Review Article

THE ROLE OF CYTOSKELETAL AND CYTOCONTRACTILE ELEMENTS IN PATHOLOGIC PROCESSES

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The Role of Cytoskeletal and Cytocontractile Elements in Pathologic Processes

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Cytoskeletal and Cytocontractile Elements: Morphologic, Biochemical, and Physiologic Aspects

IN RECENT YEARS it has been realized that the cytoplasmic matrix displays specific patterns of organization, depending on the particular activities of the cell (eg, changes in shape, cytokinesis, intracellular streaming, endo- and exocytotic processes). Evidence that three major fibrous systems (microfilaments, microtubules, intermediate filaments) represent the cytoarchitectural basis of most nucleated cell types has steadily accumulated, and extensive biochemical and morphologic studies have led to the conclusion that the dynamic state of these fiber systems determines the shape and movement of a cell.

Once microtubules could be preserved for electron microscopy,[1-3] they were considered to be the main cytoskeletal elements in forming and maintaining asymmetric cellular processes.[4-7] However, in the last few years, it has been proposed that intermediate filaments[8] might serve as the mechanical integrators in the cytoplasmic space and thus fulfill a crucial cytoskeletal role (for a review see Lazarides[9] and Anderton[10]). At the same time, biochemical studies on whole cell extracts (for a review see Pollard[11]) showed that the cytoplasm is viscoelastic rather than fluid and that its consistency is regulated—among other factors—by calcium and adenosine triphosphate (ATP), both known to be required for functional actin-myosin interaction. Actin and myosin, as well as other actin-associated muscular proteins (α-actinin, tropomyosin) are indeed present in nonmuscle cells. Moreover, it has become clear that certain contractile proteins also exert a cytoskeletal function[11]; for instance, actin is able to polymerize reversibly into a meshwork of thin filaments, thus forming a solid gel of the cytoplasmic matrix. Proteins that regulate polymerization (eg, profilin) and depolymerization of actin (eg, gelsolin, actin-destabilizing factor) have been recently described.[12,13] A cytoskeletal role for actin may also be inferred from the high actin content of some nonmuscle cells (10–15% of total proteins), which is much more than needed for motile force generation.[14]

Analysis of the cytoplasmic matrix has been refined by the use of immunochemical methods, microinjection of tracer molecules, application of specific drugs, and microcinematography. The combination of some of these techniques has made it possible to correlate, for example, the distribution of actin and myosin with motile activities of the living cell.[15] In addition, improvement of electron-microscopic techniques has allowed the acquisition of new information on the organization of the fibrous elements and their interaction among themselves and with other organelles. By combining mild extraction procedures with different preparation techniques for transmission and scanning electron microscopy, a framework of various intracellular filaments, termed the cytoskeleton, has been visualized. This cytoskeleton may form the structural backbone of the cell, to which other, more easily extractable proteins and organelles are associated, forming what is called a "microtrabecular network."[16,17]

All three fiber systems, microfilaments, microtubules, and intermediate filaments participate in the formation of the cytoskeleton proper. In addition, a fourth class of filaments with a diameter of 20–30 Å has recently been described.[18] These filaments appear to function as "linkers" between various fibers. The individual fibrillar systems are in close contact with each other, and there is evidence that such interactions influence the course of a given type of fiber.[18] At the present time, the main focus of research is characterizing such interactions and the respective linker proteins involved. Linker molecules have a specific capacity to associate with actin, tubulin, or

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constituents of the intermediate filaments. Likewise, microtubule-associated proteins (MAPs) are thought to bridge microtubules and microfilaments.[19] There is also much evidence that connections exist between neurotubules and neurofilaments,[20-22] as well as between microtubules and intermediate filaments in nonneuronal cells, exemplified by the collapse of intermediate filaments containing desmin and vimentin, when the microtubules are disrupted by colchicine.[23] However, in most cases, it has not yet been possible to attribute a specific biologic function to such associations, and their exact nature still needs to be defined, as for instance in interactions between the cytoskeletal framework and specific organelles such as mitochondria,[24] mRNA,[25,26] and secretory granules.[27] A peculiar case worth mentioning is the binding of the complement subcomponent Clq to intermediate filaments and the subsequent activation of the complement cascade, interpreted as an antibody-independent recognition mechanism leading to the elimination of poorly soluble cytoskeletal residues from damaged cells.[28]

Other important interactions occur at the cell periphery between cytoskeletal elements and membrane components. Numerous investigations have detailed situations where cell surface molecules are connected in some way to cytoskeletal components.[29-36] Such interactions were postulated to explain the capping phenomenon of surface molecules,[37,38] cell adhesion to substrate,[39-41] and cell surface motility as it occurs in microvilli.[42] Of particular interest, in this context, are the surface-mediated motile phenomena observed in platelets. When platelets are stimulated by thrombin, their shape changes from smooth disks to spiky spheres. Simultaneously, a dramatic change in the cell surface properties takes place, and surface glycoproteins become attached to cytoskeletal proteins.[43,44] Rapid polymerization of actin takes place,[45-47] and the peripheral microtubular ring, thought to sustain the discoid shape of resting platelets, collapses. The cytoskeletal organization in red blood cells is different from other cells, in that a submembranous meshwork of cytoskeletal proteins interacts firmly with membrane proteins and membrane lipids (for a review see Branton et al[48]). In this way, the erythrocyte membrane is stabilized in its characteristic biconcave shape. The red blood cell cytoskeleton is a spectrin–actin lattice and is linked to a membrane glycoprotein. Upon dissolution of the membrane bilayer by non-ionic detergents,[49] the insoluble cytoskeleton retains the original shape of the intact cell, which illustrates its major structural role in erythrocytes.

The application of new methods in cell biology and biochemistry has offered the opportunity to study cytocontractile and cytoskeletal events involved in pathologic processes. If one takes into account the complex spatial interactions that are involved in cytoarchitecture and cell movement, as we have briefly tried to describe in the preceding paragraphs, it becomes understandable that visible changes in a cellular element need not necessarily represent a primary defect. The abnormal behavior of any particular cytoskeletal element may, of course, be the target of a genetically defined disorder. This explanation is accepted, for instance, for congenital ciliary diseases. In most pathologic situations involving cytoskeletal changes as they occur during inflammatory responses, wound healing, or tumor invasion, the primary cause is not known and might reside in a cellular element that only indirectly affects the architecture of the cell. Still, the manifest changes in cytoskeletal and cytocontractile structures may give new insights into the mechanism and development of such pathologic phenomena.

Before discussing in detail the role of cytoskeletal and cytocontractile elements in pathologic processes, we shall briefly describe the three major cytoplasmic fibrillar systems.

Microfilaments are thin filaments with an average diameter of 60 Å. Bundles of microfilaments, called stress fibers, are typical for cells in culture that exhibit little or no locomotion.[15] Stress fibers exert a structural role by anchoring the cytoplasmic matrix to the substrate, even though their capacity to contract has been demonstrated.[50,51] It should be noted at this point that while more or less developed stress fibers are typical of most substrate-attached cultured cells, these structures are only observed *in vivo* under exceptional conditions (see page 377). Alternatively, microfilaments occur in smaller bundles, as in microvilli, or as a diffuse network commonly located in ruffling membranes and at the cell periphery in general. The microfilamentous network seems to be responsible for the gel-like consistency of the marginal cytoplasm.[52] Thus, this meshwork either is characteristic for compartments that display active motility and movement[15] or retains the shape of the cell.[52]

After interaction with the head fragment of myosin (heavy meromyosin), microfilaments form arrowheads.[53] This indicates that they consist of at least actin and that they are polarized with a preferential direction of polymerization.[54]

The presence of various actin-associated proteins in nonmuscle cells, forming structural complexes with actin, may explain the diversity of actin-containing structures (for a review and references see Schliwa[12] and Weeds[13]). Some of these actin modu-

lators regulate the rigidity of cytoplasmic gels, formed by F-actin and any of the gelation factors, either by decreasing the viscosity of F-actin and preventing gelation (eg, gelsolin, villin) or by increasing viscosity and cross-linking actin filaments (eg, actin-binding protein, filamin). Moreover, spectrin, myosin, tropomyosin and probably also α-actinin stabilize and reinforce actin polymers. Profilin and "actin inhibitor," on the other hand, slow down or inhibit the assembly of actin monomers into microfilaments and thus are responsible for the existence of appreciable amounts of unpolymerized actin in nonmuscle cells. Finally, a number of actin-destabilizing proteins (ADF, see page 374; brevin[55]) have been found in the blood of several mammalian species. Although these components have similar molecular weights and share other biochemical characteristics, it is not clear whether they are identical: ADF has been reported to promote transition from F- to G-actin, whereas brevin shortens actin filaments without depolymerizing them.

Microtubules are hollow, noncontractile structures with an exterior diameter of 24 nm and of variable undefined length. Disassembly into their subunits (tubulin dimers) as well as reassembly can occur very rapidly, giving rise to a dynamic cellular scaffold rather than a rigid skeleton. The scaffolding by microtubules can be spacially and temporally limited. Microtubules are involved in various types of cell movement, such as ciliary beating, phagocytosis, movement of secretory granules and other organelles, as well as mitosis. Microtubules are sensitive to high hydrostatic pressure, low temperature, and high concentrations of calcium, which may be of physiologic importance in the regulation of assembly. A number of microtubular poisons have been used in cancer chemotherapy (eg, vinblastine, vincristine), in the therapy of gout (colchicine) and against certain forms of thrombocytopenia (vincristine). Most of these drugs act as mitotic poisons and are therefore potentially toxic. If we keep in mind the large spectrum of cellular processes in which microtubules are involved, a positive therapeutic effect obtained with such drugs is not necessarily solely a consequence of antimitotic activity (see p 372).

Microtubules are often found in association with specific proteins (MAPs). Under their influence, microtubules can modify their characteristics and become tissue-specific. In some cases, MAPs render microtubules resistant to drugs. At the present time, however, only a limited number of these interactions are known. One class of MAPs is high molecular weight proteins (HMW). They are tissue-specific and co-purify stoichiometrically with tubulin. Reconstitution experiments *in vitro* have shown[19,56] that actin filaments may interact with microtubules through the intermediate of MAPs, giving rise to a network consisting of microtubules cross-linking microfilaments. In association with other yet unknown proteins, microtubules form complex organelles, such as mitotic spindles, centrioles, cilia, and flagella. In cilia and centrioles, two of these associated proteins have been defined, the ATPase dynein, which allows the sliding movement of microtubules along each other,[57] and nexin,[58] which acts as a structural protein and keeps microtubular doublets and triplets together. Microtubules in neurons (neurotubules) have a great tendency to associate with other structures, in particular with neurofilaments and mitochondria. It is likely that interaction with neurofilaments is provided by the binding capacity of MAPs (HMW and tau protein[59,60]) to both microtubules and neurofilaments.[61]

Intermediate filaments are tubular structures with a diameter of 7–11 nm. They show a tendency to fasciate and to associate with other cellular structures, such as microtubules, membranes,[62] polyribosomes, and specific proteins.[28,63] They are insoluble under physiologic conditions, which indicates that they are biochemically related. In fact, numerous attempts to produce antibodies (poly- and monoclonal) against individual polypeptidic constituents have demonstrated that intermediate filaments are immunologically related and share antigenic determinants.[64,65] Strong evidence for the existence of common antigenic determinants has also recently come from patients with Waldenström macroglobulinemia[66] producing monoclonal IgMs with autoantibody activity against intermediate filaments of various cell types. However, unique antigenic domains exist, because antibodies recognizing only one specific polypeptide have been produced. Thus, the subunit structure of intermediate filaments defines different classes of filaments[67] that are characteristic for various tissues (Table 1) and, moreover, may serve as differentiation markers for the identification of cells of unknown origin, since it has been observed that, in general, neoplastic and fast-growing tissues (see page 373) maintain the intermediate filament proteins of the tissues they stem from.

Intermediate filaments are either homopolymers or heteropolymers composed of up to 10 different polypeptides.[68] Homopolymers are formed by vimentin (Mr 58,000), desmin (Mr 53–56,000), glial fibrillary acidic protein (GFAP; Mr 51,000). Vimentin, typically found in mesenchymal cells, and desmin, found in muscle cells, share marked biochemical similarities,[69–71] including their peculiar clumping upon treat-

Table 1—Tissue Specificity of Intermediate Filaments in Higher Vertebrates

Cell type	Polypeptide component(s) (approx. Mr)				
	Vimentin 58,000	Desmin 55,000	Cytokeratins 40-68,000	Glial fibrillary acidic protein 51,000	Neurofilaments 68,000; 150,000; 200,000
Mesenchymal cells					
Fibroblasts	+	–	–	–	–
Endothelial cells	+	–	–	–	–
Chondroblasts	+	–	–	–	–
Pigment cells	+	–	–	–	–
Muscle					
Parenchymal smooth muscle	–	+	–	–	–
Vascular smooth muscle	+	+	–	–	–
Cardiac smooth muscle	– (+)	+	–	–	–
Striated smooth muscle	– (+)	+	–	–	–
Epithelial cells					
Striated squamous epithelia	–	–	+	–	–
Epidermal appendages	–	–	+	–	–
Mesothelium	–	–	+	–	–
Cultured epidermal cells (including hepatocytes)	+	–	+	–	–
Glial cells	+	–	–	+	–
Neurons	–	–	–	–	+

Data based on references Lazarides[9], Franke et al[67], and Franke et al[84] and unpublished observations.

ment with colcemid.[23,72] Moreover, these two proteins were found to coexist in some muscle cells, such as vascular smooth muscle[73,74,75] and possibly striated muscle[76] The coexistence of vimentin with GFAP was reported for astrocytes and ependymal cells,[77] as well as for glioma cells.[78] On the other hand, the simultaneous presence of vimentin and other intermediate filament proteins in cultured cells appears to be due to *in vitro* conditions, irrespective of the tissue from which the cells were originally derived.[79]

Heteropolymers constitute the tonofilaments of epithelial cells and the neurofilaments of neuronal cells. The alpha fibrous component of keratin of the epidermis is characteristic for true epithelia, for nonfunctional epithelia, such as carcinomas, and for cultured epithelial and carcinoma cells. Such cytokeratin filaments are composed of a family of polypeptides of Mr between 40,000 and 68,000.[80-82] They include basic components that are unique for intermediate filaments.[83] The polypeptides occur in cell-specific patterns or, at least, are similar in groups of developmentally related epithelial tissues.[83] Tissue diversity is more pronounced than species difference.[83] It is of interest that mesothelial cells as well as epithelial cells of the collecting ducts and the Bowman capsule of the kidney contain tonofilaments composed of cytokeratins,[84] despite their mesodermal origin (Figure 1), indicating that specific function, in addition to embryologic origin, influences the composition of intermediate filaments in a given cell.

Neurofilaments of the central and peripheral nervous system are composed of a polypeptidic triplet displaying species-specific variation of the molecular weight[85] (approximately Mr 200,000, 150,000, 68,000). These polypeptides appear to be antigenically related but are immunologically and biochemically distinct from GFAP.[86] Using antibody decoration, Willard and Simon[86] recently analyzed the physical arrangement of the three polypeptides within the neurofilament: a central core comprises the component of Mr 68,000, whereas the component of Mr

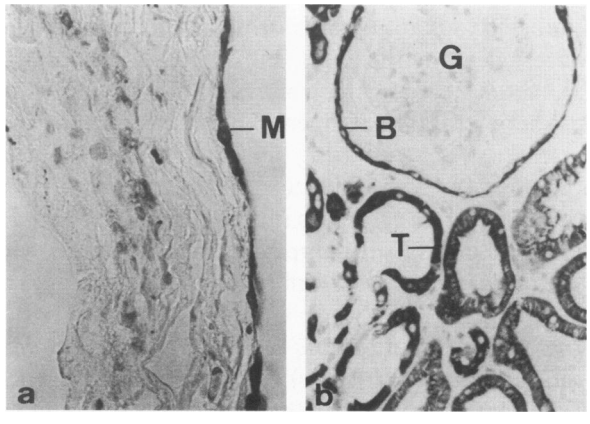

Figure 1—Immunocytochemical (biotin-avidin peroxidase) localization of prekeratin in human mesothelial cells of pleura and epithelial cells of renal tubules. **a**—Lung periphery with thickened pleura showing intensely positive staining in the mesothelial layer (*M*). The black spots within the tissue are carbon deposits. (×400) **b**—Kidney cortex showing prekeratin staining in tubular epithelial cells (*T*) and in the epithelium of Bowman capsule (*B*); glomerular structures (*G*) are negative. (×150) (Courtesy of Dr. A. Gown, University of Washington, Seattle, Washington)

200,000 (component "H") appears to be more peripherally located. It is likely that this protein is a candidate bridging adjacent filaments; moreover, it might in some way be involved in the intracellular transport system.[20] On the other hand, the phosphorylation of particular cytoskeletal elements may offer a mechanism for the regulation of interactions between such elements. For instance, a cyclic adenosine monophosphate (AMP)-dependent protein kinase has been reported to copurify with the microtubule-associated protein MAP2 and to be located on the microtubular arm.[87] Also, neurofilament subunits themselves, predominantly the 150,000 dalton species, are phosphorylated in a cyclic AMP-dependent manner.[88]

Pathologic Situations Related to Altered Function of Cytoskeletal and Cytocontractile Elements

Microtubular Diseases

Abnormal Cilia and Spermatozoa

Malfunction of ciliated epithelia, leading to sinusitis and bronchiectasis and other related diseases of the respiratory tract was noted before electron-microscopic evidence regarding its structural aspects was available. It was reported that chronic respiratory infections occurred frequently in patients with situs inversus cordis, or transposed viscera.[89,90] Then, Arge[91] noted male sterility to be a component of this syndrome as well.

With the introduction of routine electron-microscopic examination in pathology, a great number of structural abnormalities in cilia and spermatozoa have been described, some of which occur occasionally in normal individuals,[92] while others are found in formerly functional epithelia[93] such as in metaplastic respiratory epithelium following smoking[94,95] or chronic infections.[96,97] Such abnormalities, however, are not exclusively related to congenital ciliary diseases: these are generally characterized by immobility of both cilia (as is demonstrated by impaired ciliary clearance of the respiratory tract) and spermatozoa.[98] It is striking that most of these syndromes are linked to a precise visible defect, namely, structural abnormalities in the axoneme. The following descriptions will focus on such ciliary dysfunctions.

The *immotile cilia syndrome*[99] comprises a number of congenital diseases leading to chronic infection of the upper and lower airways as well as of the middle ear.[100] Mucociliary transport is very slow or even absent, as measured by tracheobronchial clearance.[101] Living and normal-appearing spermatozoa are totally immotile.[102,103] Upon electron-microscopic examination, the basic defect of these dysfunctions appears to consist of abnormalities of the dynein arms of the axonemal outer doublets (Figure 2). Both dynein arms,[103,104] the inner arms,[105] or the outer arms[106] may be affected. Other cases have been reported, showing unusually prominent[107] or abnormally short dynein arms[100] or the absence of radial spokes[108] and hence a defective microtubular arrangement in the axoneme. Furthermore, disorientation of the central pair of microtubules[109,110] may provoke a general disorientation of the individual cilia within an epithelium,[110] which accounts for unefficient mucociliary clearance.

Lack of the doublet arms on the axonemal peripheral microtubules is thought to be due to a recessive genetic defect in the synthesis of the enzyme dynein with ATPase activity.[99,103] Indeed, recent biochemical studies[111] on human spermatozoa regarding the presence of dynein normally occurring as four electrophoretically distinct forms have revealed that when the doublet arms are absent, three electrophoretic variants of dynein are missing. Moreover, the absence of central structures of the axoneme coincides with a reduction of one electrophoretic variant.[111] This illustrates the presence of dynein in outer doublet arms and in central structures. On the other hand, abnormal spermatozoa still contain a modified dynein species.[111] These findings may thus well explain the results of Forrest et al[112] and Rossman et al[113] who showed that immotile cilia could be activated by the addition of exogeneous ATPase *in vitro* and *in vivo*. Furthermore, immotile cilia could also be stimulated to generate movement when high doses of ATP were added,[112,113] suggesting that there is residual endogeneous ATPase available and functionally active in the cilia. This was indeed demonstrated by gel electrophoretic analysis.[111] The residual ATPase may be sufficient to generate beating without further addition to exogenous ATP, since Rossman et al,[114] showed that cilia of patients with the immotile cilia syndrome may beat, although abnormally. Abnormal motion was recorded in up to 40% of the cells, the remainder being totally immotile. No planar coordination or metachronicity was found. On the basis of these observations, some authors suggested the name "immotile cilia syndrome" be replaced by "dyskinetic cilia syndrome"[114] or "ciliary dyskinesia."[115]

A special form of the immotile cilia syndrome is referred to as *Kartagener's syndrome*.[89,90] Here bronchiectasis, typical chronic infections of the entire respiratory tract, male sterility, and situs inversus cordis are combined (for a recent review see Rott[116]). Visceral asymmetry appears to be determined by a

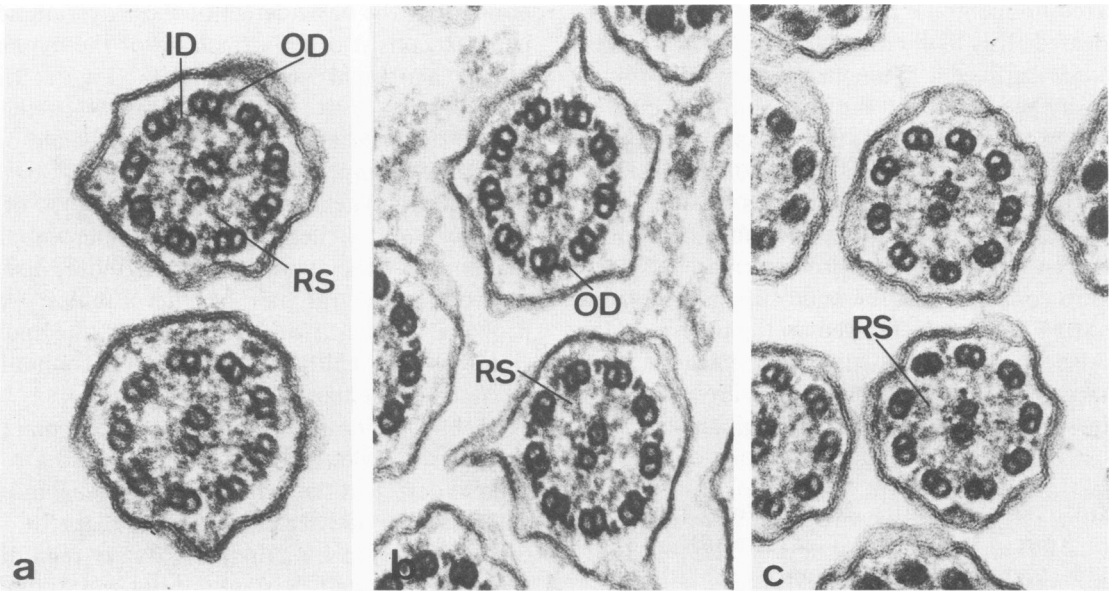

Figure 2 – Electron micrographs of sections through normal and diseased human bronchial cilia. **a** – Normal pattern with inner (*ID*) and outer (*OD*) dynein arms extending from the A-subunit of each of the nine microtubular doublets. **b** – Inner dynein arms are missing. **c** – Both inner and outer dynein arms are missing. Biopsy from a person with the immotile cilia syndrome. Note the presence of radial spokes (*RS*) in all three biopsy specimens. (× 108,000) (Courtesy of Dr. M. Baud, University of Geneva, Geneva, Switzerland)

fixed beat direction of the cilia in embryonic epithelia, provoking a rotation of the embryonic archenteron.[103] If the ciliary beat is missing, incomplete, or uncoordinated, there are equal chances of dextrorotation or levorotation, resulting, in the latter case, in the development of a situs inversus viscerum. This probability of 50% of levorotation explains why siblings of patients with the Kartagener's triad (chronic sinusitis, bronchiectasis, situs inversus) suffering from the typical nasal and bronchial symptoms, may not necessarily show situs inversus. These cases are also classified under Kartagener's syndrome.

Retinitis pigmentosa comprises a probably heterogeneous group of eye disorders. The main symptoms are constriction of the field of vision and loss of night vision. On the basis of the fact that the outer limbs of the photoreceptors are modified cilia,[117] one could argue that some form of retinitis pigmentosa might be associated with a general defect in ciliary structure. With this view, a number of patients with retinitis pigmentosa were examined, and it was found that ciliary abnormalities in their nasal mucosa were more frequent than in controls.[93,118] Nevertheless, whether there is a direct relationship between this eye disease and ciliary abnormalities has to be further substantiated.

Young's syndrome[119] comprises sinusitis, bronchitis, bronchiectasis, and idiopathic obstructive azoospermia. Electron-microscopic examination of cilia and spermatozoa have revealed a normal 9 + 2 microtubular arrangement in the axoneme as well as the presence of dynein arms.[120] Cilia are oriented correctly within an epithelium. Young's syndrome thus clearly differs from the immotile cilia syndrome, and the primary defect remains to be elucidated.

Abnormal Leukocytes

Polymorphonuclear leukocytes (PMNs) are highly motile cells. Their main function consists in locomotion toward infectious sites and phagocytosis of invading pathogens. Directed locomotion and accumulation of large pools of PMNs not only imply their response to humoral signals but also depend on a highly efficient locomotor apparatus. Crawling and phagocytosing PMNs display a characteristic polarized shape with a posterior tail and an anterior pseudopod in the direction of movement, which, during phagocytosis, embraces the prey.[121] The pseudopod consists of well-organized cortical cytoplasm in which a network of microfilaments can be visualized by electron microscopy.[122,123] The network comprises abundant actin[124,125] but also contains actin-binding protein (ABP)[126,127] and myosin.[127,128] ABP is known to cross-link actin filaments[129] and myosin to cause ATP-dependent contraction.[130] Therefore, it is probable that the interaction of these contractile proteins generates ameboid movement and phagocytosis.[131]

The abnormal behavior of neutrophilic PMNs, including bacterial infections, neutropenia, and defective locomotion in a context of recurrent bacterial

infections,[132] has been repeatedly reported. Some of these cases were classified as the *"lazy leukocytes" syndrome.*[133-135] In one case, an extensive biochemical analysis permitted better understanding of the possible disease mechanisms.[136] In this patient PMNs were produced normally and generated chemotactic activity but showed defective locomotion and phagocytosis. Although actin was found in normal amounts, its capacity to polymerize under *in vitro* conditions was defective, suggesting either abnormal actin molecules or abnormal factors controlling polymerization of actin.

Cytoplasmic microtubules also participate in the regulation of leukocyte movement,[137,138] even though they are not contractile organelles. Administration of drugs that bind to tubulin, as, eg, colchicine,[139,140] and compound R 17934,[141] was used in the study of the locomotor behavior of leukocytes in the absence of functional microtubules. Treated cells could still orient themselves and migrate directionally but did so less precisely than untreated controls. Furthermore, it was shown that in normal PMNs the number of cytoplasmic microtubules increased when the cells were exposed to chemotactic agents,[142] and that microtubules reoriented within the cells when PMNs were subjected to reversing gradients of cytotoxins.[143] This leads to the conclusion that microtubules have a role to play in translating membrane signals into locomotor responses.

Leukocytes from patients suffering from the *Chédiak-Higashi syndrome* (CHS) resemble in many respects leukocytes treated with antimicrotubular drugs. CHS is an autosomal recessive disorder in man and, analogously, in other animals such as mice ("beige mouse"), cats, mink, and cattle (for references see Oliver et al[144]). It is characterized by pale skin, hair, and eye color and recurrent pyogenic infections. The advanced phase is diagnosed as a lymphomalike malignancy leading to death during childhood. A number of neutrophil dysfunctions have been described, such as impaired chemotaxis[145,146] and a defective system of lysosomal degranulation.[147] The latter dysfunction may be involved in the increased susceptibility to bacterial infections and in the reduced process of pigmentation. The presence of enlarged lysosomes and giant secretory and pigment granules without any specific storage material in granule-containing cells and a perinuclear concentration of lysosomes are characteristic of CHS.[146] However, the basic defect of the syndrome is not yet known. Earlier studies suggested that abnormal microtubule assembly could account for such cytoplasmic dysfunction.[148-150] Evidence came from experiments on granulocytes of CHS patients, which displayed spontaneous capping of concanavalin A (Con A) receptors, as it occurs in normal cells only after pretreatment with colchicine.[151] Moreover, administration of cyclic GMP and cholinergic agonists, agents that raise cellular levels of cGMP and increase the assembly of microtubules,[152] corrected the spontaneous capping of Con A receptors in granulocytes of CHS patients. In normal cells, the same treatment antagonized the effect of colchicine[148,149] and also corrected leukocyte function.[153] Finally, it was reported that cultured fibroblasts from CHS patients displayed abnormally low rates of vinblastine-induced microtubular paracrystal formation,[154] suggesting a lower requirement for vinblastine in paracrystal formation.[155] Recently, however, evidence has accumulated that causes us to question the hypothesis of abnormal microtubule assembly in CHS. There is no difference in the presence and number of centriole-associated microtubules in CHS platelets, monocytes, or lymphocytes.[157] No abnormal pattern of microtubules could be detected by immunofluorescence microscopy in skin fibroblasts and macrophages in mice[158] or in man.[159] The quantitation of tubulin by a colchicine-binding assay gave normal values,[159] and other elements of the cytoskeleton (microfilaments) and its functional integrity, measured by cell deformability, appeared to be normal.[159] There is a possible explanation for the conflicting results obtained regarding the assessment of microtubular function in CHS cells. It was found that lysosomes in CHS patients are abnormally permeable to dyes[160,161] and that cell membrane fluidity is increased.[162] It might therefore be proposed that CHS cells exhibit a defect in the lysosomal membrane that might result in a greater instability of the related microtubular system.[159]

Neurofibrillary Diseases

In a number of neuropathies affecting the *peripheral neuronal system* (giant axonal neuropathy, infantile neuroaxonal dystrophy, amyotrophic lateral sclerosis), neurofilaments are heavily accumulated in axons or neuronal cell bodies. Such a proliferation of normal 10-nm filaments is also found in response to neurotoxins, as in the case of neuropathies induced by aluminum,[163,164] iminodipropionitrile (IDPN),[165] or methylbutylketone, n-hexane, or acrylamide (for a review see Selkoe et al[166]). Furthermore, accumulation of neurofilaments occurs through agents that selectively disrupt microtubules, such as colchicine, vinblastine, and podophyllotoxine.[167,168] The cause of spontaneous neurofilament accumulation has not been determined, but Shelanski et al[169] discuss the

possibility that the primary pathologic process in all these disorders is the excessive proliferation of 10-nm filaments, thus distorting the normal relationship between the elements of the cytoskeleton, preventing their physiologic interaction, and eventually inhibiting normal axonal transport.

Another group of neurologic diseases in which cytoskeletal elements may be implicated comprises *Alzheimer's disease* (presenile dementia), dementia of the Alzheimer type, as well as senile disorders occurring in normal aged persons. Before a selective degeneration of transmitter-specific neurons in the forebrain (nucleus basalis of Meynert) were described,[170] the classic morphologic features in the brain of the Alzheimer type were the presence of neurofibrillary tangles and neuritic (senile) plaques (Figure 3). Both fibrillary tangles and senile plaques are restricted to the central nervous system and imply abnormalities of the fibrous neuronal proteins.

Fibrillary tangles are found predominantly in the cerebral cortex (hippocampus),[171] and their concentration appears to correlate with the degree of dementia.[172] Tangles are entirely intracellular and consist of abnormal fiber bundles that are morphologically different from normal neurofilaments and neurotubules. Abnormal fibers are composed of two filaments, each about 10 nm in diameter, twisted helically around each other with a distance of 80 nm between the nodal points.[173-175] These paired helical filaments (PHFs[173]) measure approximately 22 nm in diameter at their widest point, suggesting that PHFs are twisted tubules.[174] However, it is now generally believed that neurofilaments alone are involved in this structure. For instance, Oyanagi[176] presented electron micrographs showing continuity of neurofilaments with PHFs. The biochemical composition, however, is still a matter of debate. It has recently been shown that PHFs are highly insoluble, probably because of nondisulfide covalent bonds that crosslink the filaments to a rigid polymer.[177] This finding prevents a clear molecular analysis and might explain the difficulties encountered in preparing pure fractions (see Shelanski et al[169]) and specific antibodies against them. Several attempts were made to show immunologic relationships of PHFs to neurotubules or neurofilaments.[178-180] However, specific antibodies raised against the P68,[169] the P150,[169] and the 210 kilodalton subunit[181] of neurofilaments, as well as against tubulin,[169,181] showed no reaction. By contrast, an antiserum against a two-cycle-purified microtubule fraction from human brain contained antibodies that bound to tangles and neurite plaques of the Alzheimer type.[181] These antibodies were probably directed against one or several minor determi-

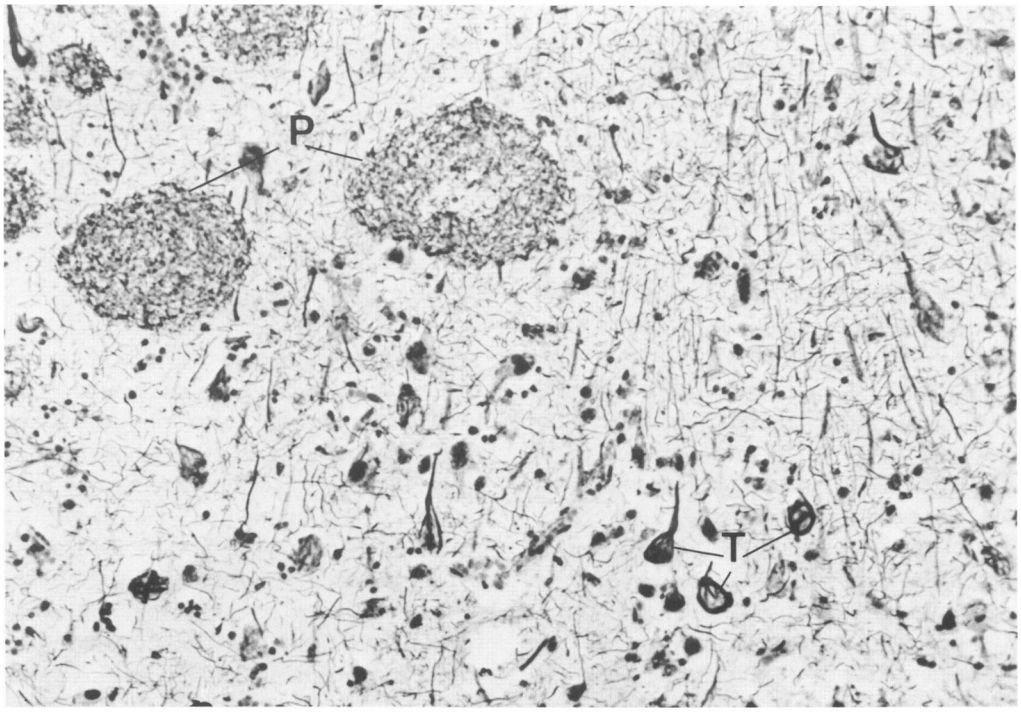

Figure 3—Alterations of the cerebral cortex in Alzheimer's disease. Cryostat section through the hippocampus of a patient suffering from Alzheimer's presenile dementia. The picture delimits a region of Ammon's horn (region $HE_1\alpha$ to $HE_1\beta$) showing typical voluminous senile plaques (*P*) and fibrillary tangles (*T*) involving the majority of the neurons. (Modified metal impregnation of Globus, ×640) (Courtesy of Dr. E. Wildi, University of Geneva, Geneva, Switzerland)

nants other than tubulin or neurofilament triplet proteins. By contrast, evidence has been presented recently[182] that neurofibrillary tangles can be visualized by the Bodian silver impregnation, a method that appears to stain selectively polypeptide components of neurofilaments. The authors conclude that Alzheimer tangles are related to neurofilaments.

Paired helical filaments are not only characteristic of neuropathies of the Alzheimer type but may also be found in other neurologic disorders with dementia as a prominent feature, such as in Guam parkinsonism–dementia complex, in postencephalic Parkinson's disease, in adults with Down's syndrome, in sclerosing panencephalitis (for a review see Wisniewski et al[183] and Iqbal et al[184]). Quantitative similarities in the degree of neurofibrillary tangle formation as were found between Down's syndrome and senile dementia of the Alzheimer type[185] may offer the possibility of a search for a common pathogenetic mechanism or, alternatively, lead to the conclusion that neurofibrillary diseases are merely of polyetiologic origin.

The senile plaque is a cortical lesion lying in an extracellular matrix. Its main constituent is a fibrous amyloid core[186] (for a review of earlier work see Narang[187]). The presence of amyloid plaques in Alzheimer's presenile dementia is not restricted to the cortex but was recently reported to occur as well in the cerebellum.[188] A characteristic senile plaque of the cerebral cortex contains presynaptic elements of degenerating neurons with neurofibrillary tangles.[189] Astrocytes and microglia at the periphery of the plaques account for the formation of glial fibrillary acidic protein in an unusually high amount.[190]

Myopathies and Muscular Dystrophy

The pathologic manifestations of muscular diseases are very complex and often involve not only the structures forming the muscle fibers but also surrounding tissues, namely, the nervous and vascular systems as well as connective tissue. Enormous difficulties are thus encountered in relating any pathologic change to a particular disease or even in defining any of these alterations as the primary cause. Candidates for such primary causes were sought among alterations of contractile proteins (see, for example, Samaha and Thies[191]), but until now these approaches did not provide useful insight into any muscle disease. Nevertheless, it has been possible to assign specific pathologic patterns for particular diseases by combining several methodologic approaches.[192]

Here we shall concentrate on three muscular diseases, two of them affecting skeletal muscle, one the myocardium. All three cases demonstrate the usefulness of following the distribution of a particular protein in biopsy material by means of immunofluorescence microscopy and of comparing these observations with the electron-microscopic view. It is to be hoped that more studies of this nature will allow a better understanding of the supramolecular organization of functionally normal and diseased myofibers.[193]

Nemaline Myopathy

This congenital, nonprogressive disease of the skeletal muscle[194] appears in most cases during childhood. It is characterized by the presence of electron-dense rods accumulating usually at the periphery of the muscle fiber (Figure 4). The rods can be up to 5 μ in length and 1 μ in diameter and are also found in other muscular disorders, namely, muscular dystrophy, atrophied muscle in denervation, polymyositis (for examples and references, see Mair and Tomé[195]). After comparison of ultrathin cross-sections of nemaline rods and Z-bands of normal myofibers, it has been proposed[196] that thin filaments are continuous throughout the rods and that, consequently, the rods represent a lateral polymer of Z-line units.[197,198] Indeed, these thin filaments forming the longitudinal backbone of the rods are composed of actin; they can be decorated with heavy meromyosin after the controlled action of a Ca^{++}-activated neutral protease.[199] Furthermore, the structural resemblance of the rodlike structures of Z-bands encouraged the search for typical Z-band proteins such as α-actinin and desmin (skeletin). Immunofluorescence microscopy using antibodies against α-actinin[200–202] unambiguously revealed the presence of α-actinin within the nemaline rods (see Figure 4). Instead, desmin (skeletin) did not bind directly to the rods, but rather to their surroundings.[192,202] The selective accumulation of α-actinin in the rods and outside the Z-bands is difficult to explain at present, but it is clear that this protein must participate in some way in the general turnover of muscle proteins after birth, since nemaline myopathy is a nonprogressive muscular disorder.[202]

Cardiomyopathy

One peculiar form of cardiomyopathy is a rare,[203,204] possibly familial[205] disease showing progressive myocardial deficiency. It is characterized by myofibrillar disruptions, by changes in the sarcoplasmic reticulum, and mainly by proteinaceous inclusions consisting of accumulated intermediate filaments.[205] By immunofluorescence microscopic examination, these inclusions stain selectively for

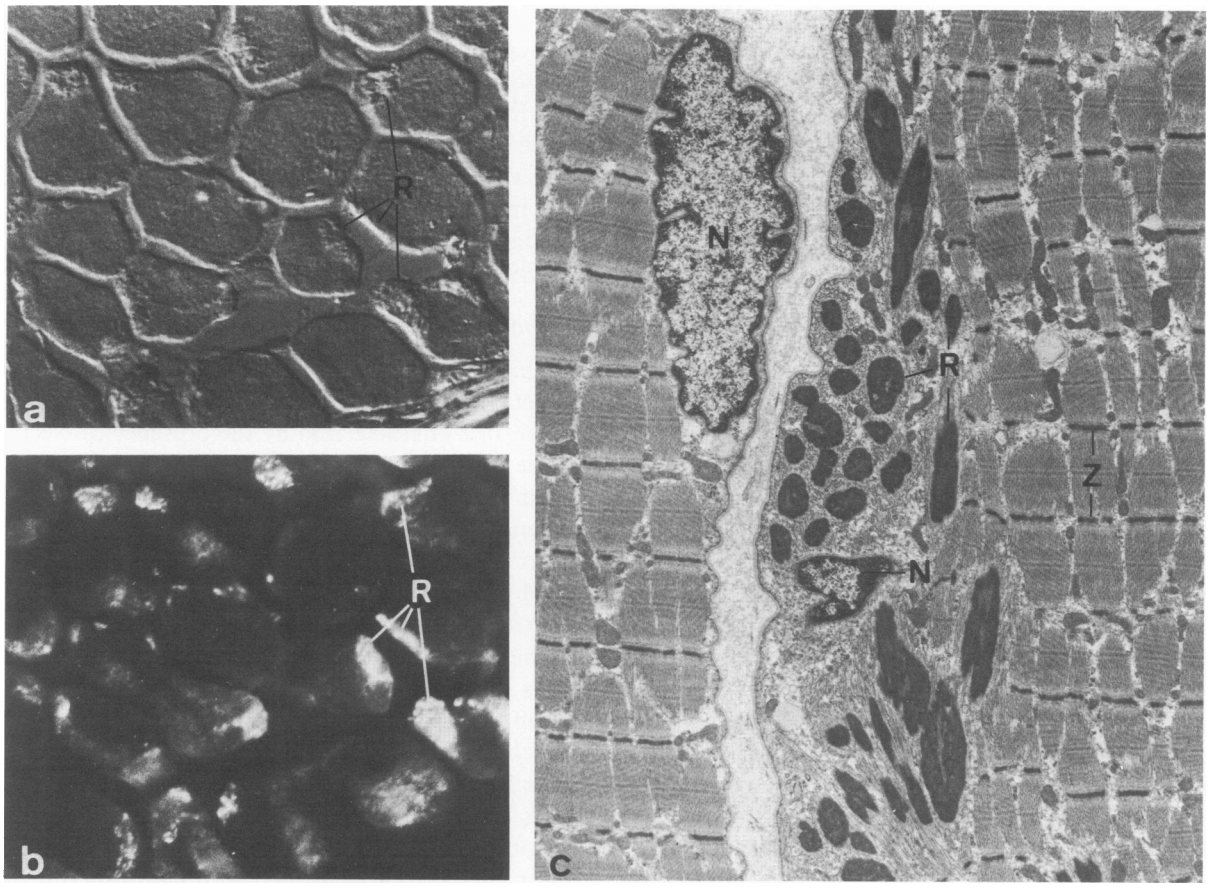

Figure 4 — Immunofluorescent and electron-microscopic characterization of a skeletal muscle biopsy from a patient with nemaline myopathy. **a** — Nomarski optics of a transverse section through affected muscle fibers showing peripherally located areas with nemaline rods (*R*), which stain brightly after decoration with anti-α-actinin antibodies (**b**). **c** — Electron micrograph of a longitudinal section through a muscle fiber with normal myofibrils and a peripheral accumulation of nemaline rods. Note the electron density of both Z-lines (*Z*) and nemaline rods. *N*, nucleus. (**a** and **b**, ×900; **c**, ×5600) (In collaboration with Dr. J. Cox, University of Geneva, Geneva, Switzerland)

desmin but not for α-actinin or for vimentin.[206] Desmin filaments are known to maintain the normal myofibrillar architecture. On the one hand, they anchor actin filaments to the Z-bands by attaching them to the sarcolemma; and on the other hand, they link actin filaments to the plasma membrane at the fasciae adherentes of the intercalated disks.[207] Therefore, the concomitant occurrence of disrupted myofibrils and desmin containing inclusions in this cardiomyopathy suggests that the inclusions derive from Z-bands.

Duchenne's Muscular Dystrophy

The primary defect of Duchenne's muscular dystrophy is still obscure. Several investigations suggested abnormalities of the muscle plasma membrane[208-210] provoking, through alterations in permeability, derangements in the ionic pump, leading to activation of proteases and finally to a breakdown of muscle fibers. In view of the relationship between plasma membrane components and cytoskeletal elements, a number of studies have been concentrated on possible changes of cytoskeletal elements from inherited dystrophic muscle explants of chickens[211,212] and cultured fibroblasts from persons with Duchenne's muscular dystrophy.[212,213] Using immunofluorescence microscopy, an initial report[211] described a lack of staining for the microtubular network in dystrophic chicken cells, interpreted as a disintegration of microtubules due to impaired regulation of the intracellular Ca^{2+} content.[214] However, these results could not be confirmed either in chickens[212] or in man.[212,213] In addition, the distribution of other cytoskeletal fibrous structures, namely, microfilaments and intermediate filaments, was found to be normal.[213] Thus, a direct correlation between the dystrophic genotype and disturbance of cytocontractile and cytoskeletal elements has, at least in cultured fibroblasts, to be questioned.

Another feature of Duchenne's muscular dystrophy is the capacity of muscle fibers to regener-

ate.[215] Nucleated myotubes containing large amounts of intermediate filaments can be observed between fully differentiated normal-sized myofibers. By immunofluorescence, they stain strongly but diffusely with antiskeletin (antidesmin) antibodies,[192] as do mature myofibers on the level of the Z-band. The immunofluorescence-microscopic technique thus offers the possibility of recognizing precisely regenerative events in diseased muscle.

Abnormally Shaped Erythrocytes

The biconcave human red cell is a highly resilient structure that owes its remarkable physical properties to a submembraneous meshwork of cytoskeletal proteins[216,217] (for reviews see Branton et al[48], Lux[218], and Gratzer[219]) consisting of a spectrin-actin lattice[220] linked to glycoprotein 3.[221,222] This cytoskeletal network can be readily isolated upon treatment of whole erythrocytes[223] or isolated membrane vesicles ("ghosts"[49]) with non-ionic detergent, a procedure that removes membrane lipids and most integral membrane proteins.[49] The cytoskeletal residue retains the original shape,[223,224] indicating that its protein constituents fulfill an important structural role in the red blood cell.

The distorted shape of many abnormal erythrocytes is also maintained in their detergent-extracted cytoskeletons.[218,225] In the case of irreversibly sickled cells, a noncovalent reorganization of the cytoskeleton[218] occurs during the sickling process, thus leading to a fixation of the abnormal cell shape.[225] Also, the shedding of membrane spicules devoid of cytoskeletal proteins that occurs upon reoxygenation of sickled cells may contribute to the fixation of the abnormal shape by reducing the membrane content of irreversibly sickled cells.[226] The cytoskeleton of sickled red cells is therefore not primarily involved in the sickling phenomenon and, rather, respond to the metabolic perturbations caused by the primary defect of the hemoglobin molecule. On the contrary, in hereditary disorders such as elliptocytosis and pyropoikilocytosis, the cytoskeleton has been shown to contain abnormally heat-sensitive spectrin,[227,228] which fails to form tetramers, thus leading to membrane skeletal instability by insufficient actin cross-linking,[229] suggesting that the primary defect in cell shape resides in the elliptocytic cytoskeleton. In hereditary spherocytosis, a similar primary defect of the cytoskeleton has been postulated, but not proven.[227] However, studies of spherocytic erythrocytes in mouse mutants have clearly shown that the amount of spectrin is markedly decreased.[230] Even though spectrin is present in normal amounts in human spherocytes, it is likely that a somehow defective spectrin molecule is responsible for the determination of the spherocyte shape.[227]

Lux[218] has recently proposed that interactions between spectrin, actin, and the spectrin-binding site to membrane glycoprotein 3 ("ankyrin")[221,231] constitute a dynamic equilibrium. Insufficient phosphorylation of the spectrin molecule would result in a sparse formation of a polymerized spectrin–actin complex, as would the lattice leading to a fragile and highly deformable membrane of the spherocytic type. On the other hand, excess cross-linking and excess spectrin–actin complex formation would be more likely to lead to the rigid echinocyte. Since much progress has been made in recent years in characterizing the elements of the erythrocyte cytoskeleton, it is quite possible that analysis of detergent-resistant cytoskeletons of pathologic erythrocytes will yield interesting material to test the predictions of the dynamic equilibrium model.

General Modifications of Cytoskeletal and Cytocontractile Elements During Pathologic Situations

Cytoskeletal and Cytocontractile Elements in Tumor Cells

Transformed cells *in vitro* and neoplastic cells *in vivo* are likely to have undergone profound alterations of their cytoarchitecture. Numerous studies have attempted to establish the biologic significance of these alterations for cultured cells,[232-236] and to a limited extent for human tumors *in vivo*.[237] In this chapter we shall first discuss the invasive character of tumor cells in relation to modifications of their cytoarchitecture by focusing on the particular aspects of 1) actin organization and 2) microtubule integrity. Finally, we shall discuss and illustrate the usefulness of intermediate filament protein characterization in determining the origin of a given tumor in order to increase the possibility of obtaining a precise and unambiguous diagnosis.

The Cytoskeleton During Tumor Invasion

One of the prominent features of malignant neoplasms is their invasiveness, which is observed histologically even when the primary tumor appears macroscopically circumscribed. Invasion by cancer cells into surrounding tissue depends on a complex interaction of several factors, such as proteolytic activity[238,239] (collagenolytic activity in particular), surface modifications rendering tumor cells independent

from the architecture of surrounding normal tissue,[240] and motile activity.[241-244] Although it has been claimed that an active locomotion of the tumor cells is not a necessary prerequisite for successful invasion,[245] there exist nevertheless some clear examples for the *in vivo* movement of tumor cells. Wood[241] has provided cinematographic documents on the penetration of V2 carcinoma cells through the walls of capillaries and veins in rabbit ear chambers. Other indirect evidence of tumor cell movement is derived from old histologic observations,[246] which have since been confirmed on several occasions.[247] This type of evidence has also been used to postulate active movement of inflammatory cells, such as granulocytes and lymphocytes.[248,249] Infiltration of tissues by leukemic cells, for example, is difficult to interpret without invoking an active locomotor behavior of the penetrating malignant cells.[250] Cytocontractile and cytoskeletal elements are instrumental in cell locomotion. Increased knowledge of their organization in cancer cells might therefore contribute to a better understanding of the mechanism of invasion.

Actin Organization in Tumor Cells: It has been shown by several laboratories,[251-254] including ours,[244,255] that in several human carcinomas, malignant cells, particularly those located at the periphery of the tumor and those invading the surrounding tissues, display pseudopodlike cytoplasmic protrusions that contain a well-developed microfilamentous network. The periphery of the same cells is strongly positive after immunofluorescent staining with actin antibodies.[244,254,256,257] The question has remained open as to whether these morphologic changes correspond to an increase of the total amount of actin in tumor cells or to an altered degree of actin polymerization. This problem has been investigated[258] in human skin tumors by the use of 1) immunofluorescent staining with actin antibodies with and without pretreatment with ADF[259] and 2) planimetric analysis of polyacrylamide gel scans in order to evaluate the amount of actin as a percentage of total protein. The results show that immunofluorescent staining with actin antibodies is more sensitive to ADF treatment in normal cells than in tumor cells (Figure 5), whereas amounts of actin present in normal and tumoral cells are similar.[258] These findings suggest that in neoplastic epithelial cells there is a change in the degree of actin polymerization rather than in the total amount of the protein when compared with their normal counterparts. The presence of increased amounts of F-actin in tumor cells suggests that actin in these cells might be organized in order to interact with myosin and participate in contractile events. An increased proportion of F-actin, as is observed in tumor cells,

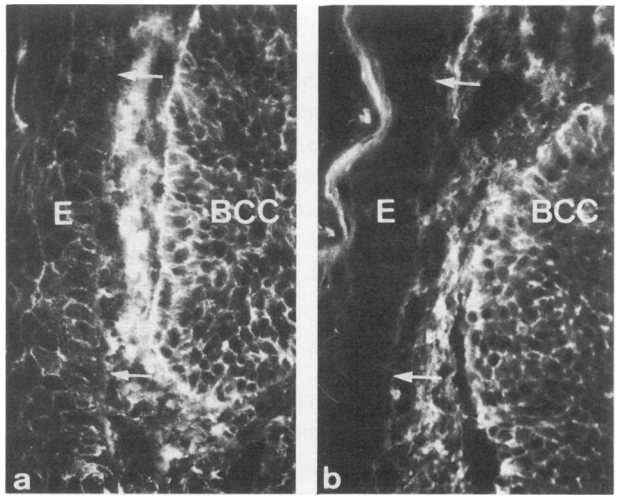

Figure 5—Adjacent cryostat sections from a surgical specimen of skin with basal cell carcinoma treated for indirect immunofluorescent staining with actin antibodies alone (**a**) or after incubation with purified ADF[258,259] (**b**). **a**—Both the non-tumoral epidermal cells (*E*) and the tumoral cells (BCC) show positive staining at their periphery. **b**—Only tumoral cells are positive. *Arrows* point to nontumoral epidermal cells. (×250) (From Low et al[258] with permission of the editor)

has also been noted in cells displaying active locomotion, such as epithelial cells moving over an open wound (see page 375) and regenerating liver cells after hepatectomy (see page 379)—indicating that changes in actin polymerization are characteristic of cell activation and movement rather than of malignant transformation.

Role of Microtubules in Tumor Invasion: Clinical data have shown[260,261] that slowly proliferating tumors can be highly invasive. Therefore, an increased mitotic activity of malignant cells may not be the only explanation of their invasiveness but, rather, might suggest that locomotion in a defined direction plays a crucial role in invasiveness.[262,263] Using organotypic tridimensional co-cultures of embryonic chick heart fragments and malignant mouse Rous sarcoma virus transformed cells, a number of antimicrotubular agents have been tested for their capacity to inhibit proliferation and invasion.[264-266] Antimetabolic mitostatic drugs (5-fluorouracil, cytosine arabinoside, bleomycine) did not produce any antiinvasive effect, whereas microtubule inhibitors (colchicine, demecolcine, vinblastine sulfate, vincristine sulfate, nocodazole) acted antimitotically and also inhibited invasion. When 5-fluorouracil (a mitostatic drug) was combined with nocodazole (which destroys cytoplasmic microtubules of interphase cells), invasion was totally abolished,[264] indicating that microtubule inhibitors (some of which are currently used in cancer chemotherapy) inhibit invasion not only through

their mitostatic effect but also through their capacity to depolymerize microtubules. These experiments emphasize the importance of an intact microtubular system for invasion. It should also be stressed that intact microtubules are essential in establishing cellular asymetry, which itself constitutes a prerequisite for directional movement.[267,268] However, it remains to be demonstrated whether the importance of intact microtubules in tumor invasion is the same *in vivo*.

Identification of Intermediate Filaments as a Diagnostic Tool

The surgical pathologist is frequently confronted with the problem of distinguishing between tumors of epithelial and mesenchymal origin. The differences in the prognosis and therapy of these tumors increase the value of a rapid and precise diagnosis. Immunologic[269] and histochemical[270] techniques as well as electron-microscopic examination of biopsy material are helpful in this context, but unfortunately there always remain cases in which a precise diagnosis cannot be made despite the use of all these tools.

As we have previously seen (see page 362), intermediate-sized filaments—fibers of similar morphologic appearance—display biochemical and antigenic differences in tissues of different embryologic origin.[9] Therefore, the constituent proteins of intermediate-sized filaments can be used as differentiation markers to identify the origin of tumor cells (Figure 6). Many recent studies point to the general conclusion that neoplastic cells maintain the characteristic intermediate-sized filament proteins of their original tissues.[271] This applies to human epithelial tumors,[272-278] thymoma,[279] and even to highly undifferentiated

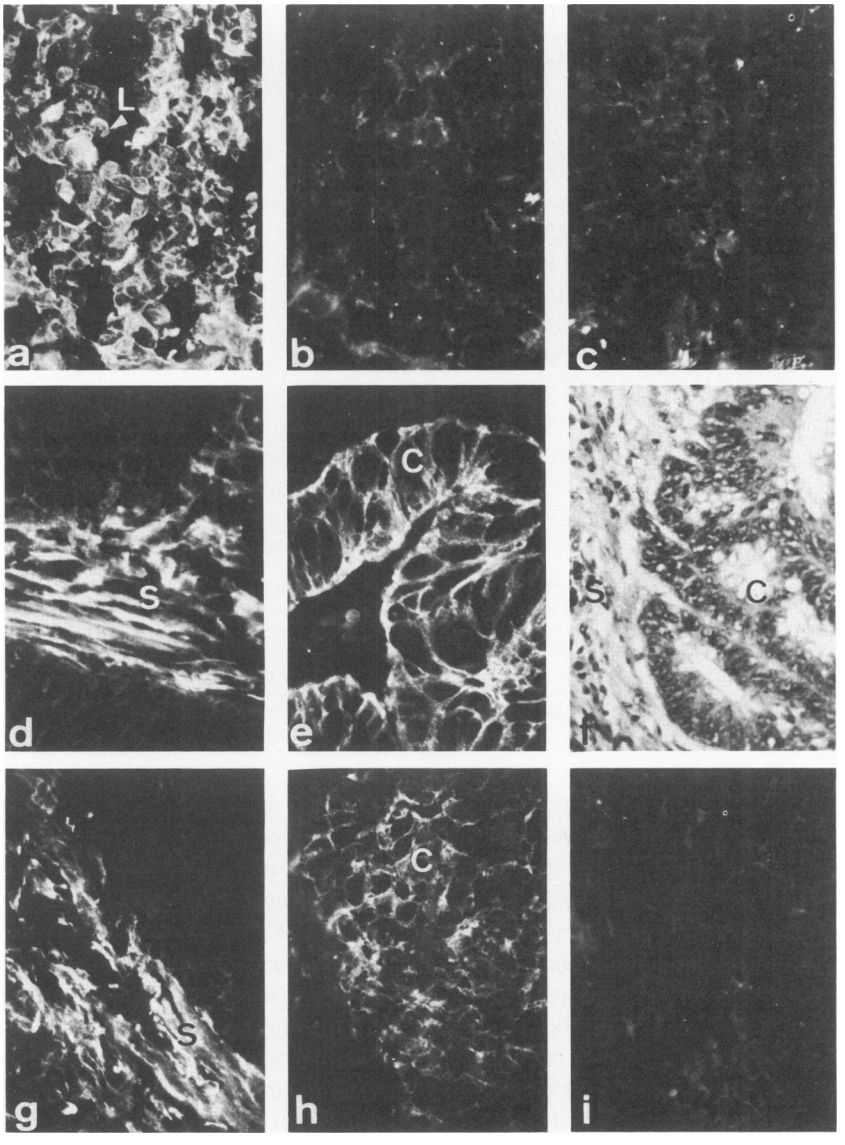

Figure 6—Indirect immunofluorescent staining of a malignant lymphoma of the lymphoblastic convoluted cell type (**a-c**), a carcinoma of the colon (**d-f**), and a carcinoma of the prostate (**g-i**). Adjacent cryostat sections were stained with guinea pig antibodies either against vimentin (**a, d, g**) or hoof prekeratin (**b, e, h**). As a control, normal guinea pig serum was used (**c, i**). Note the bright staining for vimentin of tumoral lymphoid cells (*L*) (**a**) and of the stroma (*S*) in carcinomas of the colon (**d**) and the prostate (**g**). However, carcinomatous cells (*C*) stain for prekeratin (**e, h**). **f**—Routine staining (H&E) of a section from the colon specimen adjacent to that used for immunofluorescence microscopy. (**a-e, g-i**, ×560; **f**, ×230) (In collaboration with Dr. Y. Kapanci, University of Geneva, Geneva, Switzerland)

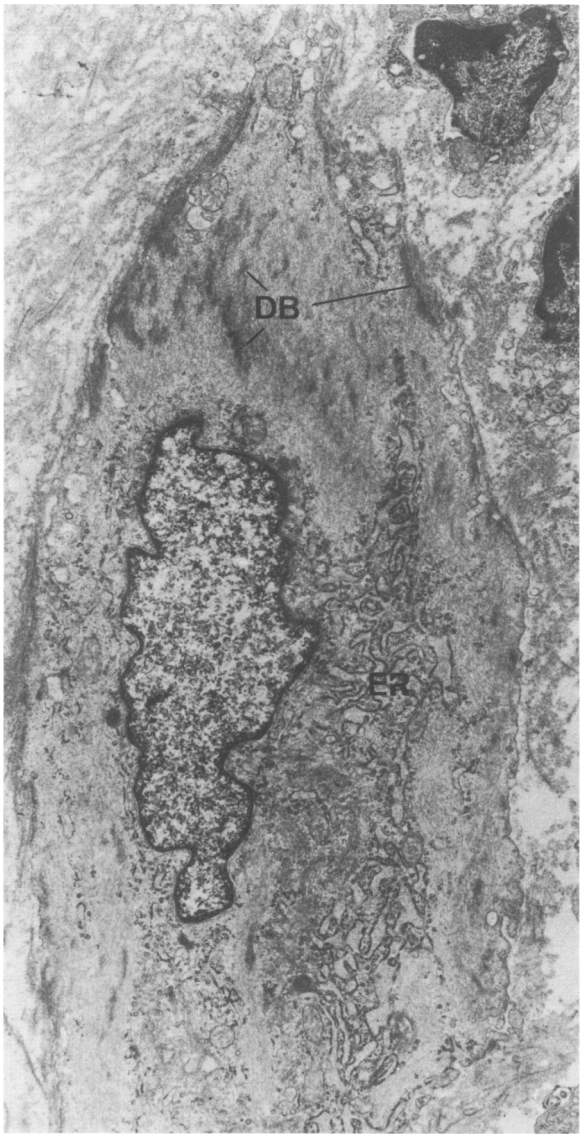

Figure 7 – Myofibroblast from the wall of a 21-day-old granuloma pouch induced in the subcutaneous tissue of a rat. Note the prominent endoplasmic reticulum (*ER*) and the well-developed microfilamentous system with dense bodies (*DB*) both within the cytoplasm and at the periphery of the cell, where they are in connection with an extracellular layer of basement-membrane-like material. (×9750) (From Gabbiani et al[292] with permission of the editor)

tumors such as anaplastic small-cell carcinoma of the lung.[271] These observations agree with findings on the specific expression of vimentin in mesenchymal neoplasms and of cytokeratin in epithelial neoplasms induced in rodent liver by carcinogen treatment.[280,281] Moreover, GFAP[282] offers a reliable marker for astrocytic cell differentiation, which is especially useful in the evaluation of mixed brain tumors.[283] The expression of this protein is also maintained in cultured glioma cells,[78] as is the expression of neurofilament proteins in cultured neuroblastoma cells.[284] Normal human melanocytes,[84,284a] as well as malignant melanomas, contain intermediate filaments of the vimentin type.[284a] These findings support a mesenchymal origin of melanocytes but do not exclude their neuroectodermal origin in an early developmental stage[284a]; they are probably useful in the differential diagnosis of melanomas from other tumors. These observations indicate that during neoplastic transformation there are no major qualitative differences in the type of intermediate filament proteins synthesized, as compared with normal tissues. However, this does not exclude the possibility that more subtle biochemical differences might exist between intermediate filament proteins of normal and neoplastic tissues, which may apply in particular to carcinomas *in vivo* and in culture, where the pattern of synthesized cytokeratins does change slightly.[83,281,285,286] It has also been shown that normal and neoplastic epithelial cells in culture contain variable amounts of vimentin, in addition to prekeratin.[79,287–289] Such a coexistence of both types of filaments has so far not been reported for neoplastic cells growing in human patients[271] and experimental animals[280] and is considered, as far as carcinoma cells are concerned, as an adaptation to culture conditions. However, it has to be noted that the situation is different for glioma cells, where GFAP and vimentin can coexist *in vivo* not only within the same cell[78] but even within the same filament.[290]

Contractile Activity of Granulation Tissue Fibroblasts (Myofibroblasts) and Motile Activity of Epidermal Cells During Wound Healing and Fibrocontractive Diseases

During wound healing two features are essential for the reconstruction of tissue continuity and repair: 1) formation and contraction of granulation tissue, and 2) movement and replication of epithelial cells. Both steps are characterized by typical modifications of the cytoskeletal and contractile elements.

The Myofibroblast

During the development of granulation tissue, fibroblasts acquire characteristics that clearly distinguish them from typical fibroblasts in normal tissue.[291] Such modified fibroblasts or myofibroblasts[292] (Figure 7) share common features with both fibroblasts (from which they, at least in part, develop) and smooth muscle cells. While the rough endoplasmic reticulum typical of normal fibroblasts remains well expressed, bundles of microfilaments appear within

the cytoplasm, and electron-opaque areas are found scattered within microfilament bundles or beneath the plasmalemma.[292] These bundles of microfilaments are similar to those observed in cultured fibroblasts and may represent the *in vivo* counterpart of the so-called stress fibers. Gap junctions develop between myofibroblasts,[293] and hemidesmosomes are found in areas where the cells are surrounded by a basal lamina.[294] These features are typical of smooth muscle cells. Moreover, when strips of granulation tissue are exposed to substances that contract or relax smooth muscle cells, contraction and relaxation is seen.[295-298] A number of agents such as 5-hydroxytryptamine, angiotensin, vasopressin, norepinephrine, bradykinin, epinephrine, and prostaglandin $F_{1\alpha}$ provoke strong contraction, whereas others such as papaverine and prostaglandins E_1 and E_2 provoke relaxation. These pharmacologic responses can be influenced by several factors, namely, aging, anoxia, or antagonists of specific drugs.[294,295] Quantification of the number and distribution of myofibroblasts in granulation tissue of porcine wounds have shown that changes in the number of myofibroblasts correlate with the rate of wound contraction.[295a]

Myofibroblasts contain considerable amounts of contractile proteins, in particular, actin and myosin. Quantitation of actin by means of densitometric analysis of gel scans has shown that rat granulation tissue contains more actin than normal skin, although the values remain lower than those of pregnant uterus (Gabbiani et al, unpublished observations). Moreover, granulation tissue yields as much extractable actomyosin as can be prepared from pregnant rat uterus.[295] The presence of actin and myosin in myofibroblasts has been directly demonstrated with the use of immunofluorescence microscopy.[293] Both proteins are distributed similarly in the cytoplasm, suggesting that actin and myosin interact to generate contraction. Actin in myofibroblasts appears to be stabilized in a polymerized form, since its sensitivity to the action of actin depolymerizing factor from human plasma is comparable to that of smooth muscle actin and is much less than that of actin in parenchymal cells. Those observations correlate with the presence of a well-developed microfilamentous apparatus in myofibroblasts visible by electron microscopy (see Figure 7).[292] It is noteworthy that after wound healing, microfilaments gradually disappear from the cytoplasm of myofibroblasts, and fixation of actin and myosin antibodies is eventually abolished.[294] These observations suggest that contraction of granulation tissue depends ultimately on the contraction of myofibroblasts.[294] This suggestion is based on the presence of contractile structures similar to those found in smooth muscle cells *in vivo* or in normal fibroblasts under culture conditions (see Kreis and Birchmeier[51] and Gabbiani and Rungger-Brändle[291]). Shrinkage of the whole tissue is thought to depend on the spontaneous or endogenously mediated contraction of myofibroblasts acting simultaneously with the deposition of collagen, thus providing a "lock-step" system.[294]

Actin Organization in Healing Epidermal Cells

In coordination with granulation tissue formation and contraction, epithelialization, namely, migration and replication of epithelial cells, is critical for the closure of a wound.[299-301] In linear wounds of the skin and in minimal trauma, epithelial regenerative processes take place before new connective tissue forms. In open wounds, mobilization and migration of epithelial cells start at the edges of the wound. In cases where the full thickness of the dermis has not been removed, cells from skin appendages, in particular, hair follicles, participate in the process of epithelialization.[300,301] It has been shown that migration plays the most important role in epithelial repair,[301,302] during which epidermal cells acquire characteristic features, such as the development of a cortical rim of microfilaments,[302,303] the loss of desmosomal complexes, and the formation of increased numbers of gap junctions.[293] The presence of an ultastructurally well-developed microfilamentous network corresponds to an intense peripheral immunofluorescent staining when one uses actin and myosin antibodies.[293] Staining for actin in migrating epithelial cells is relatively stable against the action of ADF,[258] whereas staining of normal cells is sensitive, although normal rat epidermis and healing rat epidermis contain comparable amounts of actin.[258] Like what we have said about cancer cells, this suggests that healing epithelial cells contain increased amounts of polymerized actin, probably representing, in part, the molecular basis for epithelial motile activity.

Fibrocontractive Diseases

The presence of myofibroblasts in granulation tissue and their possible role in wound contraction has prompted many authors to look for similar cells in pathologic situations characterized by connective tissue retraction. Myofibroblasts have been described in hypertrophic scars, fibromatotic lesions (particularly in the nodules of Dupuytren's disease), pathologic retractions of parenchymatous and connective tissues, in cirrhosis of the liver in man and experimental

Table 2—Myofibroblasts in Different Pathologic Situations

Pathologic situation	Reference
Normal wound healing	291, 297, 299, 306, 307-320
Hypertrophic scars	321-326
Retractions of parenchymal or connective tissues	298, 327-336
Liver cirrhosis	337-340
Dupuytren's disease and fibromatosis	309, 341-354
Tumors	304, 305, 355-371

animals, and in sarcomatous tumors. It is noteworthy that the stromal reaction observed during invasive canalicular carcinoma of the breast consists mostly of typical myofibroblasts.[304,305] A summary of the various fibrocontractive lesions in which myofibroblasts have been described and the respective references are given in Table 2. The broad range of examples demonstrates that the myofibroblasts, in addition to providing a beneficial action during wound healing, can be responsible for several important pathologic phenomena.

Modifications of Cytocontractile and Cytoskeletal Elements of the Vessel Wall in Relation to Pathologic Stimuli

The cells of the vascular wall are submitted to mechanical stresses that constantly alter their shape and intercellular relationships. It is likely that the cytoskeleton of these cells contributes in maintaining the normal multilayered organization of the vessel wall and in helping cellular adaptation to pathologic situations such as hypertension.

All cells of the vascular system are of mesenchymal origin, but they display, once established as differentiated cells in defined layers, heterogeneity of their cytoskeletal and cytocontractile elements. At present this heterogeneity concerns intermediate filaments and actin.

In mammals, cells of the endothelial layer and of the adventitia contain intermediate filaments composed exclusively of vimentin. In the media the situation is more complex: although medial cells are morphologically similar among themselves[372] and to smooth muscle cells of various parenchymatous organs (eg, gastrointestinal tract, uterus, trachea), they are heterogeneous as far as the composition of their intermediate filaments is concerned. Three types of cells can be distinguished, which are differently distributed according to species and vessel topography: one type contains only vimentin filaments, the second vimentin and desmin filaments, and the third only desmin filaments.[73,373-375] Human thoracic aorta contains practically only vimentin, whereas rat thoracic aorta contains both vimentin and desmin. In the rat, all three types of cells coexist as subpopulations in the medial layers,[376] whereby vimentin and desmin are coexpressed not only within the same cell but also within the same filament.[377] The cells containing desmin apparently are not distributed evenly throughout the aorta but follow a gradient; they are scant in the proximal segment and increasingly abundant toward the bifurcation of the vessel, as has been shown by immunofluorescent staining.[74] Further studies are needed to substantiate the possibility that the distribution of the two polypeptides is correlated with specific physiologic functions.

The situation is different in birds, where cells with the typical characteristics of smooth muscle coexist with cells of fibroblastlike appearance[378] within the same vessel. Immunofluorescent staining of avian arterial smooth muscle shows that the first type of cells stains with desmin and vimentin antibodies, while the second type stains only with vimentin antibodies.[73]

Actin, the most abundant contractile protein in vascular smooth muscle, is present in the form of microfilaments, and myosin in the form of thick filaments 150–180 Å in diameter, which can be clearly distinguished from intermediate filaments.[379] A number of studies have been directed toward the characterization of actin in vascular smooth muscle.[373,380,381] The predominant actin type is a specific α-type smooth muscle actin, which is different from the γ-type of parenchymal smooth muscle in its isoelectric point and amino acid sequence. The second most frequent actin present is the β-actin-like nonmuscle type. Besides these, two other actins, γ-like smooth muscle and γ-nonmuscle, are minor constituents. The fact that a tissue-specific ratio exists between the four different types of nonskeletal actins may demonstrate not only a large diversity of actins but also specificity of gene expression in diverse types of muscle and nonmuscle tissue (see Vandekerckhove and Weber[381]), although the functional meaning of these differences is yet unknown.[373]

Arterial Endothelial Cells During Hypertension

During several types of experimentally induced hypertension in the rat, the endothelial layer of the aorta undergoes extensive remodeling,[382,383] showing 1) increased volume and cell density; 2) increased permeability to horseradish peroxidase; 3) increased replication rate, and 4) the presence, in the cytoplasm, of numerous microfilament bundles with interspersed dense bodies (Figure 8). These changes

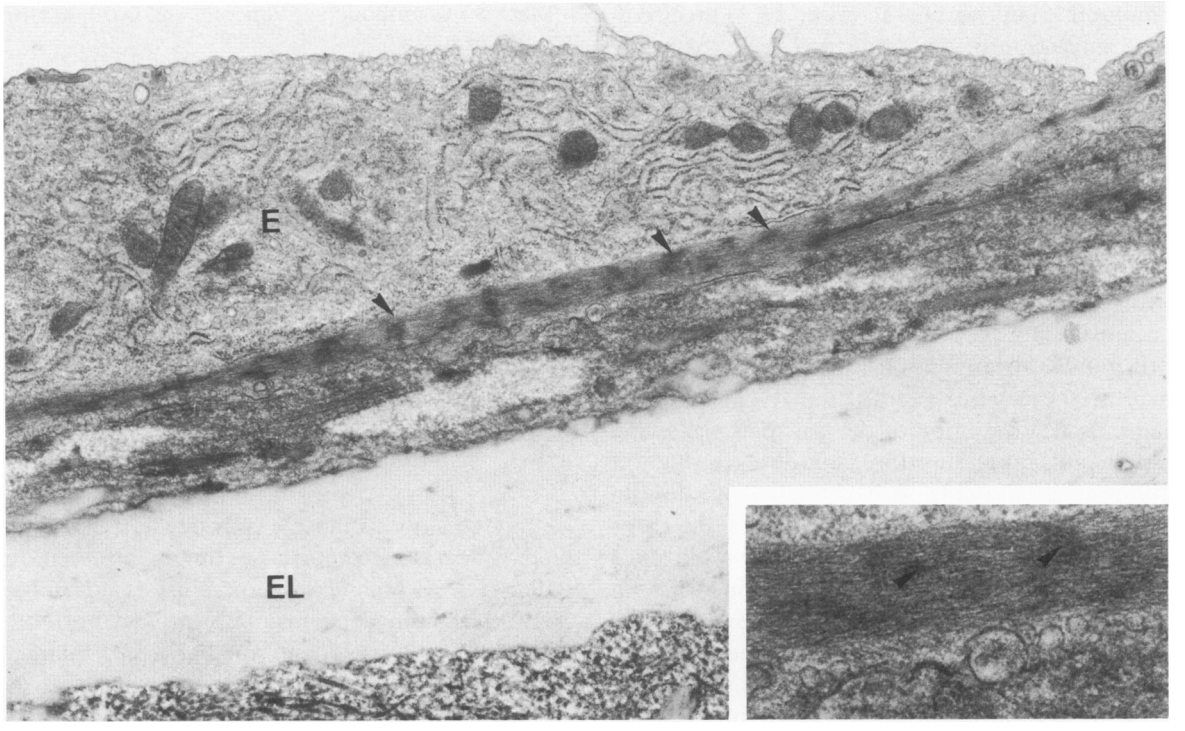

Figure 8 – Endothelial cell (*E*) of the thoracic aorta in a hypertensive rat after uninephrectomy and administration of desoxycorticosterone and salt-rich diet for 10 days. Microfilaments (**inset**) form a thick bundle immediately beneath the plasmalemma. Note the electron-dense areas (*arrowheads*) throughout the microfilament bundles. *EL*, internal elastic lamina. (× 18,000; **inset**, × 31,400)

vary according to the type of hypertension, being maximal during the early phase (7–10 days) after ligature of the aorta between the renal arteries (resulting in a Goldblatt type of hypertension) and during the early (7–10 days) and late (40 days) phases of uninephrectomy (with 0.9% NaCl given as drinking fluid together with subcutaneous administration of desoxycorticosterone acetate). The appearance of microfilament bundles reminiscent of stress lines, which are observed in cultured endothelial cells,[384] coincides with a strong staining of endothelial cells by actin antibodies.[385] The function of these microfilament bundles is unknown, but in analogy with stress lines, they could be involved in the attachment of endothelial cells to their basement lamina.[386] They are particularly abundant in situations of high endothelial cell density, increased permeability to horse radish peroxidase, and high levels of exogenous or endogenous circulating mineralocorticoids.[382,383] Recent work, using immunochemical techniques, has shown that stress fibers containing actin, myosin, and α-actinin are present in some normal aortic endothelial cells, namely, those submitted to high shear stress.[386a–386c] They become common in endothelial cells during regeneration (eg, after ballooning induced endothelial denudation).[386c] These results show that stress fibers are cellular organelles present *in vivo* that can modulate during endothelial adaptation to unfavorable or pathologic stimuli.

Intermediate-sized Filaments of the Media During Increased Blood Pressure

In spontaneously and experimentally hypertensive rats an increase in total muscle cell mass of the aortic wall has been observed.[387,388] This is due to smooth muscle cell hypertrophy rather than hyperplasia,[389] since hypertensive smooth muscle cells contain generally more total proteins and DNA, indicating that the increase in cell size results from an increased ploidy of these cells.[390] The ultrastructural image of experimentally induced, hypertrophied smooth muscle shows a massive increase in the number of intermediate-sized filaments, whereas the number of actin filaments increases proportionately to the cell volume.[379] The number of myosin filaments, on the other hand, remains unchanged. These changes may be related to the general decrease in active force generation observed in hypertrophied smooth muscle.[391]

Intermediate-sized Filaments of the Media During Experimentally Induced Intimal Thickening

Atheroma formation in the arterial intima is the leading cause of death in Western countries. The

atheromatous plaque is characterized by 1) proliferation and migration of smooth muscle cells from the media into the intima, and 2) accumulation of lipids in smooth muscle and/or phagocytic cells, as well as in the extracellular space.[392-394] Since smooth muscle proliferation in the arterial wall is one of the initial steps of atheroma formation, the question arises as to whether a particular subclass of cells, as far as intermediate-sized filaments are concerned, is responsible for intimal smooth muscle accumulation. One of the most useful experimental models for intimal smooth muscle accumulation (intimal thickening) is removal of the arterial endothelium.[395,396] We have studied intimal thickening of the rat aorta following arterial denudation induced by ballooning, using immunofluorescent staining with vimentin and desmin antibodies and electrophoretic analysis of the cytoskeletal filament proteins of the arterial wall. This study has shown that after denudation, smooth muscle cells proliferating in the intima are predominantly positive by decoration with vimentin antibodies[397] (Figure 9). Vimentin content as well as the vimentin to desmin ratio are increased in segments containing denuded areas, when compared with normal segments (Table 3). These data suggest that vimentin-rich cells are responsible for intimal smooth muscle cell accumulation after endothelial injury. However, we cannot exclude the possibility that these

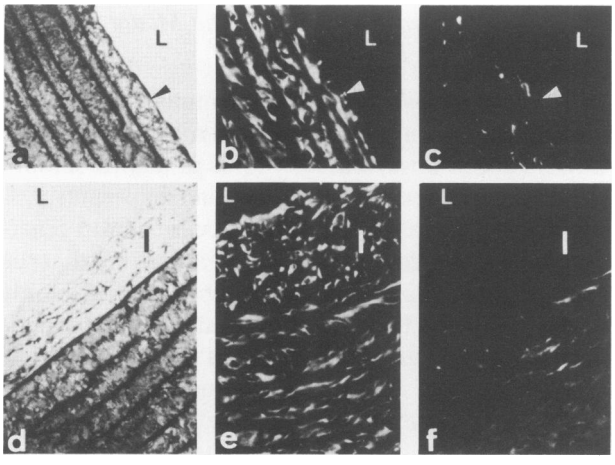

Figure 9 – Thoracic wall of the aorta of a normal rat (**a–c**) and an animal whose endothelial layer had been mechanically removed (**d–f**). Thick sections demonstrate the abundance of intimal smooth muscle accumulation within 14 days after endothelial denudation (**d**), compared with a normal specimen (**a**). Double immunofluorescence staining was done for vimentin (**b** and **e**) and desmin (**c** and **f**) (see Gabbiani et al[397]). Normal aorta shows positive reaction for vimentin in the endothelium (*arrowheads*) and in smooth muscle cells of the media (**b**). Desmin-positive cells are scattered throughout the media (**c**). In denuded aorta, vimentin-positive cells are found in the media and in the intimal thickening (**e**), whereas desmin-positive cells are not visualized in the intima (**f**). *L*, aortic lumen; *I*, intima. (**a** and **d**, × 380; **b**, **c**, **e**, and **f**, × 310) (From Gabbiani et al[397] with permission of the editor)

Table 3 – Quantitation of Vimentin and Desmin in Normal Thoracic Aorta and in Regions with Myointimal Thickening*

Area	% Vimentin (Mean ± SE)	% Desmin (Mean ± SE)	% Vimentin/ % desmin (Mean ± SE)
Normal aorta (n = 5)	1.68 ± 0.10	1.09 ± 0.09	1.56 ± 0.11
	$P < 0.001$	$P > 0.05$	$P < 0.01$
Thickened area (n = 6)	2.70 ± 0.15	1.36 ± 0.08	2.00 ± 0.07

* Coomassie-blue-stained gels were planimetrically measured, and the amount of vimentin and desmin are expressed as the percentage (%) of total protein. The Student *t* test was used. For further details, see Gabbiani et al.[397]

activated smooth muscle cells develop vimentin filaments *de novo* and correspondingly lose their desmin filaments, although this possibility is not consistent with the finding that proliferating and tumoral cells maintain the type of intermediate-sized filaments of their original tissue.[271] Vimentin filaments are typical of fibroblastic cells, and it is known that during the healing of an open wound fibroblasts acquire features that are compatible with motile and contractile activities (myofibroblasts; see page 374). The morphologic characteristics and the high vimentin content of vascular smooth muscle cells migrating into the injured intima suggest some analogies of structure and behavior between myofibroblasts and these intimal smooth muscle cells. Further work is needed to characterize the mechanisms of smooth muscle cell mobilization after endothelial injury. Compatible with both the endothelial injury hypothesis[393] and the monoclonal hypothesis[398] of atheroma formation, these findings indicate that one predominant subtype of vascular smooth muscle cells, ie, vimentin-containing cells, is responsible for the initial intimal accumulation of smooth muscle after endothelial injury.

Changes of Cytocontractile and Cytoskeletal Elements in Hepatocytes During Pathologic Situations

The various activities displayed by hepatocytes have made them a useful model for studying the cytoskeleton in functions such as the maintenance of cell shape and intracellular movements.[399] We have already mentioned that myofibroblasts are present in fibrotic tissue of liver cirrhosis (see Table 2 and Bhathal,[337] Grimaud and Borojevic,[338] Irle et al,[339] and Rudolph et al[340]) and shall now discuss some aspects of the cytoskeleton during liver regeneration,

bile secretion, and formation of alcoholic hyalin deposits.

Liver Regeneration

During the first 7 days after partial hepatectomy in the rat, hepatocytes display thickening of their peripheral microfilamentous network, particularly around the bile canaliculi.[303] The appearance of increased amounts of microfilaments coincides with an increased staining with actin antibodies and a diminished sensitivity to the depolymerizing action of ADF.[258] Concomitantly, it has been shown that the amount of polymerized actin is substantially increased in regenerating liver tissue, whereas the amount of total actin remains practically unchanged (Table 4; see Low et al[258]). Such an increase in the amount of F-actin may be instrumental in rearranging the normal architecture of the lobules.

Bile Secretion and Cholestasis

Involvement of pericanalicular microfilaments in normal bile flow has been suggested by several studies.[400-403] In cultured hepatocytes, dynamic contraction of bile canaliculi takes place, which supports the possibility that the peripheral microfilamentous network facilitates bile flow.[404] On the other hand, administration of cytochalasin B to isolated rat liver cells reversibly stops bile flow.[401] Intravenous administration of phalloidin to rats (one of the toxins of the mushroom *Amanita phalloides*), which increases the rate of intracellular actin polymerization and decreases the degradation of F-actin,[405] results in accumulation of actin filaments in hepatocytes.[406,407] In a first step, this accumulation takes place around bile canaliculi; later on and progressively, it occurs on the entire periphery of the cell. Under such conditions, microvilli disappear, bile canaliculi become dilated, and bile flow decreases.[406,408] It is noteworthy that both loss of microvilli and canalicular dilatation are characteristic features of most forms of human and experimentally induced cholestasis.[409,410]

Since most forms of cholestasis are accompanied by a general increase of microfilaments in hepatocytes, particularly around the bile canaliculi,[400,409] it is possible that alterations in microfilament formation is related to the development of human cholestasis. A familial disease displaying cholestasis and development of cirrhosis has been described in Indian children from Canada.[411] In these patients, accumulation of important amounts of pericanalicular microfilaments are observed.

It is not yet clear which role the microfilaments play in the development of cholestasis, but the implication of microfilaments has given new insight into the interpretation of the pathogenesis of this disease.

Alcoholic Hyalin

Alcoholic hyalin (Mallory bodies) has been considered pathognomic of alcoholic liver disease and is still useful in the diagnosis of various diseases,[392] such as alcoholic liver changes,[412,413] primary biliary cirrhosis,[414] Indian juvenile cirrhosis,[415] Wilson's disease,[416] hepatoma,[417,418] and abetalipoproteinemia.[419] In experimental animals, hyalin inclusions have been produced by long-term treatment with griseofulvin,[420,421] a fungistatic agent that affects microtubules.

Hyalin deposits are intracytoplasmic accumulations of randomly oriented, densely fimbriated rods about 200 nm in length and 14–20 nm in diameter. A considerable number of morphologic, immunologic, and biochemical studies[422-431] have revealed the relatedness of Mallory body constituents to proteins in intermediate filaments of the cytokeratin type. In particular, it has been shown that antibodies prepared against mouse liver cytokeratin polypeptides bind to Mallory bodies in murine and human liver tissue[431] (Figure 10), although the binding to the indi-

Table 4—Actin Content of Normal and Regenerating Liver

Fraction	% Actin of total protein			% Increase in actin content per equivalent amount of liver
	Control	Regenerating	% Increase	
Total extract	6.9 ± 0.4 (n = 9)	8.0 ± 0.6 (n = 9)	15.6 ± 3.6 (n = 9)	15.3 ± 3.8
120,000 g supernate	8.5 ± 1.2 (n = 9)	9.5 ± 1.4 (n = 9)	10.5 ± 4.1 (n = 9)	10.2 ± 4.2
120,000 g pellet	4.2 ± 0.4 (n = 7)	5.9 ± 0.7 (n = 7)	42.7 ± 5.8 (n = 7)	47.8 ± 6.9

Each value is the mean ± SD of data from three separate experiments. Planimetric analyses were made on gel electrophoretic scans. For further details see Low et al.[258]

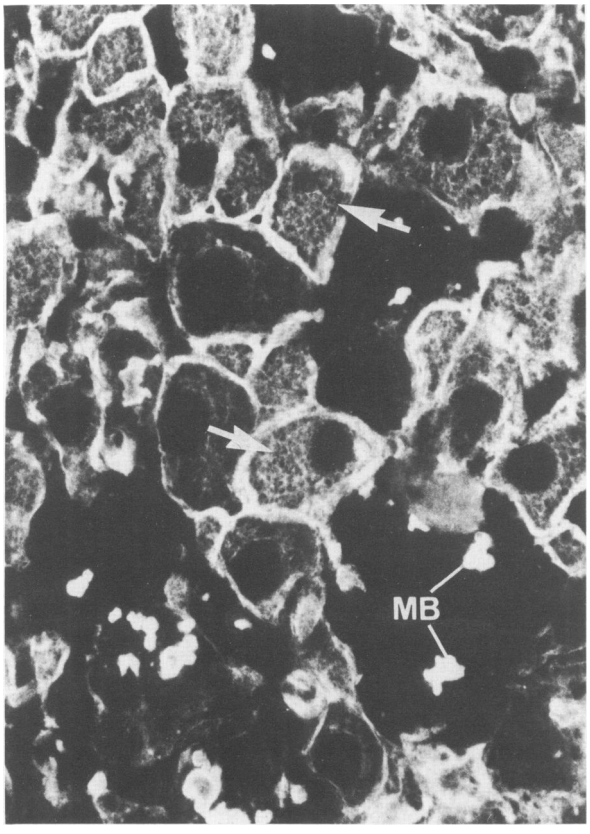

Figure 10 – Cryostat section of liver tissue of a griseofulvin-treated mouse after preparation for indirect immunofluorescence with the use of guinea pig antibodies against cytokeratin-polypeptide D. At times of intoxication strongly stained Mallory bodies (*MB*) are found beside still normal hepatocytes displaying the typical cytokeratin meshwork (*arrows*). (×550) (Courtesy of Dr. W. W. Franke, German Cancer Research Institute, Heidelberg, Federal Republic of Germany)

vidual polypeptides is somehow different when compared with normal liver cytokeratins. This may indicate either reduced accessibility or antigenicity of antigenic determinants during the development of Mallory bodies.[431] Besides polypeptides, which are more characteristic for epidermal cytokeratins than for hepatocyte cytokeratins,[431] Mallory bodies contain other, noncytokeratinous components.[424,430] Progressive derangement of the hepatocyte cytokeratin meshwork and its incorporation into Mallory bodies suggests that alcoholic hyalin may be regarded as the result of a specific alteration of the hepatocyte cytoskeleton.[431]

Conclusions

We have seen that cytocontractile and cytoskeletal elements, forming (in analogy with anatomic terminology) what could be called the cytolocomotor apparatus, not only participate in normal cellular activities such as locomotion or mitosis but probably also play a major role in several pathologic processes.

Typical alterations of cytolocomotor elements have been observed in several diseases; however, it is difficult to know whether these alterations are the primary cause of pathologic manifestations. The absence of dynein arms in the immotile cilia syndrome (page 365) is one of the few examples of a cytoskeletal alteration directly related to pathologic changes. In other situations the primary defect affects a noncytoskeletal cellular component but leads, through mechanisms that are not always clear, to typical changes of the cytoarchitecture – as is the case for the mutant β-globin molecule in sickle cell anemia (see Lux et al[225]). Most often, however, changes in the organization of cytolocomotor elements during pathologic situations may reflect the degree of cellular adaptation to pathologic stimuli. Many disease processes affect the pattern of such general cell activities as migration or proliferation; those changes in turn may profoundly modify the organization of the cytolocomotor apparatus. Thus, it appears that certain important pathologic processes (eg, tumor invasion) are, at least in part, dependent on changes in cytolocomotor elements.

Further work is needed to establish precise relationships between cytoskeletal and contractile activities and pathologic changes. However, the work summarized in this review has already contributed to the understanding of important aspects of pathologic situations. The origin of a tumor cell may be identified from its intermediate filament constituents because neoplastic cells maintain the type of intermediate filament proteins expressed in their tissue of origin. Until now, this work has been performed with polyclonal antibodies[78,271-281,283,284] which allow a clear but gross distinction among different classes of intermediate filaments. It is conceivable that the use of monoclonal antibodies and of conformation-specific monoclonal antibodies[432] will furnish new information on more subtle differences between cytoskeletal constituents of neoplastic and normal cells concerning amino acid sequence, conformation, and association with specific proteins. Evidence has already been presented of new cytokeratin molecules synthesized by epithelial tumor cells,[83,286] and in some cases it has been suggested that these newly synthesized proteins may be regarded as markers for neoplasia.[433]

The usefulness of intermediate filament proteins as cellular markers is also exemplified by the recent findings that 1) smooth muscle cells in the arterial wall are heterogeneous as far as these proteins are concerned[73,373,374] and 2) vimentin-rich smooth muscle cells colonize the aortic intima after endothelial denudation in the rat (page 378; see Gabbiani et al[397]). This suggests that vimentin-rich smooth muscle cells

are related to the proliferative response after injury and may help in the understanding of the mechanism of atheromatous plaque formation. Recent preliminary work has indicated that smooth muscle cells of the arterial media are heterogeneous also as far as their myosin composition is concerned.[434]

Little is known about the variations of the molecular type of actin and myosin during pathologic processes. It has been reported that the myosin of hypertrophic myocardium shows altered antigenicity, compared with its normal counterpart.[435] More information has also been obtained on the changes in the degree of actin polymerization during pathologic situations. Cell activation *in vivo* does not generally appear to change the amount of total actin but is accompanied by an increase in the relative amount of F-actin. This has been shown to occur during the acrosomal reaction in invertebrate sperm,[436] in stimulated platelets,[45,46] in epidermal cells healing over a wound (page 375), during rat liver regeneration after partial hepatectomy (page 379), and in carcinomatous cells, compared with their normal counterpart (page 372; see Low et al[258]).

Newly acquired motile and contractile activities by fibroblastic cells (myofibroblasts) may be the basis of several pathologic manifestations, such as contraction of granulation tissue, formation of hypertrophic scars, retraction of grafts, fibromatosis, and many types of fibrotic lesions, including liver cirrhosis (for references see Table 2). Pharmacologic agents influencing motile and contractile activities of myofibroblasts may be of use in the elucidation of the basic mechanisms of these pathologic phenomena and may also help to control their evolution.

Research on cytoskeletal and cytocontractile elements is relatively new and only in the last few years has an important progress been achieved in understanding of how such elements participate in normal physiologic processes. It is evident, therefore, that this review had to show, with few exceptions, the limits of the present knowledge on the participation of the cytolocomotor system in pathologic phenomena. Nevertheless, we hope to have stressed the enormous potential of studying cytoskeletal and cytocontractile structures for an understanding of basic changes implicated in pathologic situations.

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