular in form; (2) a distinct and different population of vesicles of relatively constant size and morphology different from intramatrical lipidic debris was not seen; and (3) calcification occurred in or on vesicular and granular bodies which ranged in size from about 50–600 nm (i.e. six times larger than the so-called 'matrix vesicles').

Similarly, in calcifying cartilage from a case of epiphyseal dystosis (*Plate 544*) I have seen (Ghadially, unpublished observation) crystalline calcium deposits in and around masses of cell debris measuring up to about 1 μm in size. It is for reasons such as this that one is unable to wholeheartedly accept the claims of those who would have us believe that a special 'extracellular organelle of calcification' exists. Be that as it may, the calcium deposits in calcifying bodies may appear homogeneously electron dense or they may have a granular or crystalline appearance. With the aid of electron-probe x-ray analysis calcium and phosphorus have been demonstrated in the calcifying bodies of epiphyseal cartilage (Hall *et al.*, 1971, 1973; Ali, 1976; Ali *et al.*, 1978). The amount of Ca and P is said to depend on the Zone in which the

Plate 544

Fig. 1. Calcifying cartilage from a case of epiphyseal dystosis. Although at times needle-like calcium deposits are associated with small vesicular structures (thin arrows) of the type described as 'matrix vesicles', they (i.e. calcium deposits) are also seen in association with much larger irregular shaped masses of cell debris (between large arrowheads). Further needle-like calcium apatite crystals are also seen amongst the collagen fibrils (thick short arrows). Conversely, several vesicular bodies (small arrowheads) morphologically acceptable as matrix vesicles do not show crystalline calcium deposits. × 57 000

Fig. 2. Calcifying zone (Zone IV) of articular cartilage from a young rabbit (three months old). A degenerating chondrocyte (C) is seen surrounded by calcified matrix (M). Punctate (arrows) and hedgehog-like calcified masses (arrowheads) lend credence to the idea that these deposits commenced in association with vesicular or granular bodies of the type described as matrix vesicles or matrical debris. × 18 000 (*From Ghadially and Roy, 1969*)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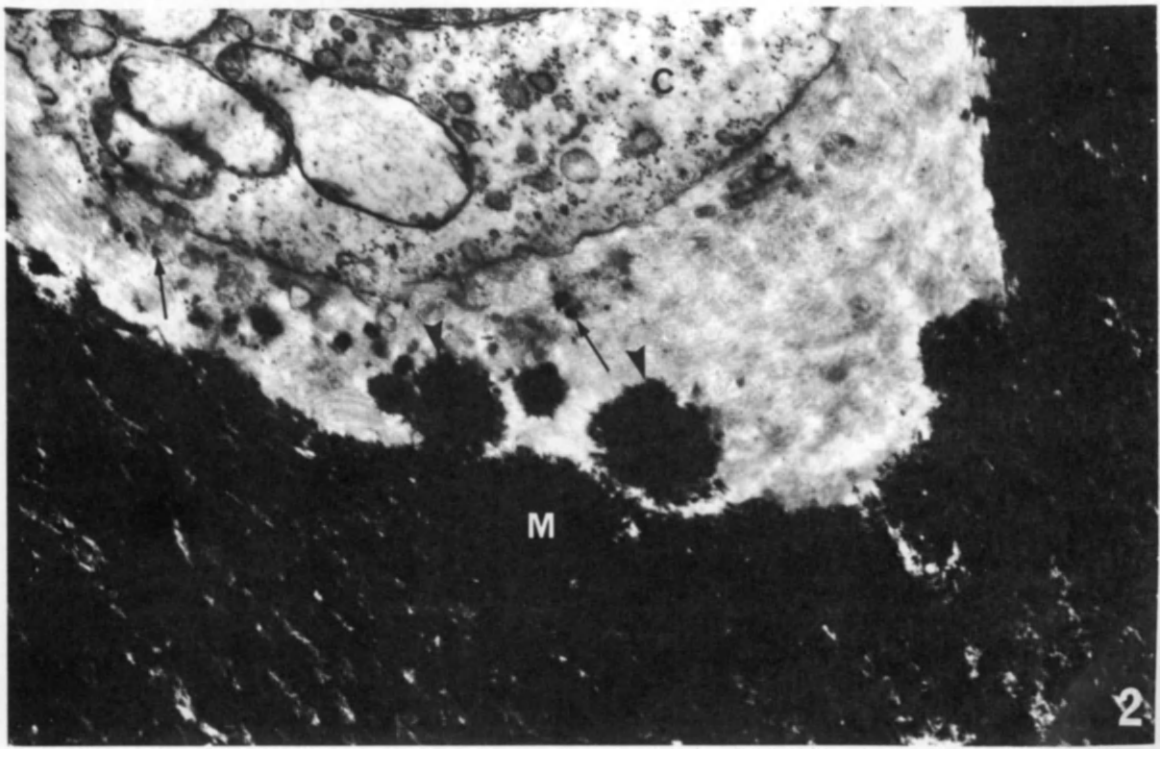
C
M
2

matrix vesicle is located. However, in vesicles containing crystalline deposits a Ca:P ratio indicative of hydroxyapatite has been found (Ali *et al.*, 1978). Calcium, magnesium and phosphorus have been found in calcifying bodies in unosmicated, unstained sections of human semilunar cartilages studied by electron-probe x-ray analysis (Ghadially and Lalonde, 1981). In osmicated material a substantial osmium peak (i.e. much higher than from surrounding matrix) was also evident, presumably reflecting the presence of lipid in these structures.

As mentioned earlier (page 1278) calcification occurs not only in association with matrical lipidic debris be it large (cell fragments) or small (so-called 'matrix vesicles') but also in association with collagen fibrils. Calcification or mineralization of collagen fibrils commences by deposition of amorphous or crystalline calcium deposits on the fibril but later the substance of the fibril also seems to be impregnated by calcium salts.

Instances where mineralization of collagen has been witnessed include: (1) calcifying Zone IV of articular cartilage (Ghadially and Roy, 1969; Ghadially, 1980); (2) Peyronie's disease (Vande Berg *et al.*, 1982); (3) idiopathic calcinosis of scrotum (Takayama *et al.*, 1982); (4) psammoma bodies of meningioma (Bennington *et al.*, 1970; Lopez *et al.*, 1974; Kubota *et al.*, 1986) (*Plate 545*); and (5) a desmoid tumour in the abdominal wall of a 6-year-old boy (Lee and Sen, 1985).

About calcification in the desmoid tumour (item 5), Lee and Sen (1985) state that 'Two forms of calcification were observed: an irregular, amorphous dystrophic calcification of collagen fibres and an organized, laminated psammomatous calcification (psammoma bodies)'. In both instances calcification was found to be a 'result of direct mineralization of individual collagen fibrils'. It is clear that matrix vesicles were not involved in this instance.

As is well known, in meningiomas (item 4) psammoma bodies form in meningiocytic whorls. The periphery of the meningiocytic whorl comprises a shell of meningiocytic cells and the centre comprises extracellular structures, such as basal lamina or fragments thereof, a whorl or a mass of collagen fibrils (this is the principal component), proteoglycan particles and occasionally also pre-elastic or elastic fibres. Besides this, on occasions a necrotic cell or large or

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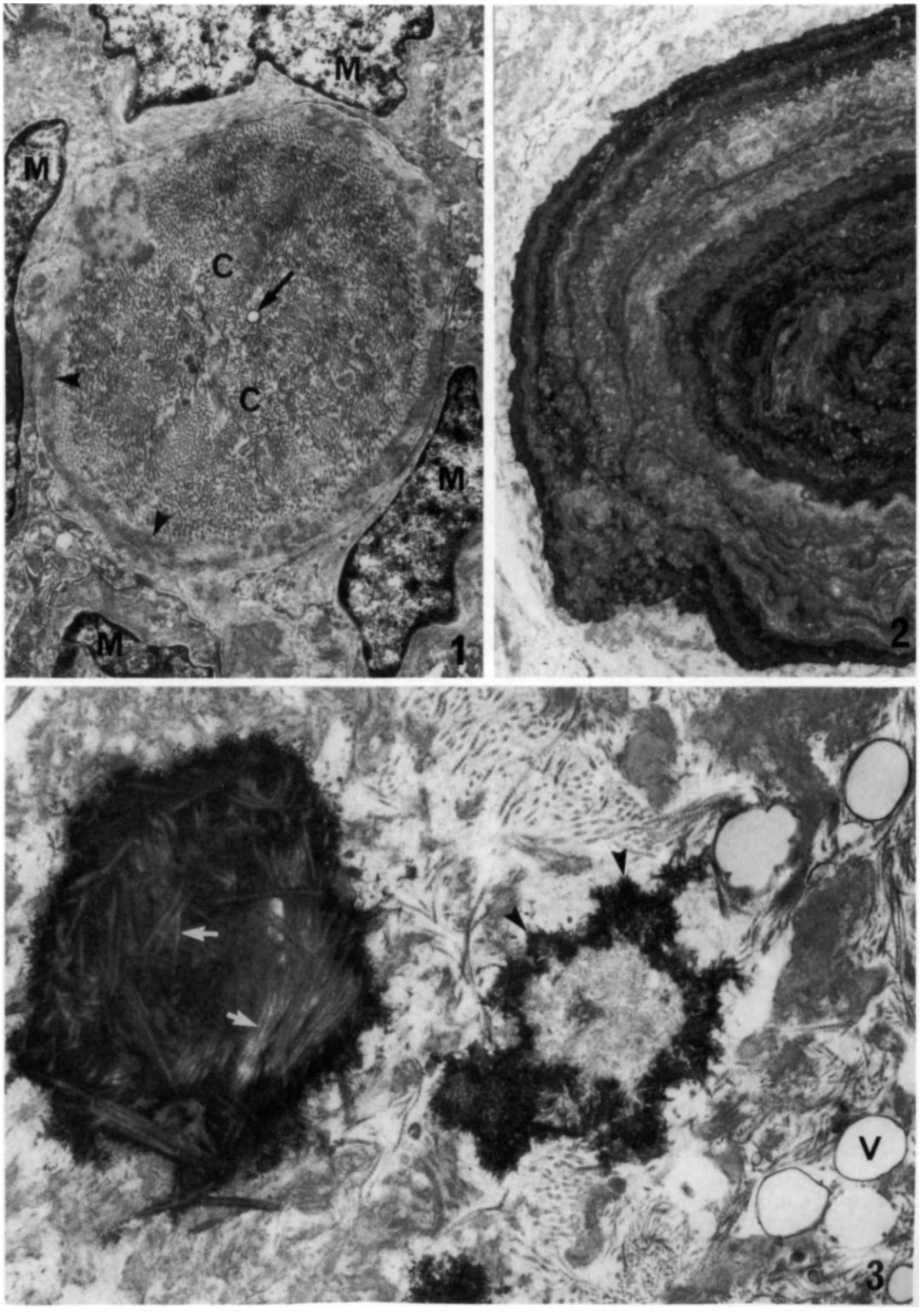
Plate 545

Meningiocytic meningioma. (*Tissue retrieved from a paraffin block supplied by Dr B. Rozdilsky*)

Fig. 1 Central region of a meningiocytic whorl. Note the numerous collagen fibrils (C), basal lamina (arrowheads), vesicle (arrow) and meningiocytic cells (M). × 9200

Fig. 2. Psammoma body showing the characteristic laminated appearance reminiscent of a transversely-cut tree trunk. The electron-dense laminae represent zones of maximum calcium salt deposition. Buried in the central electron-dense mass one can just discern electron-lucent collagen fibrils. × 5200

Fig. 3. Seen here is a calcified mass derived by deposition of calcium salts on collagen fibrils (arrows) and another where one can just discern needle-shaped hydroxyapatite crystals (arrowheads). Several membrane-bound vacuoles (V) showing no evidence of calcification. × 15000


M
M
C
C
M
M
1
2
V
3

small cell fragments are also seen. There is little doubt that the bulk of the calcium deposition during psammoma body formation occurs by calcification of collagen fibrils and sometimes this seems to be the only mechanism. However, appearances have been seen suggesting that calcification of cell debris also occurs, and according to Kubota *et al.* (1986) it is the cell debris in the form of large vacuoles (up to several μm in diameter) and not the small vesicles (i.e. matrix vesicles) which calcify. These large vacuoles they call 'matrix giant bodies'.

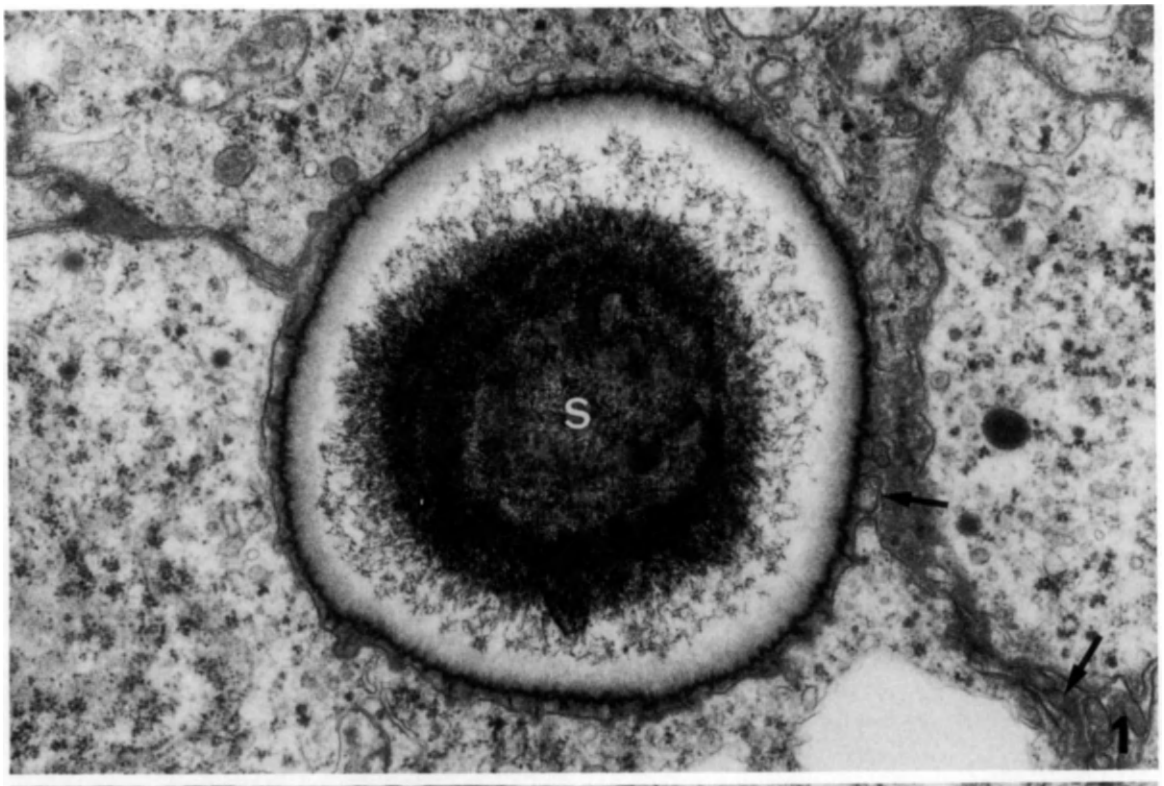
Finally, it is worth noting that calcified spherules are at times seen in pools of secretion produced by cells, particularly in the abundant mucoid secretions sometimes produced by mucinous adenomas and adenocarcinomas (Ghadially, 1988). Since cell debris (vesicular or otherwise) is also often present in such pools, one may wonder as to whether calcification commences in the lipidic debris or in the secretory product itself. Probably both phenomena occur, because in a carcinoma of the appendix I have seen calcification clearly occurring in or on cell debris (Ghadially, 1988) but in an oncocytic carcinoid (*Plate 546*) of the lung the situation was ambiguous (Ghadially and Block, 1985). In the latter tumour several calcified bodies were found in intercellular spaces or lumina bounded by cells (but not in the fibrovascular matrix) but no cell debris, vesicles or vacuoles accompanied them. On a few other occasions islands of secretory product containing vesicular and vacuolar structures were seen but no calcification was evident in or around them. However, in one instance (*Plate 546, Fig. 2*) three calcifying bodies were seen in a pool of medium density material (presumably secretory product) in company with vesicles, and a vacuole which was associated with or contained a calcifying body. Images which could be regarded as an early stage of mineralization of a vesicle or vacuole were not found. These findings lead one to suspect that often the secretory product itself may have provided the foci for calcium deposition. Since neuroendocrine cells pour their secretion into the circulation and not into intercellular lumina it is difficult to suggest what this 'secretory material' could be.

Plate 546

Oncocytic carcinoid of the lung (*From Ghadially and Block, 1985*)

Fig. 1. A calcified spherule (S) is seen lying in an expanded intercellular space. Note also the microvilli (arrows) in the intercellular space. × 25 000

Fig. 2. In an invagination of the cell membrane near an intercellular space lies some medium density material containing three calcified bodies (A, B and C) and some vesicles (v) and a vacuole (V). The vesicles show no sign of calcification. However, one of the calcified bodies (C) either lies within the vacuole or abuts the wall of the vacuole (V). × 51 000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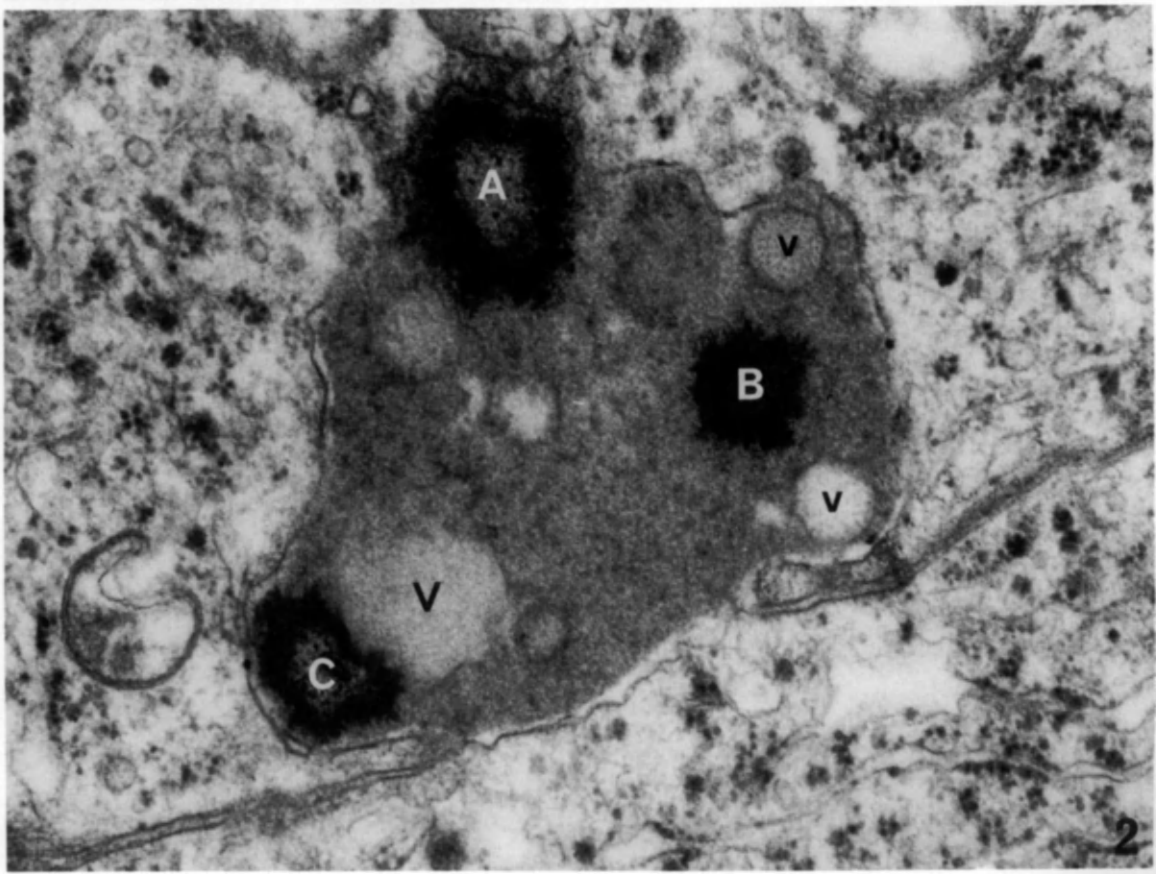
A
v
B
v
V
C
2

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