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CELLULAR DYNAMICS OF VIMENTIN FILAMENTS
AND THEIR SPATIAL RELATIONSHIP
TO MICROTUBULES IN LYMPHOCYTES

by

Micheline Paulin-Levasseur

A thesis submitted
to the School of Graduate Studies and Research,
University of Ottawa,
in partial fulfillment of the requirements
for the degree of
Doctor in Philosophy
in the
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À Pierre, Hugo et Julie

Pour la complicité, l'humour et la poésie

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ABBREVIATIONS

ADP	: adenosine diphosphate
anti-Ig	: anti-immunoglobulin
ATP	: adenosine triphosphate
αMM	: α-methyl-D-mannoside
Ca ²⁺	: calcium
Cd ²⁺	: cadmium
cAMP	: cyclic adenosine monophosphate
cDNA	: complementary DNA
cGMP	: cyclic guanosine monophosphate
CHQ	: Chinese hamster ovary
CLL	: chronic lymphocytic leukaemia
Con A	: concanavalin A
DMSO	: dimethylsulfoxide
DNA	: deoxyribonucleic acid
D ₂ O	: heavy water
EDTA	: ethylene diamine tetra-acetic acid
EGTA	: ethylene glycol bis (β-aminoethylether) N, N, N', N'-tetra-acetic acid
GDP	: guanosine diphosphate
GTP	: guanosine triphosphate
HBSS	: Hank's balanced salt solution
HEPES	: (n-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid)
HMW	: high molecular weight
hrRNA	: heterogeneous RNA
IL	: interleukin
K ⁺	: potassium
La ³⁺	: lanthanum
MAP	: microtubule associated protein
Mg ²⁺	: magnesium
Mn ²⁺	: manganese
mRNA	: messenger ribonucleic acid
MTOC	: microtubule organizing center
MW	: molecular weight
Na ⁺	: sodium
PBS	: phosphate buffered saline
PEG 6000	: polyethylene glycol 6000
PHA	: phytohaemagglutinin
PIPES	: piperazine N, N'-bis (2-ethane sulfonic acid)
poly (A) ⁺	: polyadenylated
PS	: post stimulation
SDS	: sodium dodecyl sulfate
sIg	: surface immunoglobulin
TL	: thymus leukemia
tRNA _{met}	: initiator-methionyl-transfer RNA

ABSTRACT

Lymphocytes offer several advantages as a model system for the analysis of dynamics and interactions of cytoskeletal components that can be related to the differentiation and function of these cells in the immune response. Upon interaction of ligands with their surface receptors, resting lymphocytes can be induced *in vitro* to undergo different processes such as redistribution of cell surface receptors (patching and capping) or stimulation, which mimicks the normal differentiation of lymphocytes and involves the activation of resting cells to enter the cell cycle and to progress through blastogenesis, DNA synthesis and mitosis.

Using immunofluorescence and electron microscopy, I have examined the organizational fate of vimentin and its relationship to the microtubule system in mouse splenic lymphocytes : a) during mitogenic stimulation by concanavalin A; b) during anti-Ig-induced redistribution of surface receptors; and c) in response to treatments that alter microtubule organization such as cold, microtubule-depolymerizing drugs (colchicine, colcemid and nocodazole) and the microtubule assembly-promoting drug taxol. The purpose of this approach was to characterize the cellular dynamics of the vimentin system in lymphocytes.

Resting lymphocytes display a complex network of vimentin filaments which is partially coincident with the radial pattern of microtubules and appears to extend from the centrosomal region. Accompanying the increase in cellular volume that occurs during blastogenesis in response to con A, there are progressive changes in centrosome organization and up to a 5-fold increase in the

numbers of microtubules assembled from the centrosome (Schweitzer and Brown, 1984). In fully stimulated cells, a vimentin network is not observed, but rather vimentin is detected as a diffuse aggregate located near the centrosome. This vimentin reorganization does not temporally parallel the progressive changes in tubulin expression but rather occurs as a late event during blastogenesis.

When stimulated lymphocytes enter mitosis, the cytoplasmic pattern of microtubules is dismantled and a mitotic spindle develops. Vimentin reappears as filamentous arrays extending from the polar centrosomes during prophase and then forms a cage-like structure that transiently encloses the chromosomes and the mitotic spindle. During cytokinesis, the polar centrosomes relocate to a position adjacent to the midbody and vimentin is observed as an aggregate, similar to that seen prior to mitosis, close to the centrosome in each daughter cell. These observations and the data obtained from exposure of resting and stimulated lymphocytes to treatments that alter microtubule organization support the view that vimentin arrangements may be spatially dependent on the microtubule organization. They further indicate that the structural integrity of the centrosome may be a critical factor in the cellular dynamics of the vimentin system.

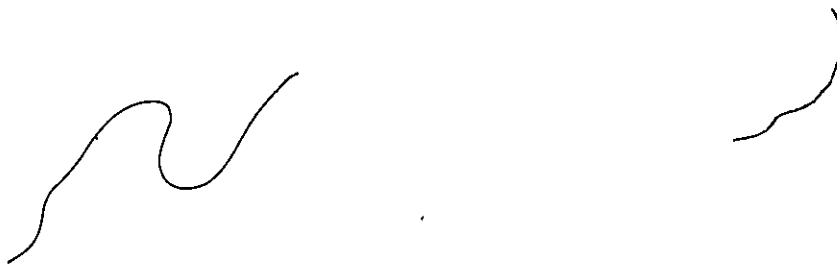
In the continued presence of colcemid during mitogenic stimulation, the entry of lymphocytes into S phase is markedly inhibited. In these cells, the centrosome/Golgi complex is disrupted upon removal of microtubules and vimentin is present in filamentous arrays through the stimulation process. In contrast, in the continued presence of taxol, stimulation of lymphocytes proceeds normally up to mitosis. Structural integrity of the centrosome/Golgi complex is not altered by taxol-induced reorganization of microtubules and vimentin shows

a sequence of rearrangements during progression in the cell cycle that resembles that observed during normal stimulation. These results support the importance of the structural integrity of the centrosome for the cellular dynamics of vimentin during mitogenic stimulation. They furthermore strongly suggest that vimentin rearrangements may be more important than changes in microtubule organization for the progression of lymphocytes through the cell cycle in response to mitogen.

Extensive rearrangements of the vimentin system also occurs during sIg cap formation on the surface of B lymphocytes. During that process, the microtubule pattern remains unaltered while the vimentin network is rapidly accumulated in a diffuse aggregate underneath the cap close to the position of the centrosome. Further observations on the cytoplasmic distribution of vimentin during sIg cap formation in cells that were treated with colcemid or taxol prior to induction of sIg redistribution indicate that : 1) accumulation of vimentin is not due to a passive trapping but represents a dynamic rearrangement of the vimentin system, and 2) vimentin filaments, like microtubules, are not directly involved in the translocation of ligand-receptor complexes but may contribute in providing directionality to the microfilament-dependent movement of such complexes. Based on the organizational fate of vimentin during sIg redistribution in normal and drug-treated cells, it is proposed that vimentin may play a role in transmitting spatial informations from the microtubule system to the microfilament contractile machinery during sIg ligand-receptor translocation to form a cap.

As the concomittant aggregation of the vimentin system and relocalization of the polar centrosomes close to the midbody observed during

cytokinesis of lymphocytes had not been previously reported in other cells, a comparative study on the organizational fate of vimentin and tubulin during division of several attached and suspension cell lines has been performed to determine if such features were peculiar to lymphocytes or of more general occurrence. This study has indicated that : 1) relocalization of the centrosomes near the midbody during cytokinesis is not peculiar to lymphocytes but may represent a more general feature of cells that grow in suspension; 2) aggregation of the vimentin system, which occurs concomittant with the relocalization of the centrosomes in lymphocytes and lymphoid cell lines, is not required for centrosome relocalization of other suspension cells. Observations on the organizational fate of vimentin during mitosis of attached and suspension cells also support a functional involvement of this filamentous system in mitotic events.



RÉSUMÉ

Les lymphocytes constituent un modèle cellulaire qui offre de nombreux avantages pour procéder à l'analyse des interactions et de la dynamique des composantes du cytosquelette pouvant être impliquées dans la différenciation et la fonction de ces cellules lors de la réponse immunitaire. On peut *in vitro*, par l'addition de certains ligands qui se fixent aux récepteurs membranaires des lymphocytes au repos, induire dans ces cellules divers processus tels que la redistribution des récepteurs (patching et capping) ou la stimulation, qui simule la différenciation normale des lymphocytes provoquant l'activation du cycle cellulaire et la progression à travers la blastogénèse, la synthèse de l'ADN et la mitose.

Par immunofluorescence et microscopie électronique, nous avons étudié les changements organisationnels de la vimentine et ses relations avec le réseau des microtubules dans les lymphocytes isolés à partir de la rate de souris, durant : a) la stimulation mitogénique par la concanavaline A; b) la redistribution des récepteurs membranaires induite par anti-Ig; et c) la modification de l'organisation des microtubules sous l'action du froid, et de drogues dépolymérisantes (colchicine, colcémide et nocodazole) ou polymérisantes (taxol). L'objectif sous-jacent à cette démarche était de caractériser la dynamique cellulaire du réseau de vimentine dans les lymphocytes.

Les lymphocytes au repos montrent un réseau complexe de filaments de vimentine qui coïncide partiellement avec l'arrangement radial des microtubules et qui semble se déployer à partir de la région centrosomique. En

même temps qu'un accroissement du volume cellulaire se produit sous l'effet de la concanavaline A lors de la blastogénèse, on note des changements progressifs dans l'organisation du centrosome et une importante augmentation (5x) du nombre des microtubules assemblés depuis le centrosome (Schweitzer et Brown, 1984). Dans les cellules totalement stimulées, on ne détecte plus un réseau de vimentine mais plutôt une agrégation diffuse située près du centrosome. Cette réorganisation de la vimentine ne se produit pas de façon concomitante aux changements progressifs dans l'expression de la tubuline, mais elle s'effectue tard au cours de la blastogénèse.

Lorsque les lymphocytes entrent en mitose, l'arrangement cytoplasmique des microtubules est démantelé et un fuseau mitotique s'établit. La vimentine apparaît en minces faisceaux de filaments déployés à partir des centrosomes polaires au cours de la prophase et forme ensuite, de façon transitoire, une structure semblable à une cage entourant les chromosomes et le fuseau mitotique. Durant la cytokinèse, les centrosomes polaires migrent près du midbody et la vimentine, accumulée près du centrosome dans chaque cellule fille, présente une organisation similaire à celle que l'on observe avant la mitose. Ces observations et celles obtenues en soumettant les lymphocytes au repos ou stimulés à des traitements capables de modifier l'organisation des microtubules appuient l'hypothèse selon laquelle l'arrangement de la vimentine pourrait être spatialement dépendant de l'organisation des microtubules. De plus, elles indiquent que l'intégrité structurale du centrosome puisse être un facteur déterminant dans la dynamique cellulaire du réseau de vimentine.

En présence continue de colcémide durant la stimulation mitogénique, l'entrée des lymphocytes en phase S est inhibée de façon marquée. Dans ces

cellules, le complexe centrosome/Golgi est altéré à la suite de la dépolymérisation des microtubules, et la vimentine est présente sous forme d'arrangements filamenteux tout au long du processus de stimulation. Par contre, en présence continue de taxol, la stimulation des lymphocytes se déroule normalement jusqu'à la mitose. L'intégrité structurale du complexe centrosome/Golgi n'est pas modifiée par la réorganisation des microtubules induite par le taxol et, durant la progression à travers le cycle cellulaire, la vimentine suit une séquence de réarrangements semblable à celle que l'on observe en conditions normales. Ces résultats viennent appuyer la suggestion selon laquelle l'intégrité structurale du centrosome jouerait un rôle important dans la dynamique de la vimentine au cours de la stimulation mitogénique. Ils suggèrent aussi fortement que les réarrangements de la vimentine puissent être plus importants que les modifications de l'organisation des microtubules pour la progression des lymphocytes à travers le cycle cellulaire engendré par le mitogène.

Des réarrangements importants du réseau de vimentine se produisent également durant la formation du cap des Ig à la surface des lymphocytes B. Au cours de ce processus, l'arrangement des microtubules ne varie pas alors que le réseau de vimentine se réorganise rapidement en une agrégation diffuse, située sous le cap, près du centrosome. Des observations additionnelles effectuées sur la distribution cytoplasmique de la vimentine durant la formation du cap sur des cellules traitées à la colcémide ou au taxol, préalablement à l'induction de la redistribution des Ig, montrent : a) que l'agrégation de vimentine n'est pas le résultat d'une accumulation passive mais qu'elle représente une réorganisation dynamique du réseau; et b) que, comme les microtubules, les filaments de vimentine ne sont pas directement impliqués dans la migration des complexes ligands-récepteurs, mais qu'ils pourraient contribuer à orienter leur déplacement

par les microfilaments. En nous fondant sur les changements organisationnels de la vimentine au cours de la redistribution de Ig dans les cellules normales et dans les cellules traitées avec drogues, nous proposons ici que la vimentine puisse jouer un rôle dans la transmission d'informations spatiales entre le réseau de microtubules et la machinerie contractile des microfilaments au cours de la migration des complexes ligands-récepteurs menant à la formation d'un cap.

Comme l'accumulation de la vimentine et la migration concomitante des centrosomes polaires près du midbody n'avait pas encore été observées dans d'autres cellules, une étude comparative a été menée sur les changements organisationnels de la vimentine et de la tubuline au cours de la division de diverses lignées de cellules cultivées en suspension ou attachées. Cette étude visait à déterminer si ces réarrangements simultanés étaient typiques aux lymphocytes ou communs à plusieurs types de cellules. Cette étude a montré que : a) la migration des centrosomes près du midbody durant la cytokinèse n'est pas unique aux lymphocytes mais qu'elle peut représenter un trait plus général des cellules cultivées en suspension; b) l'accumulation de la vimentine qui se produit simultanément à la migration des centrosomes dans les lymphocytes et les lignées de cellules lymphoïdes, n'est pas nécessaire pour la migration des centrosomes dans d'autres cellules qui croissent en suspension. Les observations effectuées sur les changements organisationnels de la vimentine pendant la mitose de cellules cultivées en suspension ou attachées appuient l'hypothèse d'une implication fonctionnelle de ce réseau de filaments durant la mitose.

*"Between the idea
And the reality
Between the motion
And the act
Falls the Shadow."*

T.S. Elliot (The Hollow Men)

FOREWORD

In almost all eucaryotic cells, the cytoplasm contains a complex lattice of actin filaments, microtubules and intermediate filaments. With their associated proteins, these three filamentous systems constitute a major proportion of total cell proteins and have collectively been termed "the cytoskeleton". Numerous studies support the general concept that the different components of the cytoskeleton may be spatially and temporally integrated to assure such diverse cellular functions as development and maintenance of cell morphology, structural organization of the cytoplasm, intracellular movement of molecules or organelles, cell locomotion, transmission of signals, mitosis, etc. (Cohen, 1979; Marx, 1983). Furthermore, several pathological disorders have been related to alterations of cytoskeletal components (for reviews, see : Weatherbee 1981; Rungger-Brändle and Gabbiani, 1983). Therefore, it is of fundamental interest and of crucial importance to understand how the cells construct their architecture and to determine the functions of the cytoskeletal components in the coordination of cell activities.

In spite of the great interest shown in the cytoskeleton during the past two decades, the characterization of its components is far from complete. For instance, little is still known about the cellular dynamics (sites of nucleation, interactions with other cellular structures, fate during the cell cycle) and cytoplasmic roles of intermediate filaments. Due to morphological criteria suitable for immunofluorescence, studies on intermediate filaments have been performed almost exclusively in well flattened cells that grow permanently in culture. It has been recently demonstrated that, in these cells, microinjection of

antibodies specifically directed against intermediate filament proteins can lead to the collapse of intermediate filaments networks without affecting cellular activities previously proposed to be mediated by cytoplasmically extended intermediate filaments. Considering this experimental fact, some investigators have suggested that the cellular dynamics and cytoplasmic roles of intermediate filaments, whose subunits are remarkably tissue-specifically expressed, may be readily observable only in cells which still perform differentiated functions (Klymkowsky, 1982).

Lymphocytes have been extensively used as a cellular system to investigate the molecular mechanisms of differentiation. Upon interaction of ligands with their cell surface receptors, isolated lymphocytes can be induced *in vitro* to undergo different processes such as redistribution of cell surface receptors (patching and capping) or stimulation, which mimicks normal differentiation of lymphocytes into competent immune cells, and involves the activation of quiescent cells to enter the cell cycle and to progress through blastogenesis, DNA synthesis and mitosis. This cellular system has been proven very useful to study the functional involvement of actin filaments and microtubules in cellular responses triggered by signals generated on the plasma membrane. It appeared, therefore, of particular relevance as the basis for this thesis to investigate the cellular dynamics and cytoplasmic roles of intermediate filaments in this cellular system.

To present a comprehensive view of my research, I will attempt in the following introduction to impart a brief survey of the literature concerning the cellular dynamics and cytoplasmic roles of the three major components of the cytoskeleton. I will also stress the particular interest of lymphocytes as a

biological system in the study of the cytoskeleton. Finally, I will define the rationale of my experimental approach.

INTRODUCTION

ACTIN FILAMENTS

Best known as the major component of the thin filaments in muscle cells, actin filaments are also basic elements of the cytoskeleton in virtually all eucaryotic cells. They essentially consist of globular monomers of actin (G-actin), with an apparent molecular weight (MW) of 42 000, assembled in a double stranded helix approximately 7nm in diameter. In all species, except in the most primitive ones such as yeast, multigene families encode slightly different actins whose expression is differentially regulated, both spatially and temporally, during development (Korn, 1982). Through analysis of actin proteins from a variety of tissues in several organisms by high-resolution two-dimensional gel electrophoresis, three isoelectric forms have been distinguished: β - and γ -actins, two cytoplasmic forms found in all cell types; and α -actin, a more acidic form present exclusively in muscle cells (Firtel, 1981). Amino acid sequences of actins from different sources have been determined (Collins and Elzinga, 1975; Vanderkerckhove and Weber, 1978; Vanderkerckhove and Weber, 1979) and the actin genes from a number of cells have been isolated (Firtel, 1981). These studies have revealed that actins are among the most conserved proteins in evolution. For example, the sequence homology between actins from sources as evolutionarily distant as the acellular slime mold *Physarum polycephalum* and the rabbit skeletal muscle has been estimated to 92% (Korn, 1982). Furthermore, considerable biochemical data have shown that actins from widely divergent species are remarkably similar to each other and to skeletal muscle actin in their structural properties.

Assembly

Readily soluble in water, the monomeric G-actin is associated with one tightly bound Ca^{2+} (calcium), which stabilizes its globular conformation, and one noncovalently bound ATP (adenosine triphosphate), which may play a determinant role during filament assembly. Aqueous solutions of G-actin can be induced to polymerize into filaments (F-actin) by simply raising the salt concentration of the solution to a level closer to that found in the cells. Although the process does not require energy, the bound ATP is hydrolyzed during polymerization (Cooke, 1975; Woodrum *et al.*, 1975). Considering the polar growth of actin filaments, Wegner (1976) has first suggested that nucleotide cleavage would, under steady-state conditions, allow a net polymerization at one end (plus end) of a filament and a corresponding depolymerization at the other end (minus end), while the average polymer length would not change. Such a steady-state flux or treadmilling of subunits along actin filaments has been clearly demonstrated *in vitro* (Woodrum *et al.*, 1975; Kondo and Ishiwata, 1976). The functional significance of this behavior *in vivo*, however, is still questioned. As it has been stressed by Kirschner (1980), the theory of treadmilling predicts that, under steady-state conditions, filaments anchored at their minus end would elongate while filaments capped at their plus end would be disassembled. Determinations of actin polarity in the cells using heavy meromyosin decoration have indicated that actin filaments are usually attached on cellular membranes by their plus end (Mooseker and Tilney, 1975; Small and Celis, 1978). This observation has therefore argued against the applicability of the treadmilling theory to living cells. However, as it is not at all clear whether cellular membranes are nucleation or insertion sites for actin filaments, a better

understanding of actin dynamic behavior *in vivo* awaits further characterization of actin nucleation sites.

Cellular Dynamics and Cytoplasmic Functions

In vertebrate skeletal muscle, actin filaments constitute with tropomyosin and troponin (a complex of three polypeptides: troponins T, I, C) the highly stable thin filaments of the sarcomere. These filaments are anchored at their plus end on the Z discs by the structural protein α -actinin (Masaki *et al.*, 1967; Schoenberg and Needham, 1976). The interaction of actin filaments with myosin stimulates the ATPase activity of this protein and generates the muscular contraction as the chemical energy is converted into mechanical work by a sliding filament mechanism (Huxley, 1969; Huxley, 1973; Huxley, 1980). Cellular integration of that specialized contractile machinery is thought to be mediated by the attachment of Z discs to the plasma membrane via the interaction of spectrin, an anchoring protein first described in erythrocytes (for a review see: Branton *et al.*, 1981), with actin filaments and/or desmin filaments (Granger and Lazarides, 1978).

Unlike the case in striated muscle cells, where the contractile machinery is arranged in a fixed paracrystalline array, the organization of actin in most nonmuscle cells is highly variable and dynamic. Actin filaments are not stable but in equilibrium with a cytoplasmic pool of monomers. Cycles of assembly-disassembly of filaments and reversible rearrangements of filaments into subcellular structures such as networks or bundles can take place very rapidly at specific stages of the cell cycle or in response to external signals. In flattened, well-anchored cells, actin filaments are observed in highly organized

bundles or stress fibers that span the entire length of the cell. When these cells enter mitosis, important shape changes occur and the actin stress fibers are dissociated into a diffuse network of individual actin filaments which will form the contractile ring during cytokinesis. Conversely, the actin stress fibers are reestablished at the end of mitosis in early G₁ daughter cells. Many investigators have taken advantage of such a dynamic behavior of cytoplasmic actins to elucidate their cellular functions. Hence, treatment of cells with different drugs that specifically displace the cytoplasmic equilibrium between G-actin and F-actin has been extensively used and still constitutes a classical approach to investigate the functional involvement of actin in different cellular processes. Among these drugs, the most familiar are certainly cytochalasins, a family of metabolites excreted by various species of molds, which induce the disassembly of actin filaments (Brenner and Korn, 1979; Lin *et al.*, 1980; Schliwa, 1982) and phalloidin, a highly poisonous alkaloid produced by the toadstool *Amanita phalloides*, which stabilizes filamentous actin and favors the polymerization of actin monomers (Wieland, 1977; Wehland *et al.*, 1978; Paulin-Levasseur and Gicquaud, 1981). It is now clear from such a pharmacological approach that, in nonmuscle cells, actin filaments fulfill both contractile and structural functions, related to cellular motility and cytoarchitecture. They have been implicated in different cellular activities including amoeboid movement (Paulin-Levasseur and Gicquaud, 1981), intracellular transport (Lasek, 1981), determination and alteration of cellular shape (Tomasek and Hay, 1984), endocytosis (Salisbury *et al.*, 1980), exocytosis (Williams and Wolff, 1971) and cell-surface mobility (Gabbiani *et al.*, 1977).

The dynamic behavior of cytoplasmic actins, on the other hand, has made difficult the study of the mechanisms modulating their assembly and

organization in the cells. For instance, little is known about the mechanisms implicated in the nucleation of actin filaments *in vivo*. It has been suggested that anchoring proteins such as nonmuscle α -actinins (Mooseker and Tilney, 1975), spectrin/ankyrin proteins (Bennett, 1982; Blikstad et Lazarides, 1983) and their non-erythrocyte analogues (Burrige *et al.*, 1982; Glenney and Glenney, 1983; Nelson *et al.*, 1983) may serve in the nucleation of actin filaments on membranous structures such as plasma membrane, endoplasmic reticulum, mitochondria and nuclear membrane (Cohen and Foley, 1980; Bennett, 1982; Glenney and Glenney, 1983) but as mentioned above, membranes might well be insertion sites for actin filaments. Nonetheless, numerous studies have indicated that the organization and functions of cytoplasmic actins may, like in muscle cells, be dependent on interaction with other proteins. It has been established that cytoplasmic myosins possess actin-stimulated ATPase activity and it is believed that a sliding filament mechanism, analogous to that of muscle, operates nonmuscle motility (Cohen, 1979). Different accessory proteins have been shown to influence both the polymeric state of actin and the interaction of actin filaments with other cellular structures (for reviews see : Schliwa, 1981; Korn, 1982; Stossel, 1984). Those include proteins which bind monomers of actin and prevent filament formation (Lazarides and Lindberg, 1974; Reichstein and Korn, 1979). Other proteins can induce fragmentation of actin filaments into G-actin or short oligomers (Hasegawa *et al.*, 1980; Yin *et al.*, 1980). Some may also favor the parallel aggregation of actin filaments into bundles or their cross-linking into networks (Otto *et al.*, 1979; Cohen and Foley, 1980; Jockush and Isenberg, 1981). Association of actin filaments with microtubules or intermediate filaments via spectrin/ankyrin proteins has been suggested (Mangeat and Burrige, 1984). As cycles of polymerization-depolymerization and reversible reorganization of actin filaments into networks or bundles have been correlated to contraction-

relaxation of cytoplasmic actomyosins (Isenberg and Wohlfarth-Bottermann, 1976; Cōndeelis and Taylor, 1977; Hellewell and Taylor, 1979) and to changes in cellular morphology (Kane, 1979; Owaribe *et al.*, 1979), these accessory proteins may be critical in the expression of the functional properties of actin in nonmuscle cells.

Regulation

Regulation of the contractile and structural functions of actin is complex and certainly involves more than one mechanism. The role of Ca^{2+} , however, is by far the best documented. In vertebrate skeletal muscle, the interaction of actin with myosin (i.e. contraction) is modulated by Ca^{2+} via its binding to troponin C (Murray and Weber, 1974; Taylor and Amos, 1981). In vertebrate smooth muscle and nonmuscle cells, Ca^{2+} also controls motility through actin- and/or myosin-linked mechanisms generally mediated by calmodulin, a Ca^{2+} -binding protein structurally and functionally very similar to troponin C (Adelstein and Eisenberg, 1980; Hartshorne, 1982; Kakiuchi and Sobue, 1983). Furthermore, Ca^{2+} has been shown to influence the polymeric state of actin in nonmuscle cells via the Ca^{2+} -dependent activity of some accessory proteins (Yin and Stossel, 1979; Hasegawa *et al.*, 1980; Yin *et al.*, 1980; Glenney and Weber, 1981). For instance, recent studies have indicated that spectrin and spectrin-like proteins which are Ca^{2+} -dependent calmodulin-binding proteins may confer Ca^{2+} -sensitivity to the submembranous organization of actin and to the interaction of actin filaments with other cellular structures such as plasma membrane, microtubules and intermediate filaments (Glenney *et al.*, 1982a; Glenney *et al.*, 1982b; Lazarides and Nelson, 1982; Carlin *et al.*, 1983; Tsukita *et al.*, 1983; Mangeat and Burridge, 1984). These results are

of particular interest considering that interactions of actin filaments with microtubules and intermediate filaments may be essential to the functional integration of the cytoskeleton.

Many accessory proteins and actin structures, however, are insensitive to Ca^{2+} and must be regulated by alternative mechanisms. There is evidence that cAMP (cyclic adenosine monophosphate), which indirectly contributes to the regulation by Ca^{2+} through its effects on membrane and Ca^{2+} transport, may be directly involved in such alternative mechanisms by causing the activation of a cAMP-dependent protein kinase and the subsequent phosphorylation of actin accessory proteins (Dedman *et al.*, 1979).

MICROTUBULES

Microtubules are ubiquitous components of the cytoskeleton as well as of specialized structures such as mitotic spindle, flagella and cilia (Porter, 1966). In all cell types and species studied, they appear as hollow cylinders 25 nm in diameter and are primarily composed of α - and β -tubulin heterodimers. The two types of tubulins are closely related globular proteins which differ only slightly in their amino acid composition (Bryan and Wilson, 1971) and can be resolved as two bands, 55 000 MW, by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis due to differences in the charge on the molecules (Feit *et al.*, 1977). Numerous studies have recently emphasized the microheterogeneity of α - and β -tubulin subunits expressed in different cells and organisms. There is abundant evidence that, in all but the simplest eucaryotes (yeast), multiple genes code for different α - and β -tubulin polypeptides (Cleveland, 1983; Cleveland and

Sullivan, 1985). Tissue-specific expression of different tubulin genes has been demonstrated in *Drosophila* and in chicken (Havercroft and Cleveland, 1984; Raff, 1984). In other cases, heterogeneity has been shown to be generated by post-translational modifications of tubulin proteins subject to strict developmental and morphogenetic controls (Nath *et al.*, 1981; Kumar and Flavin, 1982; L'Hernault and Rosenbaum, 1983). The functional significance of this diversity in tubulin subunits is unclear. It has been proposed that different tubulins may be used to build functionally different types of microtubules but there is still no definitive evidence to support this idea originally termed the "multitubulin hypothesis" by Fulton and Simpson (1976). Moreover, like actins, tubulins are most remarkable in the degree to which their properties have been conserved, as judged both from the high homology of primary sequences and from virtually identical structural characteristics of microtubules in widely divergent species (Kirschner, 1978).

Assembly

In solution, the tubulin dimer is stabilized by its binding to one molecule of Mg^{2+} (magnesium) and two molecules of GTP (guanosine triphosphate). Under appropriate conditions, tubulin dimers can be induced to spontaneously assemble *in vitro* into threadlike structures or protofilaments composed of α - and β -subunits alternately aligned on a row. Microtubule walls are usually formed by the staggered arrangement of 13 such protofilaments side by side around a central core. As in the assembly of actin, nucleotide binding and hydrolysis appear to be of crucial importance during polymerization of tubulin. The tubulin dimer has two nucleotide binding sites, one of which is non-exchangeable and invariably contains GTP in some structural role (Weisenberg

et al., 1968; Spiegelman *et al.*, 1977). The exchangeable site can bind both GTP and GDP (guanosine diphosphate), but the dimer will only polymerize readily into microtubules if liganded with GTP (Weisenberg, 1972). When the tubulin dimer polymerizes, the GTP is hydrolyzed to GDP although the energy released by this hydrolysis is not necessary for assembly (Penningroth *et al.*, 1976). Experimental and theoretical studies have first suggested that nucleotide hydrolysis would allow the treadmilling of microtubules under steady state conditions *in vitro* (Margolis and Wilson, 1978). Most recently, GTP hydrolysis has been related to an alternative form of dynamics *in vitro* called "dynamic instability" where growing and shrinking populations of microtubules co-exist at steady state (Carlier *et al.*, 1984). According to this model, a GTP cap is necessary to stabilize a microtubule and its loss leads to depolymerization. The applicability of such *in vitro* models to living cells has been recently examined. Based on kinetic data, Kirschner (1980) has proposed that treadmilling would render free cytoplasmic microtubules highly unstable and could therefore be used by the cells to selectively favor the growth of microtubules attached at their minus end. Evidence in favor of this proposal has been obtained by several laboratories (Heidemann *et al.*, 1980; Euteneuer and McIntosh, 1981). Recent observations on interphase and mitotic BSC1 cells, on the other hand, have shown that behavior of individual microtubules *in vivo* can be both rapid and asynchronous as predicted by the dynamic instability theory (Schulze and Kirschner, 1986; Wadsworth and Salmon, 1986). Further studies will be necessary to elucidate the exact mechanisms of microtubule assembly *in vivo*.

Cellular Dynamics and Cytoplasmic Functions

The dynamic nature of microtubules and the broad spectrum of cellular structures in which they are involved suggest that the cellular level of tubulin as well as the growth of microtubules are controlled both temporally and spatially in the cells. Cellular level of tubulin synthesis appears to be regulated by an autoregulatory mechanism which measures the pool of unpolymerized tubulin subunits and which adjusts tubulin mRNA (messenger ribonucleic acid) levels in response to suboptimal levels of subunits (Ben-Ze'ev *et al.*, 1979; Cleveland *et al.*, personal communication; Rosenbaum *et al.*, personal communication). The control of microtubule growth is believed to be exerted by specialized foci termed MTOCs (microtubule organizing centers) (Pickett-Heaps, 1969), the most familiar are basal bodies and centrosomes (Brown *et al.*, 1983). In flagella and cilia, axonemes are constituted of a sheaf of nine doublet microtubules arranged in a ring around a pair of single microtubules. These highly ordered arrays of microtubules are built using the ninefold symmetry of basal bodies as a template and are further stabilized by various accessory proteins, making them relatively permanent structures. In most mammalian cells, microtubules are arranged into a complex network extending from a juxtannuclear focus to the cell periphery during the interphase (Weber *et al.*, 1975a; Weber *et al.*, 1975b). When the cells enter mitosis, this cytoplasmic network is dismantled and replaced by another assemblage, the mitotic spindle, which corresponds to a completely different arrangement of microtubules (Brinkley *et al.*, 1975; Brinkley *et al.*, 1981). Reciprocally, at the end of mitosis in late telophase-early G₁, spindle microtubules are disassembled and a cytoplasmic microtubule network is reorganized in each daughter cell (Weber *et al.*, 1975a; Weber *et al.*, 1975b). From electron microscopic observations, it has been

demonstrated that the interphase network and the mitotic spindle are usually organized from discrete structures such as centrosomes, spindle poles, and kinetochores of metaphase chromosomes (Brinkley *et al.*, 1981; Brenner and Brinkley, 1982).

Bornens and Karsenti (1984) have recently reviewed the morphological changes observed in the centrosome as a function of the cell cycle. Briefly, the centrosome which is most often closely associated with the nucleus consists of a pair of centrioles surrounded by an osmiophilic pericentriolar material and dense aggregates termed satellite bodies. Three major events characterize the centriole changes prior to mitosis: 1) nucleation of daughter centrioles during late G₁/early S phase; 2) elongation of daughter centrioles throughout S and G₂ phases; and 3) separation of both pairs of centrioles with the onset of mitosis. The pericentriolar material exhibits a parallel cyclic pattern: it increases in size during G₂ and separates as dense halos associated with the centrioles during prophase. Those cyclic coordinate changes occurring in the centrosome therefore generate two functional mitotic centers which serve as anchoring sites for microtubules that form the spindle apparatus. Through mitosis, the centrosomes are equally segregated to daughter cells. In this way, a template with the capacity for organizing microtubule arrays similar to those of the parent cell is contributed to each daughter cell. From *in vitro* assembly of microtubules on centrosomes, it is now clear that both cytoplasmic and spindle microtubules are nucleated on the pericentriolar material (Gould and Borisy, 1977). The contribution of centrioles to the organizing capacity of the centrosomes is thought to be both kinetic and informative (Pickett-Heaps, 1969) but remains a subject of controversy (Bornens and Karsenti, 1984).

Ultrastructural examination of the mitotic apparatus has also stressed the interaction of spindle microtubules with kinetochores of chromosomes. Based on assembly assays *in vitro* (Snyder and McIntosh, 1975; Gould and Borisy, 1978) and drug recovery *in vivo* (Witt *et al.*, 1980; De Brabander *et al.*, 1981a), kinetochores have been suggested to function as second major microtubule centres during mitosis. Although interesting, this proposal is quite problematic from a theoretical viewpoint. Numerous studies have demonstrated that all the microtubules of the half-spindle have the same intrinsic polarity, indicating that microtubules are attached by their plus end on the kinetochores. This problem has been recently overviewed by Pickett-Heaps *et al.* (1982) in a comprehensive critique of models and concepts concerning kinetochore functions.

In contrast to the static arrangement of microtubules in axonemes, cytoplasmic and spindle microtubules are not stable but are in dynamic equilibrium with a cytoplasmic pool of heterodimers (Inoué and Sato, 1967; Rubin and Weiss, 1975). The only striking exception is the microtubule array of animal midbodies which does not separate in half-spindle (Byers and Abramson, 1968) and may not undergo a dynamic subunit flux (Pickett-Heaps *et al.*, 1982). Consequently, exposure of cells to different treatments that alter the dynamic equilibrium of tubulin has become a classical approach in the investigation of the cellular functions of microtubules. Depolymerization can be obtained by exposure to low temperatures (Ostlund *et al.*, 1980), Ca^{2+} (Schliwa *et al.*, 1981) or different drugs such as colchicine (Wilson *et al.*, 1974), colcemid (Osborn and Weber, 1976) and nocodazole (De Brabander *et al.*, 1976). Increased polymerization can be induced by D_2O (heavy water) (Sato *et al.*, 1982) or the drug taxol (Geuens *et al.*, 1983; Herman *et al.*, 1983). Based on such an approach, microtubular structures have been implicated in both structural and motile

functions such as development and maintenance of cell morphology (Tucker, 1979), positioning of organelles (Wehland *et al.*, 1983), intracellular transport of molecules and organelles (Hyams and Stebbings, 1979), ciliary movement (Warner, 1979), and segregation of chromosomes during mitosis (Pickett-Heaps, 1969). Furthermore, the microtubule system has been shown to play an important role in the organization and function of the Golgi complex in different mammalian cells (for a review see Thyberg and Moskalewski, 1985).

There is considerable evidence that different accessory proteins may contribute by their association with microtubules to the expression of such diverse functions. ATPase activity of dynein in axonemes and of dynein-like proteins in mitotic spindle (Warner and Mitchell, 1980) transduces the chemical energy of ATP into mechanical force for ciliary movement (Satir *et al.*, 1981) and possibly for pole-to-pole separation during anaphase (Cande and Wolniak, 1978). The so-called high molecular-weight (or HMW) microtubule associated proteins, frequently referred to as MAP-1 and MAP-2, have been shown to co-purify with microtubules from different sources and promote their assembly *in vitro* (Bulinski and Borisy, 1980; Vallee *et al.*, 1984). These proteins co-localize predominantly with microtubules in living cells (Scherson *et al.*, 1984) and are believed to mediate the association of microtubules with other cellular structures such as membranous organelles (Sherline *et al.*, 1977), actin filaments (Sattilano *et al.*, 1981) and intermediate filaments (Bloom *et al.*, 1985).

Regulation

Among the different factors which alter the equilibrium between polymerized microtubules and soluble tubulin dimers, Ca^{2+} and MAPs are the

only ones that may play a physiological role on microtubule structures. Evidence supporting Ca^{2+} -dependency of microtubule processes *in vivo* has so far been circumstantial (Schliwa, 1980). Microinjection of Ca^{2+} into dividing sea urchin eggs has been shown to induce a transient reduction of spindle fiber birefringence (Kiehart, 1981). Likewise, ionophore-mediated Ca^{2+} influx has been reported to reversibly affect the microtubule system of cultured cells (Fuller and Brinkley, 1976) and helizoan axopodia (Schliwa, 1976). Numerous studies have further suggested that the effects of Ca^{2+} on microtubules are mediated by the Ca^{2+} -binding protein calmodulin (Cheung, 1980; Schliwa, 1980; Burke and De Lorenzo, 1981; Schliwa *et al.*, 1981). The presence of calmodulin has been described in basal bodies (Means and Dedman, 1980) and axonemes of cilia (Jamieson *et al.*, 1979). Immunolocalization of calmodulin in mitotic cultured cells has demonstrated the association of this protein with spindle poles, chromosome-to-pole microtubules and midbody microtubules (De Mey *et al.*, 1980; Means and Dedman, 1980; Willingham *et al.*, 1983). Phosphorylation of tubulin by Ca^{2+} -calmodulin kinase has been reported by several laboratories (Burke and De Lorenzo, 1981; Wandosell *et al.*, personal communication). These observations suggest a calmodulin-mediated regulatory role of Ca^{2+} on microtubule functions.

As mentioned above, MAPs can promote the assembly of microtubules *in vitro*. The functions of these proteins *in vivo* is still unclear. Schliwa *et al.* (1981) have shown that MAPs could prevent the Ca^{2+} /calmodulin-induced disassembly of microtubules in an *in vitro* cell system and suggested that these proteins could act as modulators in the regulation by Ca^{2+} . The recent report that MAP-2 is phosphorylated by a Ca^{2+} /calmodulin-dependent protein kinase (Schulman, 1984) has further raised the possibility that the cells may use such a

mechanism to confer Ca^{2+} -sensitivity to interaction of microtubules with other cellular structures (Selden and Pollard, 1983).

Cyclic nucleotides may also influence the assembly of microtubules in some cells. cAMP has been reported to promote microtubule polymerization in Chinese hamster ovary (CHO) cells (Rubin and Weiss, 1975) and some transformed cell lines (Puck, 1977; Tash *et al.*, 1980). In contrast, it has been shown that cAMP induces microtubule disassembly while cGMP (cyclic guanosine monophosphate) stabilizes microtubules in lung mastocytes (Kaliner, 1977). These observations have led to the suggestion that protein phosphorylation by cAMP-dependent kinase may play a role in the regulation of microtubule initiation and elongation (Jameson *et al.*, 1980; Tash *et al.*, 1980).

INTERMEDIATE FILAMENTS

First observed in the middle 1960s (Biberfeld *et al.*, 1965; Sternlieb, 1965; Ishikawa *et al.*, 1968), intermediate-sized filaments (10 nm in diameter) have been considered as sub-products of either actin filaments (7 nm) or microtubules (25 nm) for several years (Buckley *et al.*, 1978; Buckley *et al.*, 1979; Rubin *et al.*, 1979). It is now well established that these filaments are composed of a distinct class of proteins (for reviews see : Lazarides, 1980, and Lazarides, 1982). Unlike actin and tubulin which are highly conserved from cell to cell and species to species, intermediate filament proteins vary considerably in their biochemical and immunological properties, showing a remarkable tissue specificity (Bennett *et al.*, 1978). They have been grouped into five subclasses : 1) a single protein vimentin (58 000 MW) in mesenchymal cells and in cultured

cells; 2) a single protein desmin (52 000 MW) in muscle cells; 3) a complex group of about 30 keratins (40 000 - 70 000 MW), subdivided into acidic type I and neutral-basic type II keratins, in epithelial cells; 4) a single protein, glial fibrillary acidic protein (55 000 MW) in astroglia; and 5) a triplet of neurofilament proteins, NF-L (65 000 MW), NF-M (about 105 000 MW) and NF-H (about 135 000 MW) in neuronal cells (Lazarides, 1980; Steinert, 1981; Zackroff *et al.*, 1981; Lazarides, 1982; Steinert *et al.*, 1984; Weber and Geisler, 1984). There is strong evidence that the developmental transitions observed during differentiation are paralleled by changes in the expression of intermediate filament proteins (Osborn and Weber, 1982; Biehl *et al.*, 1985; Ngai *et al.*, 1985). In some instances, co-expression of two different intermediate filament proteins has been reported in the same cell, both *in vitro* and *in vivo*, suggesting that the distinction of cell type-specific subunit proteins may not be as simple as generally assumed (Franke *et al.*, 1979; Gard *et al.*, 1979; Yen and Fields, 1981). All intermediate filament genes studied to date appear to exist in only one copy per haploid genome (Quax *et al.*, 1983; Zehner and Paterson, 1983; Lehnert *et al.*, 1984; Lewis *et al.*, 1984; Marchuk *et al.*, 1984; Quax *et al.*, 1984; Bloemendal *et al.*, 1985; Krieg *et al.*, 1985; Zehner and Paterson, 1985). One exception is the human 67 000 dalton (type II) keratin gene, which may be present in more than one copy (Johnson *et al.*, 1985). Therefore, intermediate filament diversity most probably originates within the germ line and is not generated by somatic rearrangements. The reasons why cells manufacture such a diversity of proteins to produce filaments of indistinguishable morphology remains obscure.

Assembly

As the diversity of intermediate filament subunits became evident, the question of how so many different proteins can form morphologically identical filamentous structures appeared of great interest. Highly insoluble under physiological conditions, intermediate filaments are usually unraveled into substructures using denaturing conditions or, in the case of non-epithelial intermediate filaments, low ionic strength buffers containing EDTA (ethylene diamine tetra-acetic acid). These substructures spontaneously reassemble into filaments when conditions are brought to physiological levels. Intermediate filaments have proved refractory to the conventional techniques used to solve the structure of helical protein polymers such as low angle X-ray diffraction and image analysis of negatively stained or shadowed specimens observed by electron microscopy (Anderton, 1981). Recently, however, biochemical characterization of subdomains together with extensive sequence data obtained by protein chemistry or DNA (deoxyribonucleic acid) technology have greatly contributed to our understanding of structural properties of intermediate filaments (Quax-Jeuken *et al.*, 1983; Quax *et al.*, 1983; Ip *et al.*, 1985; Steinert *et al.*, 1985; Weber and Geisler, 1985).

Hence, Weber and Geisler (1985) have proposed a general topographical model of subunit structure that accommodates the morphological uniformity of intermediate filaments. The unifying theme of this model is a central α -helical rod domain of 311-314 amino acids which has a highly conserved secondary structure and is flanked by two terminal non- α -helical domains (head- and tail-pieces) of hypervariable sequence and length. The basis for the morphological uniformity of intermediate filaments appears to reside in the

conserved structure of the rod domain (Steinert and Parry, 1985; Weber and Geisler, 1985). The difference in the size and properties of intermediate filament proteins would be due almost entirely to the variability of the terminal domains (Steinert and Parry, 1985). These domains which are highly sensitive to proteolytic digestion are believed to protrude from the intermediate filament core and to be intimately involved in specifying the functions of intermediate filaments (Steinert *et al.*, 1980; Steinert *et al.*, 1983; Steinert and Parry, 1985; Steinert *et al.*, 1985). There is also some evidence that at least part of the terminal domains are required for assembly *in vitro*, a feature lost by the isolated rod domain (Geisler and Weber, 1982; Geisler *et al.*, 1982; Sauk *et al.*, 1984; Geisler and Weber, 1986).

Interestingly, a similar structure has been recently predicted for two typical components of the nuclear lamina of mammalian somatic cells, lamins A and C (McKeon *et al.*, 1986). Deduced from human cDNA (complementary DNA) clones, the amino-acid sequences of these proteins comprise a region of about 300 amino acids near their amino terminus that shares striking high homology with the rod domain characterizing the intermediate filament proteins. This region would be flanked by a non- α -helical domain at the carboxy terminus of the lamins. Together with the demonstration that the lamina of *Xenopus* oocytes consists of filaments with a diameter of 8-10 nm (Aebi, personal communication), these data suggest that the lamins may represent an unrecognized class of intermediate filament proteins.

Different *in vitro* studies have recently indicated that the formation of a tetrameric protofilament consisting of a pair of double-stranded coiled-coil subunits may be a critical step in the assembly of intermediate filaments (Geisler

and Weber, 1982; Weber and Geisler, 1984; Woods and Inglis, 1984; Ip *et al.*, 1985; Parry *et al.*, 1985). The way in which these coiled-coil structures would align to form the tetramer is uncertain but is most probably based on structural restrictions (Steinert *et al.*, 1985). Keratin intermediate filaments are obligate heteropolymers and their assembly requires one type I and one type II subunits (Steinert *et al.*, 1982a). In contrast, non-epithelial intermediate filaments are homopolymers, but they can form facultative heteropolymers among themselves (Henderson and Weber, 1981; Steinert *et al.*, 1981; Sharp *et al.*, 1982; Granger and Lazarides, 1983; Quinlan and Franke, 1983; Tokuyasu *et al.*, 1985).

Cellular Dynamics and Cytoplasmic Functions

While the biochemical characterization of intermediate filaments is progressing quite rapidly, little is known still about their cellular dynamics (i.e. sites of nucleation, interactions with other cellular structures, fate during the cell cycle) and their cytoplasmic functions. Resistant to extraction with non-ionic detergents or high salt concentrations (Anderton, 1981), intermediate filaments are the most stable components of the cytoskeleton (Lazarides, 1980; Lazarides, 1982). No equilibrium between polymerized and non-polymerized forms has yet been reported *in vivo*. It is likely that intermediate filament proteins are entirely assembled into filaments in the cytoplasm (Fulton *et al.*, 1980; Renner *et al.*, 1981; Blikstad and Lazarides, 1983). Consequently, the information obtained from *in vitro* assembly-disassembly studies of different types of intermediate filaments (Starger *et al.*, 1978; Zackroff and Goldman, 1979; Renner *et al.*, 1981; Steinert *et al.*, 1981; Stromer *et al.*, 1981) cannot be readily extrapolated to an understanding of the cellular dynamics of these structures. Moreover, no drug has been identified that exhibits a specific action on intermediate filaments. It is

therefore impossible to specifically investigate their function *in vivo* with pharmacological tools as it has been done extensively for microtubules and actin filaments.

Fortunately, immunofluorescence and electron microscopy have allowed the observation of the cytoplasmic distribution of intermediate filaments in many cells, particularly in well flattened cells. Generally, they appear to extend as a complex filamentous network between the nucleus and the cell surface. Based on these microscopic observations, it has been proposed that intermediate filaments might primarily function as a structural framework for the positioning of cellular organelles (Lehto *et al.*, 1978; Virtanen *et al.*, 1979; Woodcok, 1980; Staufenbiel and Deppert, 1982; Summerhayes *et al.*, 1983) and possibly for the transmission of signals from the cell surface to the nucleus (Metuzals and Mushynski, 1974; Goldman *et al.*, 1985; Jones *et al.*, 1985; Traub, 1985). Although intermediate filaments may appear at first as static structures, it is clear that they are also dynamic elements which can undergo important cytoplasmic rearrangements during different cellular processes. Welch *et al.* (1985) have shown that physiological stress causes a rapid aggregation of vimentin filaments in rat embryo fibroblasts. Eckert and Caputi (1985) have provided evidence of substantial reorganization of keratin filaments in PtK1 cells during shape changes and locomotion. Extensive reorganization of intermediate filaments has been reported to occur during mitosis in different cultured cells and appears to follow two basic scenarios. In both cases the interphase intermediate filament network is altered, either to form a cage-like structure surrounding the mitotic apparatus (Blose, 1979; Aubin *et al.*, 1980; Zieve *et al.*, 1980; Blose, 1981; Blose and Bushnell, 1982; Capco *et al.*, 1982; Celis *et al.*, 1983; Franke *et al.*, 1984; Celis *et al.*, 1985) or to transiently disintegrate into non-filamentous, electron-

dense amorphous structures (Horwitz *et al.*, 1981; Franke *et al.*, 1982; Jones *et al.*, 1985). Furthermore, anti-microtubule drugs have been demonstrated to induce a striking reorganization of intermediate filaments, particularly of vimentin type (Ishikawa *et al.*, 1968; Bennett *et al.*, 1978; Hynes and Destree, 1978; Starger *et al.*, 1978; Knapp *et al.*, 1983; Celis *et al.*, 1984).

Cellular mechanisms responsible for such rearrangements remain uncertain. The possible involvement of intermediate filament-associated proteins in the establishment of organized filament networks is a current area of research. There is now evidence that these proteins may function as linkers of intermediate filaments. They have been broadly subdivided into two categories: those bridging intermediate filaments with themselves and those associating intermediate filaments with surrounding cellular structures (Wang, 1985). Proteins of the first category comprise filaggrins which are responsible for lateral aggregation of filaments into bundles in epidermal cells (Dale *et al.*, 1978), paranemin (280 000 MW) and synemin (230 000 MW) which interact with intermediate filaments in muscle cells (Price and Lazarides, 1983), a protein of 66 000 MW found associated with all five classes of intermediate filaments in a variety of cells (Pruss *et al.*, 1981), and a protein of 50 000 MW, p 50 or epinemin, interacting with vimentin-type filaments only (Wang, 1985). Proteins of the second category include desmoplakin which links intermediate filaments to desmosomes (Thornell *et al.*, 1985), the actin-accessory protein spectrin which may link intermediate filaments to membrane (Mangeat and BurrIDGE, 1984; Thornell *et al.*, 1985), and the microtubule-associated protein MAP-2 which may mediate the interaction of intermediate filaments with microtubules (Bloom *et al.*, 1985).

However, the functional significance of cytoplasmic rearrangements of intermediate filaments as well as their interaction with other cytoskeletal proteins is not clear. It has been suggested that intermediate filaments may be involved in locomotion (Felix and Straüli, 1976; Eckert *et al.*, 1980; Dulbecco *et al.*, 1983), changes in cell shape (Goldman and Knipe, 1972; Horwitz *et al.*, 1981; Eckert *et al.*, 1980; Eckert and Caputi, 1985), intracellular transport of molecules and organelles (Wang *et al.*, 1979), cell response to adverse growth conditions (Welch *et al.*, 1985), adequate partitioning of chromosomes and organelles during mitosis (Aubin *et al.*, 1980; Capco and Penman, 1983), and formation of the midbody during cytokinesis (Capco and Penman, 1983). An organizational and functional relationship between intermediate filaments and microtubules has also been proposed and supported in different cultured cells by the coincident distribution of the two filamentous systems (Wuerker and Kirkpatrick, 1972; Geiger and Singer, 1980; Maro *et al.*, 1983) and the presence of intermediate filaments in close proximity with MTOCs (Goldman *et al.*, 1980; Celis *et al.*, 1984). Nevertheless, this relationship and the requirement for intermediate filaments in cellular functions are still questioned. The microinjection of intermediate filament-specific antibodies in a few permanent cell lines can induce the aggregation of intermediate filaments without affecting the distribution of microtubules or interfering with cell morphology, locomotion or mitosis (Gawlitta *et al.*, 1981; Klymkowsky, 1981; Lane and Klymkowsky, 1981; Lin and Feramisco, 1981). Considering the tissue-specific composition of intermediate filaments, such observations have led to suggestions that intermediate filaments may have roles in differentiated cells and tissues that are not evident in permanent cell lines (Klymkowsky, 1982).

Regulation

To allow extensive cytoplasmic rearrangements of intermediate filaments, cellular mechanisms must exist that overcome the structural stability of these cytoskeletal components. Unfortunately, very few studies have examined that question. Intermediate filament proteins are known to be subject to extensive phosphorylation by cAMP-dependent and -independent protein kinases (Lazarides, 1980; O'Connor *et al.*, 1981), and may be phosphorylated in some cells in a Ca^{2+} /calmodulin-dependent manner as well (Spruill *et al.*, 1983). However, the functional role of phosphorylation in the modulation of intermediate filament functions is not known. Celis *et al.* (1985) have examined the correlation between phosphorylation and transient disintegration of keratins during mitosis of He La cells. Their results indicate that phosphorylation is not directly implicated in keratin aggregation but may play a role in modulating the association of keratin filaments with other cellular structures. Likewise, Wang (1985) has suggested that phosphorylation of intermediate filaments may affect the binding interaction between intermediate filament-associated proteins and intermediate filament-core proteins. For instance, it has been shown that phosphorylation of the core-neurofilament protein NF-H influences its association with the neurofilament protein NF-L (Wong *et al.*, 1984).

The presence of Ca^{2+} -activated neutral thiol proteinases showing a high substrate affinity for intermediate filament proteins, particularly desmin and vimentin, has been reported in various mammalian cells (Traub and Nelson, 1981; Vorgias and Traub; 1986). It has been demonstrated that degradation of vimentin and desmin by these Ca^{2+} -activated proteinases proceeds in the form of a limited proteolysis which alters two known properties of these proteins : 1) the

assembly of 10-nm filaments and 2) the binding of the subunit proteins to nucleic acids (Nelson and Traub, 1983). Considering the fact that intermediate filaments are often associated with Ca^{2+} -sequestering membranes *in vivo* (Granger and Lazarides, 1982; Granger *et al.*, 1982; Ramaekers *et al.*, 1982; Tokuyasu *et al.*, 1985), Traub has suggested that limited digestion of intermediate filaments by these proteinases may occur in response to signals inducing an influx of Ca^{2+} into the cytoplasm and therefore may constitute an important regulatory mechanism of intermediate filament functions. Jones *et al.* (1985) have recently argued the possible involvement of such proteinases in the inclusion of intermediate filaments during mitosis but this proposal awaits further evidence.

LYMPHOCYTES AS A BIOLOGICAL SYSTEM

Lymphocytes represent the major functional group of white blood cells or leukocytes. Because of their essential role in the immune response of higher vertebrates, these cells have been the object of great scientific interest and of intensive research (for review see : Kaplan, 1979b; Hume and Weideman, 1980). They are responsible for the primary recognition of foreign molecules or antigens and also function as the specific effector cells in the elimination of infectious agents, grafts and tumours. Widely distributed in the body, these cells are found in large numbers in the blood, lymphatic fluid, lymph nodes, thymus, spleen and bone marrow. Lymphocyte populations are very heterogeneous in their embryonic origin, functional activities and cell surface properties. They have been broadly grouped into two major classes : T lymphocytes, thymus derived cells responsible for cell mediated immunity, and B lymphocytes, bone marrow derived cells which are primarily involved in the production of antibodies or

humoral immunity. The two classes are quite easily distinguishable by differences in the composition of their plasma membrane proteins. Two of the most useful markers are the Thy-1 glycoprotein, common to all T cells but not to B cells, and surface immunoglobulins (sIg) which are characteristic of B cells only. The use of antibodies against these markers has made it possible to remove or purify one or the other cell class from a mixed population and has therefore been important in the characterization of these cells.

When isolated from blood, spleen or lymph nodes, mixed populations of T and B lymphocytes consist of small non-dividing cells (G_0 cells), 5-8 μm in diameter, which have a large condensed nucleus and a scanty rim of cytoplasm with few organelles. In culture, G_0 cells remain quiescent, showing little metabolic activity. Upon addition of multivalent cell surface ligands, i.e. specific antigens or polyclonal mitogens, a proportion of these resting cells can be induced to undergo processes such as redistribution of cell surface receptors (patching and capping), stimulation, proliferation and development of specific effector functions. These processes have been extensively investigated not only because of their importance in the expression of lymphocyte immune functions but also because of their possible relevance to the understanding of cellular mechanisms involved in the transmission of signals generated at the plasma membrane.

Patching and Capping

Redistribution of cell surface receptors can be routinely observed upon exposure of B lymphocytes to anti-immunoglobulin (anti-Ig) antibodies at 37°C. sIg are the integral membrane homologues of soluble antibodies found in the serum and are membrane receptors of prime importance to B lymphocyte

functions. At 37°C, as first observed by Taylor *et al.* (1971), anti-Ig antibodies cross-link these specific receptors uniformly distributed on B lymphocytes surface, forming ligand-receptor complexes. Those complexes first redistribute spontaneously into patches on the cells, presumably by the passive diffusion of the cross-linked receptors in the plane of membrane, and then migrate by an energy-dependent mechanism towards one pole to form a cap over the centrosome region (Stackpole *et al.*, 1974; de Petris, 1975). The requirement for the initial cross-linking of the receptors by the ligand in patching and capping has been demonstrated by the use of monovalent antibody fragments. Such monovalent fragments, usually generated by proteolytic digestion of antibodies, still bind to the receptors but fail to induce their redistribution (Taylor *et al.*, 1971). On murine lymphocytes, the redistribution of sIg occurs very rapidly and is completed within 7 minutes after addition of the antibodies (Rogers *et al.*, 1981). Formation of the cap is usually followed by a period of increased cell motility and by the removal of ligand-receptor complexes from the cell surface by endocytosis.

The series of events described above is not peculiar to sIg but has been shown for other membrane molecules (Braun *et al.*, 1978a; Braun *et al.*, 1978b). Histocompatibility molecules, TL (thymus leukaemia) antigen, β_2 -microglobulin, concanavalin A (con A) and F_c receptors, and other surface determinants of lymphocytes have all demonstrated the capacity to aggregate in the membrane, when cross-linked by the appropriate ligands under the appropriate conditions. However, marked differences in the capping behavior of these various molecules have been reported and include variability in the site of cap formation, the kinetics, the efficiency (use of one or two ligands, fraction of responding cells), the response to drugs, and the relationship to cell motility (Schreiner and Unanue, 1977; Braun *et al.*, 1978a; Braun and Unanue, 1983). Several investigators have

suggested that such variability may reflect fundamental differences in the mechanisms involved in the redistribution of these various molecules, whereas others (Bourguignon and Singer, 1977) have argued that the differences observed are more quantitative than qualitative and, therefore, proposed that all capping processes occur by a basically similar mechanism.

• Mechanisms of Capping

The mechanisms underlying the capping process are not yet fully understood. sIg capping has certainly been most extensively studied. There is considerable evidence that sIg cap formation is essentially modulated by the interactions of cytoskeletal elements (for reviews : Weatherbee, 1981; Braun, 1983; Braun and Unanue, 1983). It is now generally accepted that actin filaments and their associated contractile machinery generate the motive force for the translocation of sIg. The initial evidence for such a direct involvement has been based on the effects of cytochalasins and Ca^{2+} -modulating agents on sIg cap formation. Cytochalasin B, which disassembles actin filaments, has been shown to cause consistently a partial and reversible inhibition of sIg capping (Taylor *et al.*, 1971; Unanue and Karnovsky, 1974; de Petris, 1975). It has been demonstrated that agents which perturb Ca^{2+} metabolism, such as the ionophore 23187 (Shreiner and Unanue, 1976a), and local anesthetics are potent inhibitors of sIg capping (Shreiner and Unanue, 1976a; Shreiner and Unanue, 1976b; Braun *et al.*, 1978b; Braun *et al.*, 1979; Woda *et al.*, 1980). Considering the regulatory role of Ca^{2+} in nonmuscle contractility, the inhibitory effects of these agents have been functionally related to the microfilament system. Such pharmacological data have been further supported by ultrastructural and immunological studies demonstrating that different contractile proteins, such as

actin (Gabbiani *et al.*, 1977), myosin (Braun *et al.*, 1978a), α -actinin (Geiger and Singer, 1979; Hoessli *et al.*, 1980), spectrin (Levine and Wilard, 1983; Nelson *et al.*, 1983), and calmodulin (Salisbury *et al.*, 1981; Nelson *et al.*, 1982), all accumulate underneath the capped sIg.

The participation of the microtubule system in sIg capping is less obvious and first became evident in studies of the effects of con A binding on the mobility of sIg. Con A is a plant lectin isolated from the jack bean which acts as a tetra~~valent~~ valent ligand for a wide variety of membrane glycoproteins and does elicit diverse responses at the cell surface of lymphocytes depending on the conditions (Bhalla *et al.*, 1979). At 37°C, the binding of high con A concentrations ($>5\mu\text{g/ml}$) does not lead to the aggregation of its own receptors and inhibits the antibody-induced redistribution of sIg in a process called "anchorage modulation" (Yahara and Edelman, 1972; de Petris, 1975; Henis and Elson, 1981). The inhibitory effect of con A can be reversed upon disruption of microtubules by colchicine (Yahara and Edelman, 1973; Yahara and Edelman, 1975) or their reorganization by the microtubule-assembly-promoting drug taxol (Paatero and Brown, 1982). These observations as well as the synergistic inhibitory effect of colchicine and cytochalasin B on sIg capping have led to the proposal that mobility of membrane receptors may be modulated by a cooperative mechanism involving microfilaments and microtubules. Using an improved fixation method for immunofluorescence, Rogers *et al.* (1981) have shown that microtubules are present at all stages of sIg redistribution in mouse splenic lymphocytes and that only subtle changes in their organization can be observed during cap formation. It now appears most likely that the microtubule system provides the spatial information to direct the microfilament-dependent movement of sIg. This concept is consistent with models suggested by Yahara and Edelman (1973) and

Bourguignon and Singer (1977), and has been further supported by the recent observations that pretreatment of lymphocytes with colchicine or taxol, before exposure to anti-Ig, disrupts the normal relationship between the position of the cap and the cytoplasmic localization of the Golgi/centrosome complex during sIg redistribution of mouse splenic lymphocytes (Rogers *et al.*, 1981; Paatero and Brown, 1982).

From electron microscopic observations, Zucker-Franklin *et al.* (1979) have first reported the presence of intermediate filaments in human lymphocytes and suggested a possible involvement of these structures in sIg cap formation. This suggestion has been recently supported by immunofluorescence observations demonstrating that vimentin-type intermediate filaments which are arranged as a meshwork in resting lymphocytes accumulate underneath the site of the cap during sIg redistribution in human and murine lymphocytes (Dellagi and Brouet, 1982). When lymphoblastoid T cells were treated with colcemid, their vimentin meshwork was reorganized into a ring-like structure encompassing the nucleus. Although not illustrated by micrographs, capping of β_2 -microglobulin on the surface of colcemid-treated cells would apparently result consistently in the reorientation of the drug-induced vimentin ring at the site of the cap. These data have led to the suggestion that the anchoring point for vimentin filaments is located within the uropod and may play a role in sIg cap formation. Similar observations have been reported by Bourguignon and Bourguignon (1981) for redistribution of con A on murine lymphocytes. The nature of vimentin involvement in the capping process is not known.

Studies of the mechanisms involved in redistribution of other surface receptors of lymphocytes are limited and often contradictory (Bourguignon and

Singer, 1977; Braun *et al.*, 1978a; Braun *et al.*, 1978b). F_c receptors and TL antigens behave similarly to sIg during patching and capping. These molecules redistribute rapidly upon interaction with a single ligand and their aggregation is accompanied by a coincident accumulation of contractile proteins to the area of the cap, by changes in cell shape, and by stimulation of motile activity. Capping of these molecules is most probably operated by the same mechanisms as in the case of sIg. In contrast, the behavior of receptors like H_2 and θ (Thy-1) alloantigens is significantly different. The redistribution of these molecules occur slowly and only if two ligands are used. Their aggregation does not elicit motile activity of lymphocytes (Schreiner and Unanue, 1975). Association of the caps with contractile proteins and mechanisms involved in the redistribution of these receptors are controversial. Bourguignon and Singer (1977) have reported the coincident accumulation of actin and myosin at the site of the cap during aggregation of H_2 and θ complexes, and proposed that interactions of these receptors with cytoskeletal elements may essentially modulate their redistribution. On the other hand, Braun *et al.* (1978a; 1978b) have failed to detect any relationship between the distribution of myosin and the site of H_2 caps, and suggested that the redistribution of these receptors may be primarily directed by a lipid flow dragging the complexes in the plane of membrane (Bretscher, 1976).

- Nature of Second Messenger in Capping

A question of prime importance to immunology and, in general, to cell biology is the nature of signals generated by the binding of the ligand on its surface receptors. The time course of sIg capping has led many investigators to consider the possible role of ions as second messengers. It has been demonstrated

that binding of anti-Ig to surface receptors induced an abrupt release of 20-30% of the exchangeable calcium pool into the extracellular medium within 2 minutes after the formation of ligand-receptor complexes (Braun *et al.*, 1979; Braun, 1983). Kinetic studies, modification of the extracellular ionic milieu, and experiments with La^{3+} (lanthanum) have further indicated that this release occurs through the mobilization of an intracellular Ca^{2+} store, and that sIg capping is insensitive to extracellular ionic milieu. As mentioned previously, substantial evidence implicates Ca^{2+} in the regulation of the three major filamentous systems of the cytoskeleton. The potential role of Ca^{2+} efflux as the discrete ionic signal influencing the modulation of sIg could be to coordinate the activity of the different cytoskeletal components so that an appropriate response is elicited.

Substantial evidence has related redistribution of sIg to motile activity of lymphocytes. The antibody induced aggregation of sIg into a cap does stimulate cell locomotion. Furthermore, during active locomotion, B cells spontaneously segregate sIg from other membrane proteins to form caps similar to those induced by antibodies (Schreiner and Unanue, 1976a; Yahara and Kakimoto-Sameshima, 1977). Based on this relationship between capping and motility, Unanue and Schreiner (1975) have suggested that lymphocyte response to the binding of anti-Ig may be mediated by non-ionic factor regulating cellular contractile activity such as cAMP. Indeed, cAMP has been found concentrated preferentially underneath sIg cap in B lymphocytes (Earp *et al.*, 1977). Agents known to increase intracellular levels of cAMP have been shown to cause a remarkable stimulation of lymphocyte receptor capping and to impair ligand-induced motility of lymphocytes (Butman *et al.*, 1981). Bourguignon and Hsing (1983) have further demonstrated that ligand-dependent and -independent

capping is accompanied by an increase in intracellular cAMP and by the coincident accumulation of adenylyl cyclase at the site of cap formation. As in the case of Ca^{2+} , cAMP has been implicated in multiple regulatory pathways of the cytoskeleton and could therefore play an important role in the coordination of cytoskeletal activities during the capping process.

- Biological Function of Capping

The capping process has been observed not only in lymphocytes but also in a variety of cell types, and it may be a general property of animal cells (Sundquist, 1972; Albertini and Anderson, 1977; Bourguignon and Singer, 1977; Bourguignon and Rozek, 1980; Heath, 1983). The functional significance of this process, however, is not known. Redistribution of surface receptors appears to be essential to some immune function such as phagocytosis by polymorphonuclear leukocytes (Oliver *et al.*, 1974) and lymphocyte-mediated cytotoxicity. The relevance of sIg capping to the initiation of antibody secreting response of B cells is controversial (Greaves *et al.*, 1974; Roberts and La Via, 1976; Schreiner and Unanue, 1976c; Pozzan *et al.*, 1981). It is interesting to point out that immune deficiencies exhibited by patients with chronic lymphocytic leukaemia (CLL), Hodgkin's disease or one of several non-Hodgkin lymphomas are characterized by abnormalities in redistribution of Ig receptors (Cohen, 1975; Splinter *et al.*, 1979; Zucker-Franklin *et al.*, 1979). However, as a defect in capping may reflect a general impairment of membrane properties, many more investigations have to be performed before the relationship between such abnormalities in receptor redistribution and the inability of affected cells to properly mature and develop immune functions can be defined.

Stimulation

Polyclonal mitogens or specific antigens can also induce lymphocytes to enter the cell cycle and to progress through blastogenesis (G_1), DNA synthesis (S phase) and mitosis as a sequence of morphological and biosynthetic changes (Oppenheim and Rosenstreich, 1976) (see Figure 1). This series of events termed stimulation is of great experimental value since it mimicks the normal differentiation of lymphocytes into competent immune cells. Lymphocyte stimulation has been extensively used as a model system to understand the general phenomenon of cellular differentiation and the mechanisms triggering lymphocyte stimulation still constitute a major area of investigation.

Lymphocytes *in vivo* respond to antigens according to a high genetic specificity so that only a small number of cell clones are stimulated by a single antigen. The *in vitro* study of lymphocyte stimulation has been facilitated by the use of polyclonal mitogens. These substances can elicit a less specific response and stimulate a significant proportion of B or T cell clones in a lymphocyte population. Even though the stimulation is less specific, it leads to the same series of events that characterizes clonal stimulation *in vivo*. The plant lectin con A has been most commonly used to induce *in vitro* stimulation of murine lymphocyte populations. This lectin binds to B and T cells but directly stimulates only certain clones within the T lymphocyte sub-population (Greaves and Janossy, 1972). The mechanisms involved in the subsequent indirect stimulation of other T and B cells in con A responding populations are not fully understood (Owens and Kaplan, 1982). They implicate the releasing of soluble factors (e.g. interleukins or ILs) and/or the establishment of cell-cell interactions. The

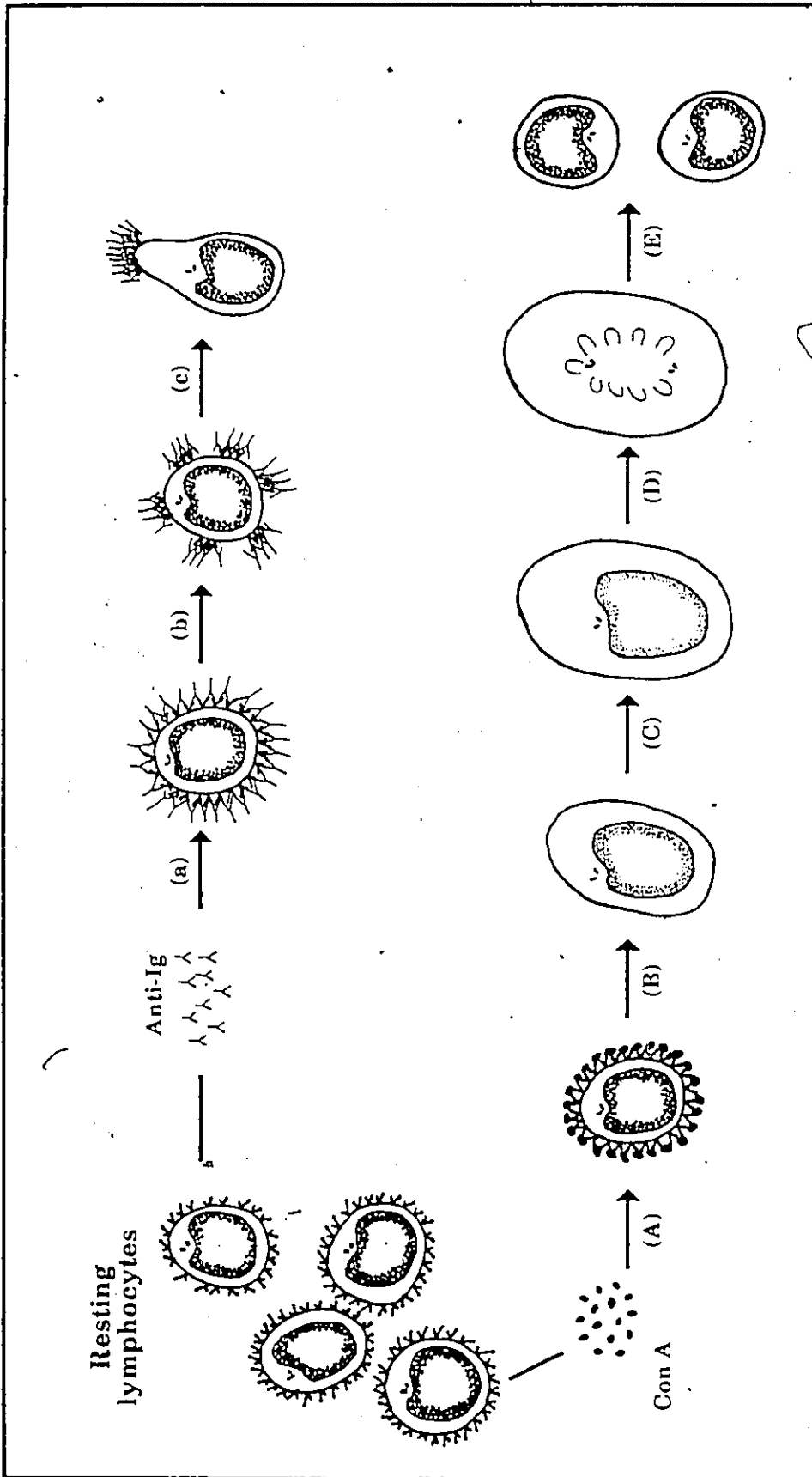


Figure 1 Schematic representation of sIg redistribution and Con A stimulation in mouse splenic lymphocytes. Addition of anti-Ig to cultures of mixed populations of lymphocytes results in: a) formation of ligand-surface receptor complexes on B lymphocytes; b) rapid (2 min) redistribution of the complexes into patches (capping); c) migration and aggregation of the patches over the centrosome region (capping). Addition of Con A results in: A) cross-linking of surface receptors by the lectin; B) biochemical and structural changes accompanying cell size increase; C) DNA synthesis; D) mitosis; and E) cytokinesis.

asynchronous response of lymphocyte populations to con A is most probably in part related to the delayed response of indirectly stimulated cells.

- Triggering of Stimulation

The critical events responsible for the progression of lymphocytes from G_0 through the cell cycle remain to be established. Upon addition of con A to lymphocyte cultures, the first critical event is undoubtedly the cross-linking of receptor molecules by the mitogen on the surface of T cells but neither rapid redistribution nor internalization of the complexes appear as obligatory requirements for mitogenic stimulation (Edelman *et al.*, 1973; Gunther *et al.*, 1974; Resch, 1976; Stenzel *et al.*, 1978). In fact, there is generally a negative correlation between the capacity of lectins to induce rapid (< 30 minutes) cap formation and their mitogenic action (Pozzan *et al.*, 1981). It is also well established that prolonged periods of contact with the mitogen are necessary for commitment of lymphocytes to DNA synthesis. If the saccharide α -methyl-D-mannoside (α MM) is used to competitively remove con A from the lymphocyte surface at early times following mitogen addition, subsequent DNA synthesis is completely abolished. The inhibition produced by α MM diminishes with increasing periods of contact with con A, and addition of the saccharide at 18-20 hours post stimulation (PS) has no effect on mitogenic response, indicating that commitment of lymphocytes does not occur as a punctual event but requires an induction period (Gunther *et al.*, 1974; Rudd *et al.*, 1979; Resch *et al.*, 1981). Observations that two pulses of con A at 0-3 hours and 15-18 hours of culture can stimulate lymphocytes to the same extent as prolonged periods of contact with the mitogen have led to the suggestion that commitment of the cells may be elicited by a dual mechanism implicating at least two signals delayed in time

(Toyoshima *et al.*, 1976). The requirement for con A induction period have been related to mitogen/antigen modulation of the so-called "lymphocyte autocrine pathway" which involves the expression of IL-2 receptors and the production and secretion of IL-2. Several studies have indicated that the progression of T cells from G₀ to DNA synthesis requires at least one initial signal resulting from mitogen binding and another later signal generated by the binding of IL-2 to its receptors (Cantrell and Smith, 1983; Cantrell and Smith, 1984; Meuer *et al.*, 1984). IL-2 is a small glycopeptide of about 15 000 MW produced by a subset of stimulated T lymphocytes, termed helper cells. Its secretion in human lymphocyte cultures reaches a peak in late G₁, 30 to 50 hours after the addition of the mitogen. IL-2 then binds and stimulates the proliferation of a subset of responsive stimulated T cells bearing IL-2 receptors on their surface (Stadler *et al.*, 1981)

• Early Events of Stimulation

Many cellular processes occur almost immediately after the addition of the mitogen to lymphocyte culture and precede the more striking biosynthetic and morphological changes generally referred to as the late events of stimulation. There is evidence that some of these early processes are related to DNA synthesis and may play an important role in the induction and regulation of lymphocyte responses to the mitogen.

For instance, an early influx of Ca²⁺ which is detected within minutes after addition of the mitogen has been shown to be a necessary event in the initiation and sustenance of mitogenic response (Raff *et al.*, 1975; Metcalfe *et al.*, 1980). Removal of Ca²⁺, using the chelating agent EGTA (ethylene glycol bis

(β -aminoethylether) N, N, N', N'-tetra-acetic acid), during the first 20 hours PS has been reported to inhibit consistently mitogen-induced DNA synthesis in a reversible manner (Whitney and Sutherland, 1972; Diamanstein and Ulmer, 1975). Mn^{2+} (manganese), which blocks Ca^{2+} currents in excitable cells, has been shown to prevent mitogenic responses if present during the initial 16 hours PS. These observations have led to the suggestion that the early influx of Ca^{2+} possibly corresponds to the initial signal generated by the binding of the mitogen and may act as second messenger in mitogenic stimulation. This point of view has been further supported experimentally by the demonstration that the Ca^{2+} ionophore A23187 can induce stimulation of lymphocytes in a Ca^{2+} -dependent manner and, more recently, by the observation that Cd^{2+} (cadmium), which does not affect the Ca^{2+} uptake of lymphocytes but potentially competes for intracellular site-binding molecules such as calmodulin, also prevents mitogenic response when added at early times PS (Scott *et al.*, 1985).

Resch and coworkers (Resch, 1976; Resch and Rode, 1979) have proposed that the early influx of Ca^{2+} may be implicated in the activation of the membrane enzyme acyl coA:lysolecithin acyltransferase, which results in the rapid turnover of the fatty acids of membrane phospholipids and the increased fluidity of the membrane observed in lymphocytes shortly after addition of the mitogen. According to these authors, increased membrane fluidity might in turn play a crucial role in mitogenic response of lymphocytes by affecting the activity of membrane bound ATPases such as the (Na^+, K^+) -ATPases, which are highly sensitive to their lipid environment. Activity of preexisting (Na^+, K^+) -ATPases has been shown to be enhanced in lymphocytes within minutes PS and to be essential to mitogenic stimulation. Inhibition of the early increase in the pump mediated Na^+ (sodium) efflux and K^+ (potassium) influx by the cardiac

glycoside ouabain has been reported to effectively prevent mitogenic stimulation (Kaplan, 1979a; Owens and Kaplan, 1979; Owens and Kaplan, 1980). It has been demonstrated that the early increase in ouabain-sensitive influx of K^+ is due to a rise in intracellular level of Na^+ and is not implicated directly in the mitogen-induced appearance of new ouabain-binding sites observed between 3 and 5 hours PS (Segel *et al.*, 1979; Severini *et al.*, 1986a; Severini *et al.*, 1986b). The early increase in K^+ influx is subsequently maintained through blastogenesis and is paralleled by an increased passive efflux of K^+ (Chandy *et al.*, 1984; De Coursey *et al.*, 1984; Matteson and Deutsch, 1984), such that intracellular levels of the ion may not be changed significantly through stimulation. In murine lymphocytes, the increase in (Na^+, K^+) -ATPase activity is accompanied by a sharp depolarization of the membrane potential within 2 hours PS, followed by a repolarization 7 hours later and a final hyperpolarization during the last 24-48 hours of stimulation. The time course of the hyperpolarization has been shown to correlate with the entry of lymphocytes into S phase (Kiefer *et al.*, 1980; Gerson and Kiefer, 1981). Although there is evidence that alterations of ion fluxes and membrane potential may be a common strategy in the stimulation of many eucaryotic cells (Epel, 1980; Robinson and McCaig, 1980), the relationship between these events and the subsequent progression of lymphocytes through the cell cycle remains unclear and has been discussed by Kaplan and coworkers (Kaplan, 1978; Kaplan, 1979a; Kaplan, 1979b; Severini *et al.*, 1986a).

In addition to changes in functions and properties of membrane, mitogens also induce rapid fluctuations of intracellular levels of cyclic nucleotides. The relevance of these fluctuations in the induction and regulation of the mitogenic response is difficult to assess as the results are often conflicting and controversial. For example, intracellular cGMP levels have been reported to

undergo a 10- to 50-fold increase within 20 minutes of mitogen addition by some investigators (Hadden *et al.*, 1972) while others have failed to observe any change (Metcalf *et al.*, 1980). Likewise changes in intracellular levels of cAMP, involving a transient increase within 30 minutes which is followed by a return to control levels 2 hours later and another increase during late G₁ which persists till mid-S phase, have been detected by some laboratories (O'Brien *et al.*, 1978; Wang *et al.*, 1978; Foker *et al.*, 1979) but have not been confirmed by others (Hadden *et al.*, 1972). In general, nevertheless, it has been found that agents which promote increase in intracellular levels of cAMP inhibit mitogenic stimulation (Diamanstein and Ulmer, 1975) whereas agents which decrease cAMP and/or increase cGMP in lymphocytes potentiate the mitogenic response (Hadden *et al.*, 1972). Although these experimental data are consistent with the Yin-Yang hypothesis of biological control proposed by Goldberg *et al.* (1973; 1974), the role of cyclic nucleotides in mitogenic stimulation has to be further ascertained (Byus *et al.*, 1977). For instance, the interdependency between Ca²⁺ and cyclic nucleotide levels remains to be defined. It is not at all clear whether cyclic nucleotide fluctuations observed in lymphocytes are a direct result of mitogen binding or secondary to Ca²⁺ influx. There is evidence that Ca²⁺ may regulate the levels of cyclic nucleotides by either inhibiting adenyl cyclases, activating phosphodiesterases or stimulating guanyl cyclases (Schultz *et al.*, 1973). Conversely, the observations that dibutyl cAMP inhibits mitogen-induced Ca²⁺ uptake while dibutyl cGMP enhances such an uptake suggest the possibility that intracellular levels of cyclic nucleotides may affect the mitogen-induced uptake of Ca²⁺ (Freedman *et al.*, 1985).

Lately, a few laboratories have noted the occurrence of early nuclear changes during mitogenic stimulation (Boumah *et al.*, 1984; Greer and Kaplan,

1986; Persson *et al.*, 1986). Repair of numerous DNA strand breaks, estimated to 3200 per diploid genome in unstimulated cells, has been reported to occur normally within 2 hours PS in mouse lymphocytes and to depend on the poly ADP (adenosine diphosphate) ribose synthetase system (Greer and Kaplan, 1986). Recent experimental data have provided evidence that DNA strand break repair is not required for the entry of lymphocytes into the cell cycle but is essential for the passage of G₁ cells into S phase. The purine analogue 8-azaguanine (8-AG) or the pyrimidine analogue 5-fluorouracil (5-FU) which would apparently increase the number of lesions and block the break repair process, have been shown to prevent irreversibly the passage of murine and human lymphocytes into S phase without affecting the cellular events that normally occur prior to that phase during mitogenic stimulation (Boumah *et al.*, 1984). Likewise, it has been reported that aphidicolin, an inhibitor of α -polymerase which prevents completely DNA strand repair without impairing ADP ribosylation, blocks the passage of murine lymphocytes into S phase but does not interfere with the early events of mitogenic stimulation (Greer and Kaplan, 1986).

Kelly (1985) has reported rapid increase, within 30 and 90 minutes, in the steady-state transcript levels of three proto-oncogenes - c-myc, c-fos and c-myb - in lymphocytes following the delivery of growth signals such as binding of mitogen or IL2. As the proteins encoded by these proto-oncogenes are localized in the nucleus, it is believed that they may play an important role in the control of lymphocyte progression through the cell cycle (Kelly, 1985; Persson *et al.*, 1986).

- Biosynthetic and Morphological Events of Stimulation

The progression of con A stimulated lymphocytes through the cell cycle is characterized by important biosynthetic activities. Protein synthesis is activated within 2 hours and continues to increase until 48 to 72 hours PS (Kay, 1968). As significant increases in incorporation of [³H]-uridine are detectable only 12 hours after addition of the mitogen, the early protein synthesis essentially results from the translation of preexisting mRNAs and may be partly due to mitogen-induced changes in the processing and cytoplasmic exportation of pre-formed mRNAs (Jagus-Smith and Kay, 1976; Mitchell *et al.*, 1978). A two fold increase in polyadenylation of heterogeneous RNAs (hnRNAs or pre-mRNAs) has been detected immediately after mitogen binding (Grunert and Schäfer, 1982). Likewise methylation of hnRNAs leading to much longer half-lives of RNA species has been reported to occur within 60 minutes PS (Grunert and Schäfer, 1982). Studies by Kay and coworkers (Jagus-Smith and Kay, 1976; Kay *et al.*, 1978) have further indicated that initiation rates of translation may be limited in unstimulated cells by a deficiency in initiation factors and/or the presence of initiation inhibitors. There is evidence that binding of the mitogen may induce higher levels of initiation factors in lymphocytes. tRNA^{met} (initiator-methionyl-transfer RNA) levels have been shown to increase immediately after mitogen addition and to correlate thereafter translation rates through blastogenesis (Cooper and Braverman, 1981). Observations supporting the presence of initiation inhibitors in unstimulated cells however have been circumstantial so far and await biochemical characterization of such substances (Burrone and Algranati, 1977; Thomas *et al.*, 1979).

In contrast to protein synthesis, there is a lag period of 6 hours before any change in RNA transcription can be observed in lymphocytes (Kay, 1968; Grünert and Schäfer, 1982; McCairns *et al.*, 1984). First appearance of polyadenylated (poly(A)⁺) transcripts in the cytoplasm of both murine and human lymphocytes is detected after 13 hours PS. Major transcription processes occur between 12 and 28 hours PS and are maintained till 48 to 72 hours PS. DNA replication commences within 24 to 36 hours PS as the cells enter S phase. Maximum incorporation of [³H]-thymidine occurs around 48-60 hours PS. Lymphocytes then proceed through cell division in a very asynchronous manner. The proliferative response rapidly dies away, and the cells return to a non-cycling state which may, as discussed by Ling and Holt (1967), correspond to a prestimulated state distinct from that of G₀ resting cells.

Concomittantly with those biosynthetic changes, the total cell size enlarges up to three-fold (within a range of 9-18 μm) its initial diameter. The ratio of cytoplasmic to nuclear volumes together with the number and activity of most cellular organelles increase. Nuclear structure is progressively modified: the nuclear volume increases, the condensed chromatin disaggregates, and the interchromatinic space enlarges markedly. Setterfield *et al.* (1983) have related these nuclear structural changes to the extent of stimulation. Unstimulated nuclei (morphotype I) are small, contain large masses of condensed chromatin, and show little interchromatinic nucleoplasm and small nucleoli. Fully stimulated nuclei (morphotype III) are big, contain small clumps of condensed chromatin distributed more or less uniformly throughout the extensively enlarged interchromatinic nucleoplasm, and usually show a structurally complex nucleolus. Partially stimulated nuclei (morphotype II) are characterized by structural changes intermediate between those of morphotypes I and III. As they

can be easily indentified in light or electron microscope sections, these morphotypes are useful parameters in attempting to relate structural and biochemical changes to functions in lymphocyte populations that undergo stimulation asynchronously.

Major changes in the expression and organization of cytoskeletal components have also been reported to occur during stimulation of lymphocytes. Resting lymphocytes are non-adherent, non-motile cells which exhibit a uniform spherical shape and possess a dense network of actin filaments underlying the plasma membrane (Fagraeus *et al.*, 1980). Upon mitogenic stimulation, lymphocytes rearrange their cytoplasmic actin to form numerous cell surface projections, so called filopodia or microvilli, each containing actin filaments organized in bundles (Otteskog *et al.*, 1982; Thortensson *et al.*, 1982). The appearance of projections on the surface of stimulated lymphocytes seems to correlate with the ability of the cells to actively locomote and spread on a surface. These structural changes are accompanied by increased levels of actin mRNAs in stimulated lymphocytes. Resting lymphocytes contain high levels of β - and γ -actin mRNAs, with β -actin mRNAs representing the major transcript species in these cells (KecsKemethy and Schäfer, 1982; McCairns *et al.*, 1984). As early as 6 hours after the addition of the mitogen, both β - and γ -actin mRNA levels are elevated by 2.5 fold. The γ -actin mRNA level then increases more than β -actin between 6 and 24 hours PS, resulting in a 3 fold increase in β -actin mRNAs, a 6 fold increase in γ -actin mRNA and therefore a reduced β/γ -actin mRNA ratio (McCairns *et al.*, 1984). Correspondingly, Waterhouse *et al.* (1983) have shown that the average concentration of actin in mouse splenic lymphocytes increases 2 fold within 24 hours of stimulation with the lectin con A.

The functional relevance of such changes in actin expression and organization for the mitogenic response of lymphocytes is not understood. Relatively high concentrations of cytochalasins have been shown to inhibit mitogenic stimulation while low doses have been reported to potentiate it (Medrano *et al.*, 1974; Greene *et al.*, 1976; Resch *et al.*, 1976). The use of cytochalasins in determining the involvement of actin filaments in mitogenic stimulation is fraught with difficulties considering the multiple effects of those drugs on stimulated lymphocytes. Apart from their effects on the microfilament system, cytochalasins have been demonstrated to induce accumulation of cAMP and to affect phospholipid turnover as well as amino acid and glucose transports in lymphocytes (Yoshinaga *et al.*, 1972; Greene *et al.*, 1976; Resch *et al.*, 1976; Hume *et al.*, 1978). The pharmacological approach is therefore not sufficient here and may not be appropriate to elucidate the role of actin filaments in lymphocyte stimulation.

Extensive changes in the expression and extent of tubulin assembly have also been observed during mitogenic stimulation of lymphocytes. Resting lymphocytes display a sparse microtubule system radiating from the centrosome and running around the cell periphery. Increases in the numbers and length of microtubules extending from the centrosome have been observed during blastogenesis of human peripheral blood lymphocytes stimulated by phytohaemagglutinin (PHA) (Biberfeld, 1971) and of mouse splenic lymphocytes stimulated by con A (Rudd *et al.*, 1979). The basic features of microtubule behavior during mitosis have been described in rat thymic lymphocytes by Murray *et al.* (1965) using electron microscopy. From direct counts of microtubules on electron micrographs of serial sections through the centrosomal region of resting and stimulated mouse splenic lymphocytes, Schweitzer and

Brown (1984) have estimated that there is up to a 5-fold increase in the microtubule numbers during con A stimulation. These changes are accompanied by increases in tubulin mRNA levels (Kecskmethey and Schäfer, 1982; McCairns *et al.*, 1984). Resting lymphocytes show low levels of both α - and β -tubulin mRNAs. Upon stimulation, tubulin mRNA levels are elevated by 10 fold after 72 hours, with most of the increases occurring between 6 and 24 hours after the addition of the mitogen as the cells start to progress from G_0 to G_1 . These data correlate well with the increase in cellular tubulin content reported by Pipeleers *et al.* (1977) for PHA stimulated human lymphocytes and by Waterhouse *et al.* (1983) for con A stimulated murine lymphocytes. Using an *in vitro* assay, Schweitzer and Brown (1984) have further tested the tubulin assembly capacities of centrosomes from resting and stimulated murine lymphocytes. Their analysis demonstrates that the centrosomes from stimulated lymphocytes are significantly more capable of initiating the assembly of microtubules. This increased assembly capacity is reflected morphologically by changes in the distribution and numbers of satellite bodies which are the major sites of microtubule assembly at the centrosome.

The exposure of lymphocytes to drugs which induce microtubule disassembly, in particular colchicine and colcemid, have been extensively used to examine the functional involvement of microtubules in mitogenic response of lymphocytes. Numerous studies have indicated that microtubule disrupting drugs inhibit the entry of mitogenically stimulated lymphocytes into S phase (Sherline and Mundy, 1977) but few have attempted to provide evidence for a direct causal relationship between removal of microtubules and alteration of lymphocyte functions (Rudd *et al.*, 1979). As in the case of cytochalasins, a major problem in the use of microtubule disrupting drugs is the multiplicity of cellular

side effects caused by these compounds. There is indeed a voluminous conflicting literature about the effects of these drugs on mitogenic responses of lymphocytes known to precede S phase. In different reports, microtubule disrupting drugs have been shown to inhibit phosphatidyl inositol turnover (Schellenberg and Gillespie, 1977), amino acid uptake (Greene *et al.*, 1976; Resch *et al.*, 1981), RNA synthesis (Hauser *et al.*, 1976; Rudd *et al.*, 1979), protein synthesis (Resch *et al.*, 1981), as well as the morphological changes accompanying the passage of lymphocytes from G₀ into G₁ (Edelman, 1976). Other studies, however, have failed to confirm these observations (Resch *et al.*, 1977; Betel and Martinjse, 1976; Cuthbert and Shay, 1983). Thyberg (1984) has recently presented a comprehensive overview of the possible role of microtubules in initiation of DNA synthesis in lymphocytes and other cells. From the work of several laboratories (Rudd *et al.*, 1979; Cuthbert and Shay, 1983), it appears now that the role of cytoplasmic microtubules is not to allow binding of the mitogen and transmission of a mitogenic signal as proposed by early models of lymphocyte activation (Edelman *et al.*, 1973; Yahara and Edelman, 1973; Edelman, 1976). Rather, microtubules are believed to ensure the functional integrity of cytoplasmic components required for appropriate cell growth and entry into S phase. This concept is based on experimental evidence demonstrating that colchicine or colcemid treatments sufficient to disrupt microtubules in lymphocytes lead to extensive disorganization of other cytoplasmic components, such as fragmentation of the Golgi apparatus and displacement of the centrosome from a nuclear cleft towards the plasma membrane (Thyberg *et al.*, 1977; Rudd *et al.*, 1979). It is also consistent with kinetic data reported by Hall *et al.* (1982) which indicate that the main effect of colchicine on mitogenic response of lymphocytes is to decrease the rate of cell entry in S phase.

Two studies have used the microtubule assembly-promoting drug taxol as an alternative approach to determine the importance of microtubule organization in mitogenic stimulation of lymphocytes (Cuthbert and Shay, 1983; Brown *et al.*, 1985). It has been reported that taxol does not affect the morphological changes or DNA synthesis that occur in human peripheral blood lymphocytes stimulated with PHA (Cuthbert and Shay, 1983) or in mouse splenic lymphocytes stimulated with con A (Brown *et al.*, 1985). In both cases, however, taxol did inhibit proliferation of lymphocyte populations by arresting the stimulated cells in mitosis. Brown *et al.* (1985) have shown that, in both resting and stimulated mouse splenic lymphocytes treated with taxol, the normal submembranous array of microtubules is reorganized into one or a few large bundles extending from the centrosome. In these cells, the centrosome is displaced from its normal location near the nucleus towards the plasma membrane but remains associated with an apparently structurally intact Golgi apparatus. From their observations, these authors have concluded that all major events of mitogenic stimulation up to the first mitosis can occur in the presence of a highly reorganized microtubule system. They could not, however, exclude the possibility that microtubules may be important for mitogenic stimulation. In fact, it is possible that the centrosome/Golgi apparatus association may be essential to mitogenic stimulation and that the taxol-reorganized microtubule system by maintaining this association still plays the same role as a normal microtubule system.

Expression of vimentin during mitogenic stimulation has been previously examined only in human peripheral blood lymphocytes by Dellagi *et al.* (1983). According to this study, T cells stimulated by alloantigens or mitogens would retain an unmodified filamentous network of vimentin while

mitogenically stimulated B cells would undergo changes in vimentin expression. Using double immunofluorescence, the authors have observed that 70-80% of stimulated B cells exhibit a perinuclear vimentin aggregate with scattered stretches of vimentin arrays extending into the cytoplasm while the remainders (20-30%) do not display any detectable vimentin. From these observations, it has been concluded that B cells tend to lose expression of vimentin during their maturation into plasma cells. Possible involvement of the vimentin system in mitogenic response of lymphocytes has not been considered yet.

QUESTIONS POSED AND RATIONALE OF THE EXPERIMENTAL APPROACH

From the above description of lymphocytes as a biological system, it is evident that the different processes induced in these cells by the interaction of ligands with their surface receptors readily allow important questions about the dynamics and cytoplasmic roles of intermediate filaments to be addressed :

- 1° Based on structural and pharmacological evidence, vimentin filaments have been shown to be spatially related to the microtubule system in well flattened cultured cells. Is there such a relationship between the two filamentous systems in lymphocytes which are primary culture cells in suspension? What is the spatial fate of the vimentin system in relation to the important changes in microtubule organization that occur during mitogenic stimulation?

2° Because they form an extensive network between the nucleus and the plasma membrane during interphase in flattened cultured cells, intermediate filaments have been suggested to play a role in the cytoplasmic integration of organelles or in the transmission of signals. Is there a correlation between the organization of the vimentin system and the increase in numbers of organelles during mitogenic stimulation of lymphocytes? Is the vimentin system involved in the short-term response of B lymphocytes to binding of anti-Ig? Does accumulation of vimentin at the site of the cap, as reported by Dellagi and Brouet (1982), occur during spg redistribution in cells treated with anti-microtubule drugs which uncouple the normal relationship between the site of the cap and the position of the centrosome/Golgi complex? Is the vimentin system involved in long-term response of lymphocytes to binding of the mitogen con A? What is the organizational fate of the vimentin system in cells stimulated in the continued presence of colcemid or taxol?

3° Extensive rearrangements of intermediate filaments, particularly of vimentin-type, have been observed during mitosis in flattened cultured cells. What is the organizational fate of the vimentin system in relation to the microtubule patterns during mitosis of lymphocytes which are primary culture cells in suspension?

Using electron microscopy and immunofluorescence techniques, I have attempted here to answer these important questions. Experiments were carried out on unfractionated mixed populations of B and T lymphocytes from spleens of

male Balb/c mice. Observations carried out during the course of this research have often raised new questions. For instance, the investigation of the mitotic fate of vimentin filaments and microtubules in mouse splenic lymphocytes have revealed interesting features not previously described during division of cultured cells. In these cells, the vimentin system is aggregated and the centrosomes are relocated to a position near the midbody during cytokinesis. To determine if such features are peculiar to lymphocytes or of more general occurrence, I have performed a comparative study of the organizational fate of vimentin filaments and microtubules during division of several attached and suspension cultured cells as well as of mouse L cells grown attached or in suspension. The results of this comparative study which provide new information about the role of vimentin filaments and their relationship with the microtubule system during cell division are also presented.

MATERIALS AND METHODS

CELL CULTURES

Mouse splenic lymphocytes

Male Balb/c mice, eight to fourteen weeks old, were killed by cervical dislocation. Spleens removed aseptically were disrupted on a wire mesh screen in RPMI 1640 (Flow). The cells were then passaged several times through a 20 gauge needle to break cell clumps. The cell suspension was layered on ice cold fetal calf serum (Gibco) for 5 min to sediment tissue debris. The suspension was removed and spun at 450Xg for 5 min in an IEC clinical centrifuge. The pellet was treated with ammonium chloride (Fischer) (0.83% in distilled water) for 10 min at 4°C to lyse red blood cells. The suspension was underlayered with fetal calf serum and centrifuged for 5 min at 450Xg. The supernatant was discarded and the pellet (95% lymphocytes) was resuspended in complete medium consisting of RPMI 1640 supplemented with 10% fetal calf serum, penicillin (100 U/ml)-streptomycin (100 µg/ml) (Difco) and buffered at pH 6.9 with 20 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid). Murine lymphocytes were cultured in complete medium at a density of 2.5×10^6 cells/ml in flasks (Corning), which were sealed and incubated at 37°C. For better cell viability over long culture time, cells were supplemented every day with fresh complete medium (10% of total volume).

Bovine lymphocytes and other lymphoid cells

Retropharyngeal lymph nodes were excised from freshly killed cows at a local meat packing plant and kept on ice until used. Fat and muscle surrounding the nodes were removed prior to surface sterilization with 70% ethanol (15-30 sec.). Sterilized nodes were pinned and an incision was made down their length to expose the internal tissue. This tissue was removed and disrupted on a wire mesh screen in RPMI 1640. The cells were gently suspended using a pipette and centrifuged at 600Xg for 10 min. The supernatant was discarded and the pellet was resuspended in 10 ml of medium with antibiotics. The cells were centrifuged as above and the washing procedure was repeated at least twice. Bovine lymphocytes were cultured in flasks at a density of 5×10^6 cells/ml in RPMI 1640 supplemented with 10% fetal calf serum, penicillin (100 U/ml)-streptomycin (100 μ g/ml) and buffered at pH 6.9 with 20 mM HEPES.

Lymphoid cells lines were obtained from the American Type Culture Collection. Suspension cultures of mouse lymphoma EL4 cells were grown at a density of 1×10^6 cells/ml in α -MEM (Flow) supplemented with 10% fetal calf serum and penicillin (100 U/ml)-streptomycin (100 μ g/ml) (37°C, 5% CO₂). Suspension cultures of mouse myeloma SP2/0 cells were grown at a density of 1×10^6 cells/ml in DMEM (high glucose) (Gibco) supplemented with 10% fetal calf serum, 0.16mg/ml sodium pyruvate (Gibco) and 2×10^{-5} M 6-thioguanine (Sigma) (37°C, 8% CO₂).

Other Cell Lines

Mouse 3T3 fibroblasts were obtained from The American Type Culture Collection and cultured as monolayer in DMEM (Gibco) supplemented with 10% fetal calf serum, 0.16 mg/ml sodium pyruvate, penicillin (100 U/ml)-streptomycin (100 µg/ml) and 0.25 µg/ml fungizone (M.A. Bioproducts) (37°C, 5% CO₂).

Rat kangaroo PtK2 cells and mouse L cells were obtained from the American Type Culture Collection. PtK2 cells were cultured as monolayer in α-MEM supplemented with 10% fetal calf serum and penicillin (100 U/ml)-streptomycin (100 µg/ml) (37°C, 5% CO₂). Mouse L cells were either grown in suspension or as monolayer in α-MEM with 10% fetal calf serum and penicillin (100 U/ml)-streptomycin (100 µg/ml) (37°C, 5% CO₂).

Con A STIMULATION

Con A (Calbiochem) was made up at 1 mg/ml in RPMI 1640 and aliquots were kept frozen at -70°C. It was thawed out immediately prior to use. In all experiments, lymphocytes were precultured overnight prior to mitogen addition to ensure constant culture conditions. Con A was added to murine and bovine lymphocytes at final concentrations of 4 µg/ml and 5 µg/ml respectively.

The extent of stimulation was routinely assayed by measuring the incorporation of [³H]-thymidine (Amersham) (2 µCi/ml, 2 h), 48 h after the addition of the mitogen (Rudd *et al*, 1979). Increase in cell diameter and nuclear

morphotype were also used as complementary parameters to evaluate the extent of stimulation.

DRUG TREATMENTS

Stock solutions of 0.4 mg/ml colchicine (Sigma) and 1 mg/ml colcemid (Sigma) were prepared in RPMI 1640. Stock solutions of 5 mg/ml nocodazole (Sigma) and 10 mg/ml taxol (Natural Products Branch, Division of Cancer Treatment, National Cancer Institute) were made up in DMSO. All stock solutions were kept frozen in aliquots at -20°C until used.

For short-term treatments, resting or stimulated populations of lymphocytes were exposed to 4 $\mu\text{g}/\text{ml}$ colchicine for 6 h, 1 $\mu\text{g}/\text{ml}$ colcemid for 4 h, 10 $\mu\text{g}/\text{ml}$ nocodazole for 4 h, and 10 $\mu\text{g}/\text{ml}$ taxol for 1 h or 4 h. For long-term treatments, resting lymphocytes were cultured for 48 h either in the presence of drug (1 $\mu\text{g}/\text{ml}$ colcemid or 10 $\mu\text{g}/\text{ml}$ taxol) only or in the dual presence of drug and con A (4 $\mu\text{g}/\text{ml}$). In all experiments with nocodazole and taxol, control cultures were exposed to equivalent DMSO concentrations.

IMMUNOFLUORESCENCE

Capping experiments

Capping of sIg was induced by incubation of resting lymphocytes with 200 $\mu\text{g}/\text{ml}$ rhodamine isothiocyanate conjugated goat anti-mouse IgG (Cappel

Laboratories) for 7 min at room temperature. The cells were then rapidly processed for immunofluorescence staining of either tubulin or vimentin. For details of procedures, see Rogers *et al* (1981).

Immunofluorescence staining

Monolayer cells were grown at sub-confluent density on coverslips. Suspension cells were plated onto poly-L-lysine (Sigma) (0.1% in distilled water) coated coverslips. Immunofluorescence staining was routinely performed as described hereafter unless otherwise specified. Samples were briefly (30 sec) fixed in 3% paraformaldehyde (J.B. EM Services Inc.), permeabilized for 1 h with 1% Triton X-100 (Sigma) in a stabilizing buffer, post-fixed 10 min in 1% glutaraldehyde (J.B. EM Services Inc.), reduced in sodium borohydride (1 mg/ml in phosphate buffered saline (PBS)) as previously described (Rogers *et al*, 1981). For single indirect immunofluorescence staining, samples were then incubated 45 min with the primary antibody, washed three times for 4 min in PBS, incubated 45 min with the secondary antibody, washed three times for 4 min in PBS, counterstained with Hoechst 33258 and mounted in glycerol-PBS containing 0.1% p-phenylenediamine (Baker Chemicals Co.). For double indirect staining, samples were placed for 20 min in 0.15% gelatin in PBS and washed three times for 10 min in PBS before applying antibodies. Antibodies were then applied as follows: first primary antibody, 45 min; three washes for 4 min in PBS; first secondary antibody, 45 min; three washes for 4 min in PBS; second primary antibody, 45 min; three washes for 4 min in PBS; second secondary antibody, 45 min; three washes for 4 min in PBS. Samples were then counterstained with Hoechst 33258 and mounted for immunofluorescence.

In specified experiments, samples were rather fixed 15 min in ice cold 95% ethanol, air dried 20 min and then incubated with antibodies as described above for single indirect immunofluorescence staining.

The immunofluorescence staining of tubulin was carried out using a murine monoclonal antibody (at a dilution 1:200) prepared against tubulin isolated from flagellar axonemes of *Polytomella agilis* (Aitchison and Brown, 1986) or an antiserum (1:25) produced in rabbit against purified bovine brain tubulin (Conolly *et al*, 1977). The immunofluorescence staining of vimentin was performed using a commercial murine monoclonal antibody (1:10) against vimentin (Labsystems), a murine monoclonal anti-vimentin (1:75) generously provided by Dr. N. Marceau, Université Laval, or an antiserum (1:30) produced in rabbit against vimentin of mouse 3T3 fibroblasts (Federoff *et al*, 1983). Immunostaining of centrioles was carried out using a rabbit autoimmune serum (1:30) (Turksen *et al*, 1982). The human autoimmune antibodies to centromeres (1:40) were kindly supplied by Dr. M.J. Fritzler, University of Calgary.

The secondary antibodies used were : a fluorescein isothiocyanate conjugated goat anti-rabbit IgG (1:150) (Miles Yeda Ltd.); a rhodamine isothiocyanate conjugated goat anti-rabbit IgG (1:75) (Zymed Laboratories); a fluorescein isothiocyanate conjugated goat anti-mouse IgG (1:100) (Cappel Laboratories); a rhodamine isothiocyanate conjugated goat anti-mouse IgG (1:100) (Cappel Laboratories); and a fluorescein isothiocyanate conjugated goat anti-human IgG (1:60) (Cedar Laboratories).

Microscopy

Preparations were observed with a Zeiss Universal microscope (Carl Zeiss Inc.) equipped for epifluorescence and phase contrast optics. A mercury HBO 50W or a Xenon XBO 75 arc lamp with a proper combination of filters was used as the light source. Photomicrographic recording was performed on Illford XPI-400 film.

ELECTRON MICROSCOPY

Capping experiments

Capping of sIg was induced by incubation of resting lymphocytes with ferritin-conjugated goat anti-mouse IgG (Cappel Laboratories) at a final dilution of 1:10 for 7 min at room temperature. Cells were pelleted by centrifugation for 5 min at 450Xg and immediately processed for electron microscopy as previously described (Rogers *et al*, 1981).

Digitonin permeabilization of lymphocytes

Lymphocytes were : 1) washed twice with Hank's balanced salt solution (HBSS); 2) washed twice with HBSS-MEP (2 mM MgCl₂, 2 mM EGTA, 33 mM PIPES, pH 7.2); 3) permeabilized with 0.005% digitonin (Aldrich) in HBSS-MEP containing 0.25% glutaraldehyde and 1.3% PEG 6000 (polyethylene glycol), for 3 min; 4) washed with HBSS-MEP; 5) fixed with 1% glutaraldehyde in HBSS-MEP for 10 min; 6) rinsed with HBSS-MEP ; and 7) further permeabilized

with 0.005% digitonin in HBSS-MEP containing 1.3% PEG 6000 for 30 min. Samples were then washed with HBSS-MEP, washed again with 0.1M sodium phosphate buffer, fixed with 4% glutaraldehyde in 0.1 M sodium phosphate buffer for 30 min, post-fixed with 1% osmium tetroxide in 0.05 M sodium phosphate buffer for 20 min and then dehydrated and embedded as described previously (Rogers *et al*, 1981).

Intact cells

Intact cells were processed for electron microscopy as described previously (Rogers *et al*, 1981; Rudd *et al*, 1979).

Microscopy

Silver sections (60-70 nm thick) were cut using a Dupont diamond knife and a Sorvall Porter-Blum MT2-B ultramicrotome. Sections were stained with uranyl acetate (2% in 50% ethanol) and lead citrate (Reynolds, 1963). Observations were carried out using a Philips EM 201C electron microscope.


RESULTS

ORGANIZATIONAL FATE OF THE VIMENTIN SYSTEM AND ITS RELATIONSHIP TO THE MICROTUBULE PATTERN DURING MITOGENIC STIMULATION

Blastogenesis

Lymphocyte populations isolated from mouse spleens consist of uniformly small spherical cells (G_0 or resting cells) with a diameter of 5 to 7 μm (Fig. 2a). Morphologically, these cells are characterized by a scanty rim of dense granular cytoplasm with few organelles and a morphotype I nucleus (Setterfield *et al.*, 1983) which contains large masses of condensed chromatin in contact with the nuclear envelope and lacks obvious nucleoli (Fig. 2b). Addition of the mitogen con A to populations of resting cells results in an asynchronous stimulation. Sixty to seventy percent of the population responds to the mitogen and the cultures show a peak of DNA synthesis at 48 hours PS. As the responding cells enter the cell cycle and proceed through blastogenesis, the volumes of the cytoplasm and nucleus increase and the total cell size enlarges up to 10-18 μm in diameter (Fig. 3a). In fully con A-stimulated cells (with a diameter $>10 \mu\text{m}$) the density of the cytoplasm, which contains increased number of organelles, is reduced and the morphology of the nucleus presents the characteristics of the nuclear morphotype III (Setterfield *et al.*, 1983) with a structurally complex nucleolus and small clumps of chromatin dispersed throughout the greatly enlarged interchromatinic nucleoplasm (Fig. 3b).

Figure 2 Microscopy of resting mouse lymphocytes



a. Phase contrast of a resting lymphocyte. The nucleus occupies most of the cell volume. — 2 000 x — b. Electron micrograph illustrating the morphological characteristics of a resting cell. The nucleus (N) shows a type I morphology. The dense granular cytoplasm contains few organelles and the centrosome (C) is located in a nuclear cleft. — 24 000 x — c. Pattern of microtubules in the cell seen in (a) as revealed by immunofluorescence with the rabbit antiserum to tubulin. Microtubules radiate from the centrosome to the cell periphery. — 2 000 x — d. Electron micrograph showing a high magnification view of the centriolar region of the section presented in (b). A satellite body (arrowhead) is seen associated with one centriole and one microtubule (arrows) appears to terminate between the two centrioles. — 62 700 x

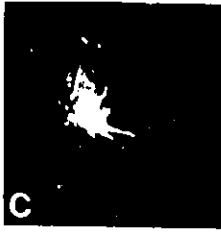
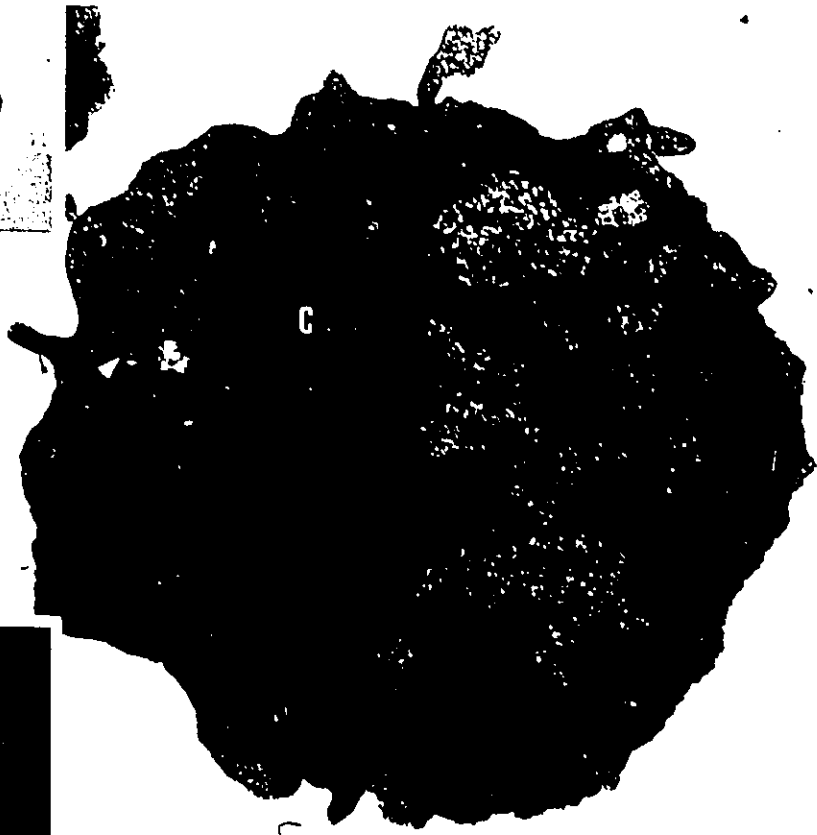
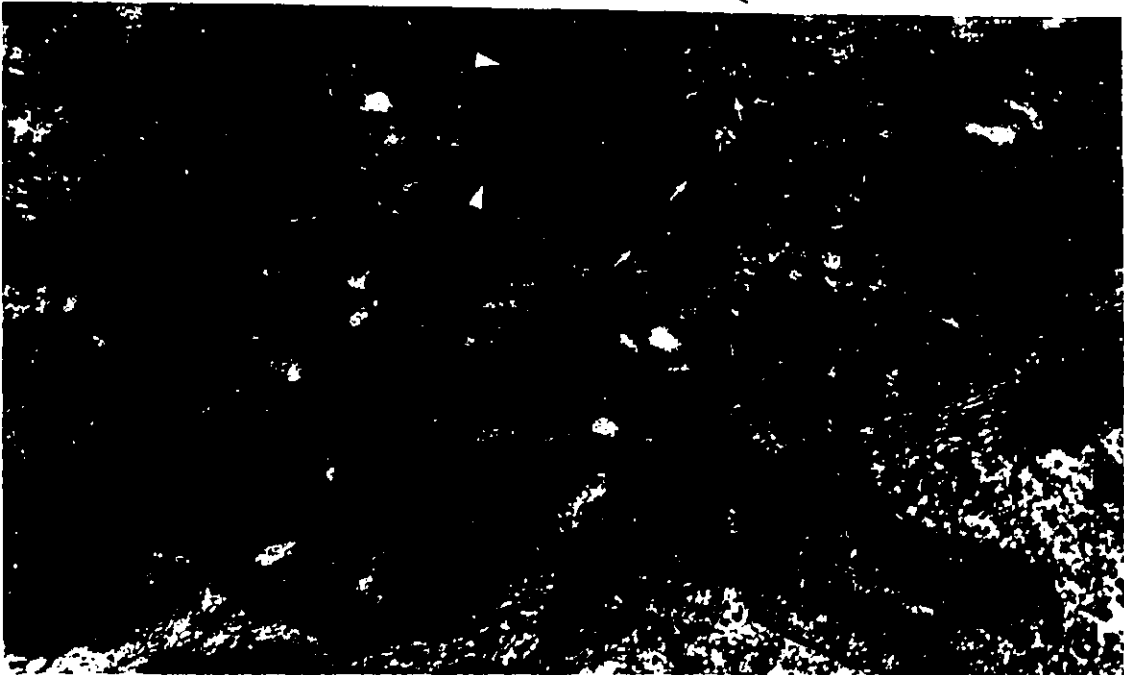
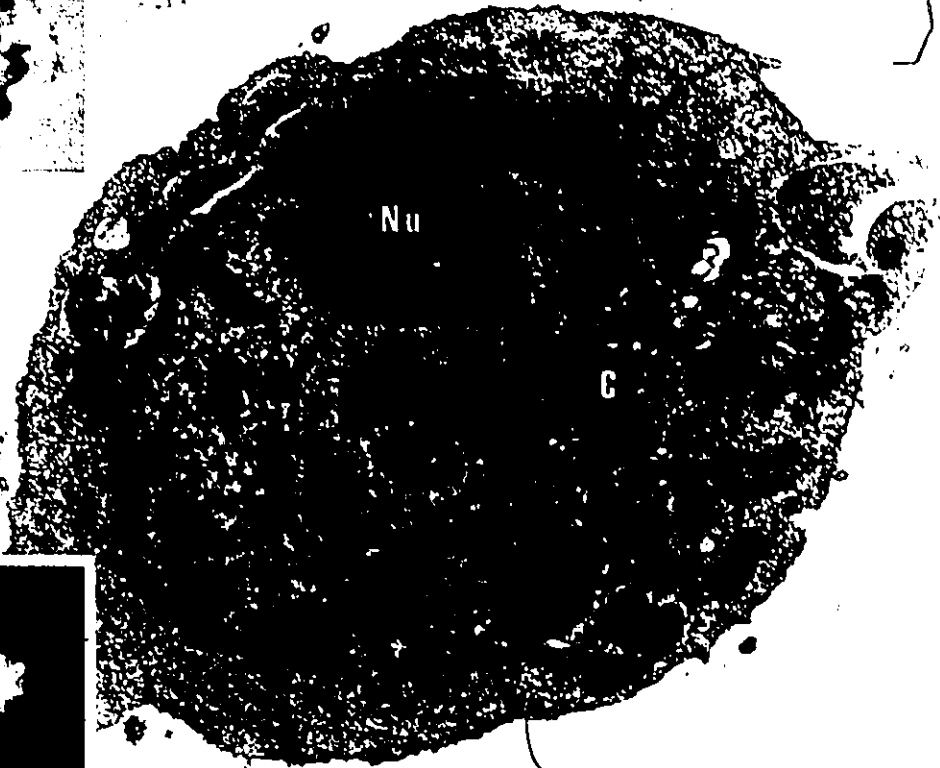


Figure 3 Microscopy of mouse lymphocytes stimulated with con A for 48 hours

a. Phase contrast of a stimulated lymphocyte. The total cell volume and the ratio of cytoplasmic/nuclear volumes have enlarged.

— 2 000 x — b. Electron micrograph illustrating the morphological characteristics of a stimulated cell. The nucleus (N) shows a type III morphology with a structurally complex nucleolus (Nu). The density of the cytoplasm is reduced and the numbers of organelles have increased. The centrosome (C) is located in a nuclear cleft.

— 15 400 x — c. Pattern of microtubules in the cell seen in (a) as revealed by immunofluorescence with the rabbit antiserum to tubulin. Numerous microtubules radiate from the centrosome to the cell periphery. — 2 000 x — d. Electron micrograph showing a high magnification of the centrosome region of the section presented in (b). Increased numbers of satellite bodies (arrowheads) are observed in the pericentriolar material. Numerous microtubules extend from the centrosome. Small intermediate filament arrays (IF) can be seen close to the mitochondria in this section. — 51 300 x

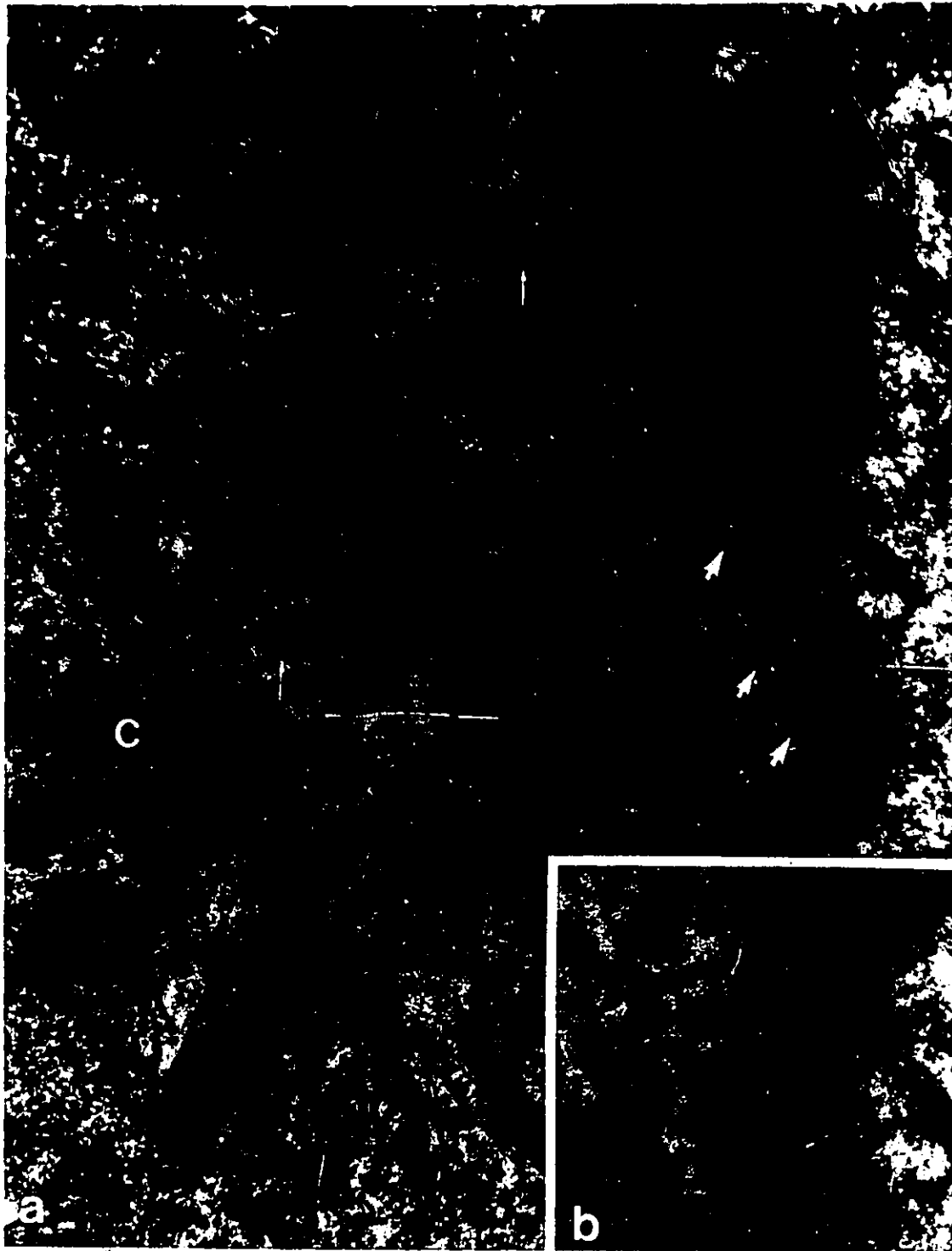


Resting and stimulated lymphocytes have one centrosome usually located adjacent to a nuclear cleft (Fig. 2b; Fig. 3b). Elements of the Golgi apparatus are localized near the centrosome and the majority of other organelles are also found on the centrosome side of the cells. The microtubule system radiates from the centrosome and extends near the plasma membrane around the cell periphery in both resting and stimulated cells (Fig. 2c; Fig. 3c). As previously described (Brown *et al.*, 1983), the microtubule pattern of resting cells is rather sparse compared to that of stimulated cells which show more numerous and longer microtubules. At the ultrastructural level, microtubules are not easily visualized in resting cells due to the limited numbers of microtubules and the density of the cytoplasm. They are most easily found in sections through the centrosomal region. Figure 2d shows a typical view of the centrosomal region of a resting cell. A few satellite bodies are associated with one of the two centrioles and one microtubule is seen that appears to terminate between the two centrioles. In contrast, the density of the cytoplasm is reduced in stimulated cells and large numbers of microtubules are usually observed in sections through the centrosomal region (Fig. 3d). Satellite bodies are also more numerous and appear to be the sites of attachment for many microtubules. As illustrated in figure 4, some of the microtubules extending from the centrosome towards the nucleus in stimulated cells seem to terminate on nuclear pores.

To observe the cytoplasmic distribution of the vimentin system in relation to the microtubule patterns in resting and 48-hour con A-stimulated lymphocytes, double immunofluorescence staining was performed using the rabbit antiserum to vimentin and the murine monoclonal anti-tubulin. (Figure 5 illustrates the overall changes in the organization of tubulin and vimentin observed during blastogenesis. In resting cells (Fig. 5a - c), vimentin is organized

Figure 4 **Microtubule system in a stimulated mouse lymphocyte**

- a. Electron micrograph of the centrosome (C) region of a stimulated lymphocyte illustrating the attachment of microtubules on satellite bodies and the apparent association of some microtubules with nuclear pores (large arrows). A few individual intermediate filaments (small arrows) are observed in the centrosome vicinity. — 51 300 x —
- b. High magnification of (a) showing the apparent association of some microtubules with nuclear pores. — 76 000 x



in a complex filamentous network which is partially coincident with the microtubule pattern and appears to extend from the centrosomal region. In the large fully con A-stimulated cells (with a diameter $> 10 \mu\text{m}$) (Fig. 5d - f), a filamentous vimentin network is not detected, but rather vimentin is observed as an aggregate located near the centrosome. A few short filamentous arrays were seen in association with the aggregate in some stimulated cells and a faint, diffuse staining was observed over other regions of the cytoplasm. Double immunofluorescence staining of resting and stimulated cells with the rabbit antiserum to vimentin and the commercial monoclonal anti-vimentin (Labsystems) showed identical patterns of organization (Fig. 7a - c; Fig. 8a - c).

In resting cells, a cytoplasmic network of vimentin was consistently detected regardless of the culture period preceding the processing for immunofluorescence (Fig. 6a - c). In stimulated populations stained 24 hours (Fig. 6d) or 48 hours (Fig. 6e) after addition of con A, a vimentin aggregate was observed only in the large fully stimulated lymphocytes (diameter $> 10 \mu\text{m}$) while a network of vimentin filaments was still present in partially stimulated cells (with a diameter intermediate between 6 and $10 \mu\text{m}$). These observations indicate that aggregation of the vimentin system is not induced by culturing the cells but rather represents a specific response of lymphocytes to the mitogen that occurs as a late event during blastogenesis.

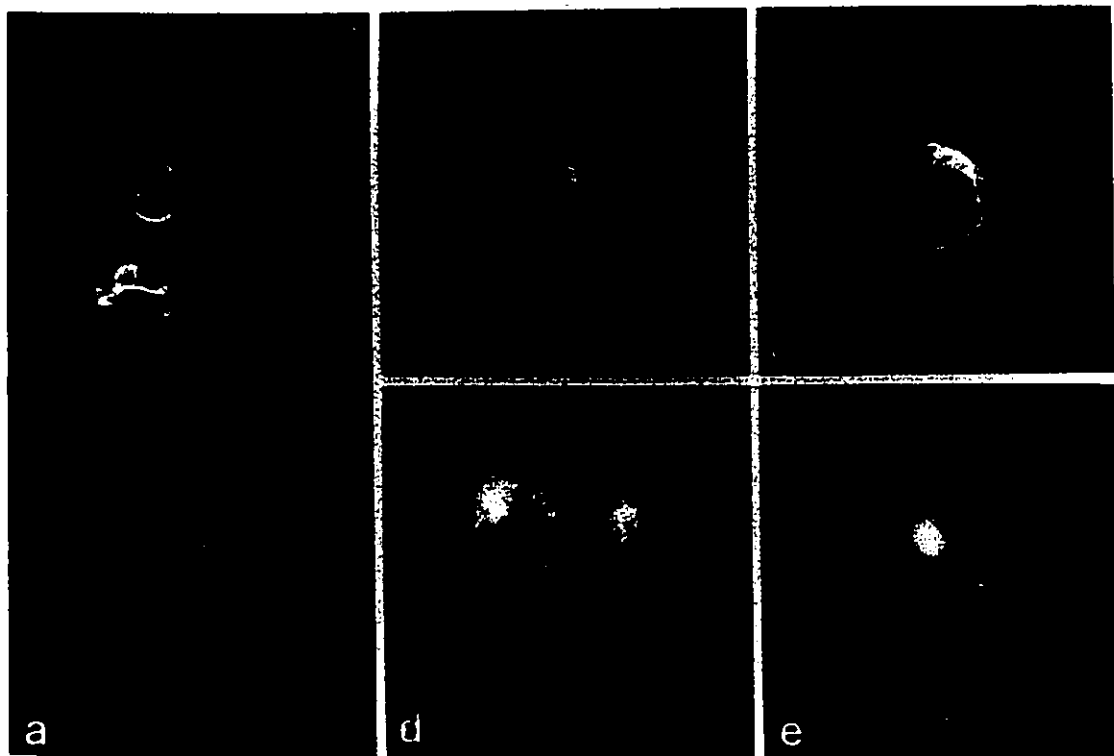
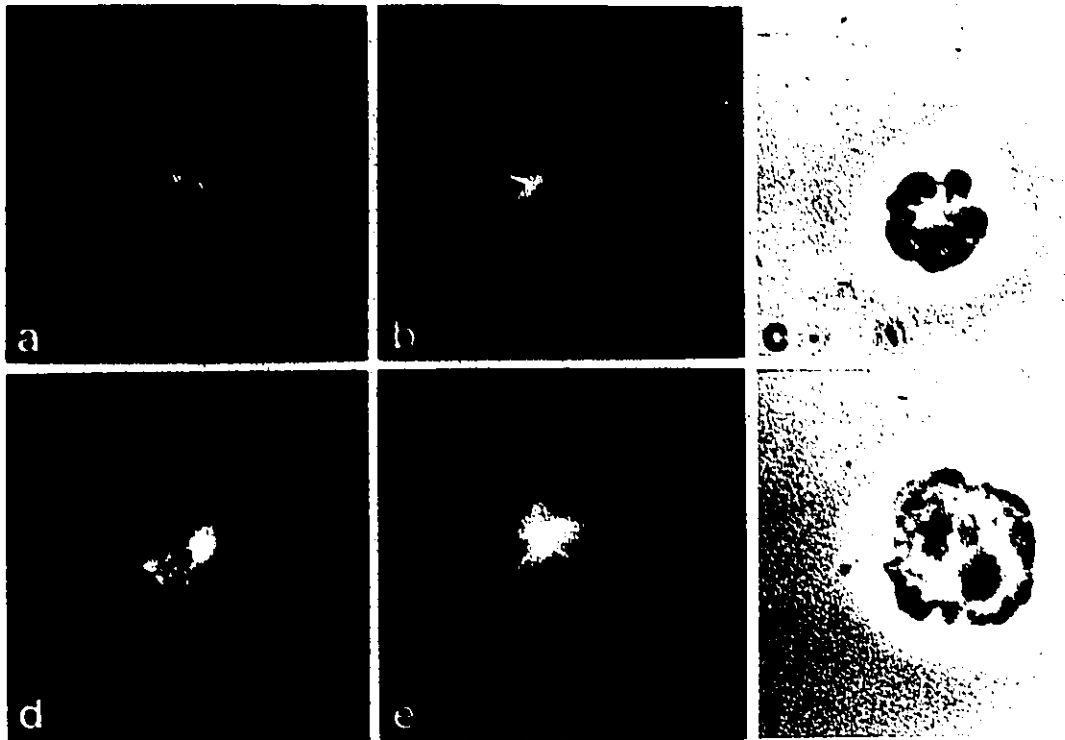
The cytoplasmic organization of the vimentin system in resting and stimulated cells was further examined at the ultrastructural level. As mentioned above, cytoskeletal components are not easily visualized by electron microscopy in the dense granular cytoplasm of resting cells. Only a few microtubules are usually found in sections of intact cells and intermediate filaments are obscured.

Figure 5 Organizational fate of vimentin and tubulin in resting and fully stimulated mouse lymphocytes

The resting and stimulated cells seen by phase contrast in (c) and (f) were double stained using the rabbit antiserum to vimentin (a, d) and the murine monoclonal anti-tubulin (b, e). In resting cells (a - c), vimentin forms a complex filamentous network which is partially coincident to the radial pattern of microtubules and appears to extend from the centrosome region. In stimulated cells (d - f), vimentin is observed as an aggregate located in the vicinity of the centrosome. — 2 000 x

Figure 6 Organization of vimentin in mouse lymphocytes after different intervals of culture in absence or in presence of con A

Patterns of vimentin were observed using the rabbit antiserum to vimentin in : a. freshly isolated resting lymphocytes; b. resting lymphocytes after 24 hours of culture; c. resting lymphocytes after 48 hours of culture; d. lymphocytes stimulated with con A for 24 hours; and e. lymphocytes stimulated with con A for 48 hours. Resting cells (a - c) exhibit a cytoplasmic network of vimentin filaments regardless of the culture period. Filamentous patterns of vimentin are retained in partially stimulated cells as illustrated by the cell at the top left in (d) and the cell on the right in (e) while vimentin is detected as an aggregate in the fully stimulated cell seen on the left in (e). — 2 000 x



To circumvent this problem, resting cells were gently permeabilized with digitonin to partially extract the cytoplasm prior to fixation. The structural organization of such extracted cells is well preserved (Fig. 7d), and intermediate filaments can be observed in arrays that are particularly evident in the nuclear cleft region (Fig. 7e). In contrast to microtubules, intermediate filaments were infrequently observed in small arrays or as individual filaments in sections of intact stimulated cells (Fig. 3d; Fig. 4a). Permeabilization with digitonin did facilitate the observation of microtubules but did not result in the detection of additional intermediate filaments in these cells. In sections of both intact and permeabilized cells a fibrogranular mass, which may correspond to the aggregate detected by immunofluorescence, was observed in the vicinity of the centrosome/Golgi complex (Fig. 8d, e). Intermediate filaments were sometimes seen at the margins of the mass but were not prominent (Fig. 9a, b).

Mitosis and cytokinesis

In populations of 48-hour con A-stimulated lymphocytes, approximately 2% of the cells are in mitosis. The typical aspect of a lymphocyte that has entered prophase is illustrated in figure 10. The nuclear envelope is intact and condensed chromosomes are found at the periphery of the nucleus. At this stage, the cytoplasmic microtubule network is dismantled and the duplication/separation of the centrosome which generates two mitotic centers is completed. Figure 11 illustrates different steps of the duplication/separation process as detected using the rabbit antiserum to tubulin. Shortly after duplication (Fig. 11a, b), the two microtubule centers are located inside the same nuclear cleft with one of them closely apposed to the nuclear surface and the other slightly displaced distally in the cytoplasm. Microtubules are preferentially

Figure 7 Organization of vimentin in resting mouse lymphocytes

The resting cell seen by phase contrast in (c) was double labelled with the rabbit antiserum to vimentin (a) and the commercial murine anti-vimentin (b). Identical staining patterns were detected with both anti-vimentins. — 2 000 x — d. Electron micrograph showing a resting cell extracted with digitonin (0.005%). Structural organization of the cell is well preserved. N, nucleus. C, centrosome. — 22 000 x — e. High magnification micrograph of a resting cell illustrating the presence of an array of intermediate filaments (IF) in the nuclear cleft region. — 74 100 x

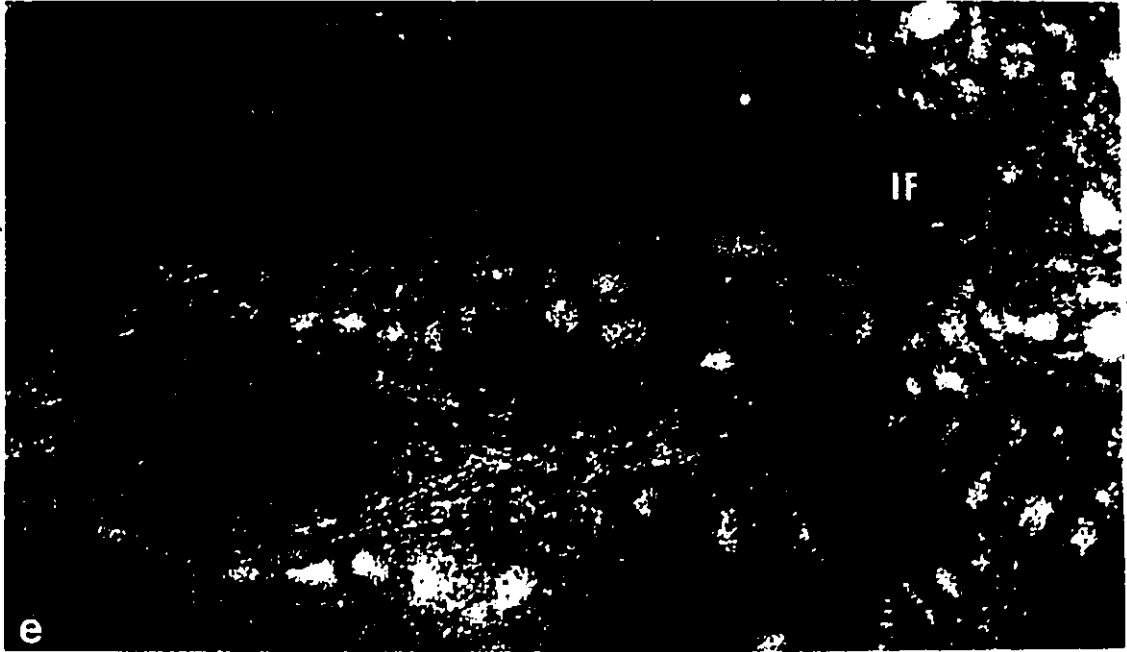
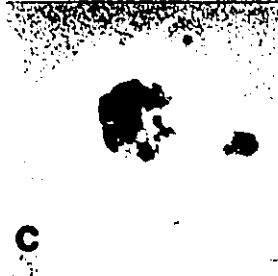
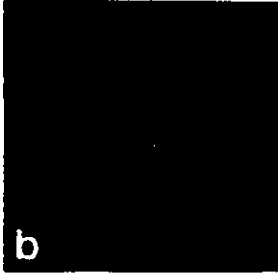
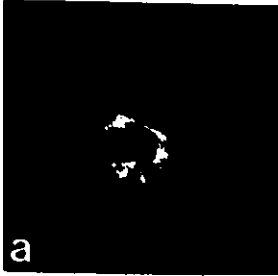


Figure 8 . Organization of vimentin in fully stimulated mouse lymphocytes

The fully stimulated cell seen by phase contrast in (c) was double labelled with the rabbit antiserum to vimentin (a) and the commercial murine anti-vimentin (b). Identical staining patterns were detected with both anti-vimentins. — 2 000 x — d. Electron micrograph of a stimulated cell showing a fibrogranular mass (M) located in the vicinity of the centrosome (C). N, nucleus.

Nu, nucleolus. — 14 000 x — e. High magnification micrograph of a stimulated cell illustrating the structure of a fibrogranular mass (M). Few intermediate filaments (arrows) are seen at the margin of the mass close to the Golgi apparatus. — 45 600 x

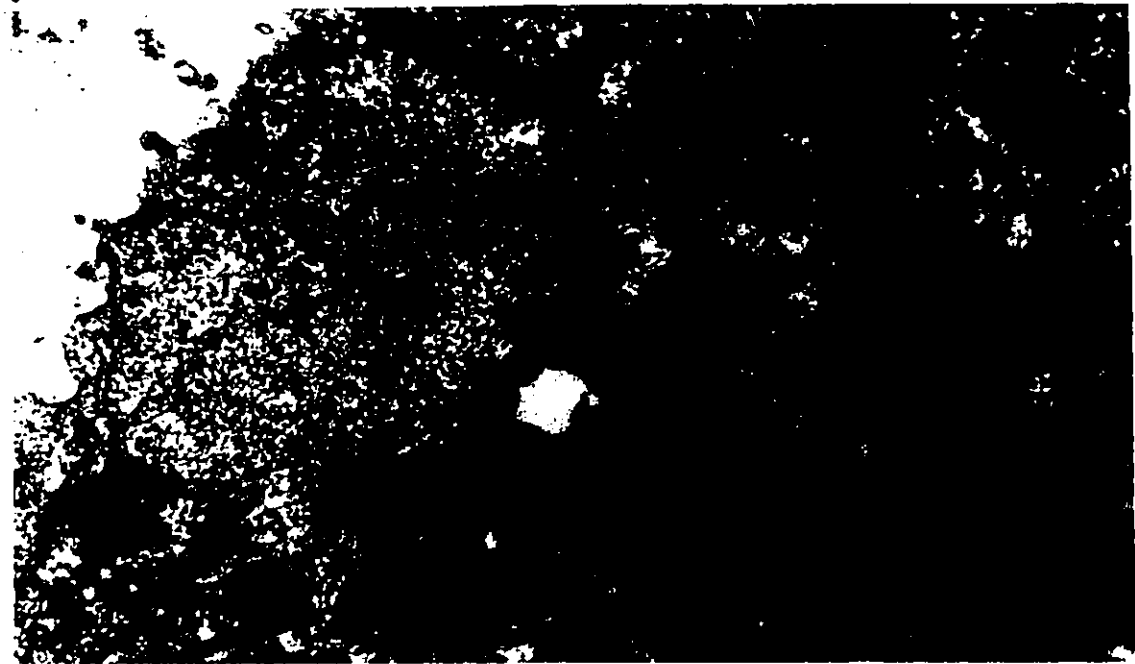
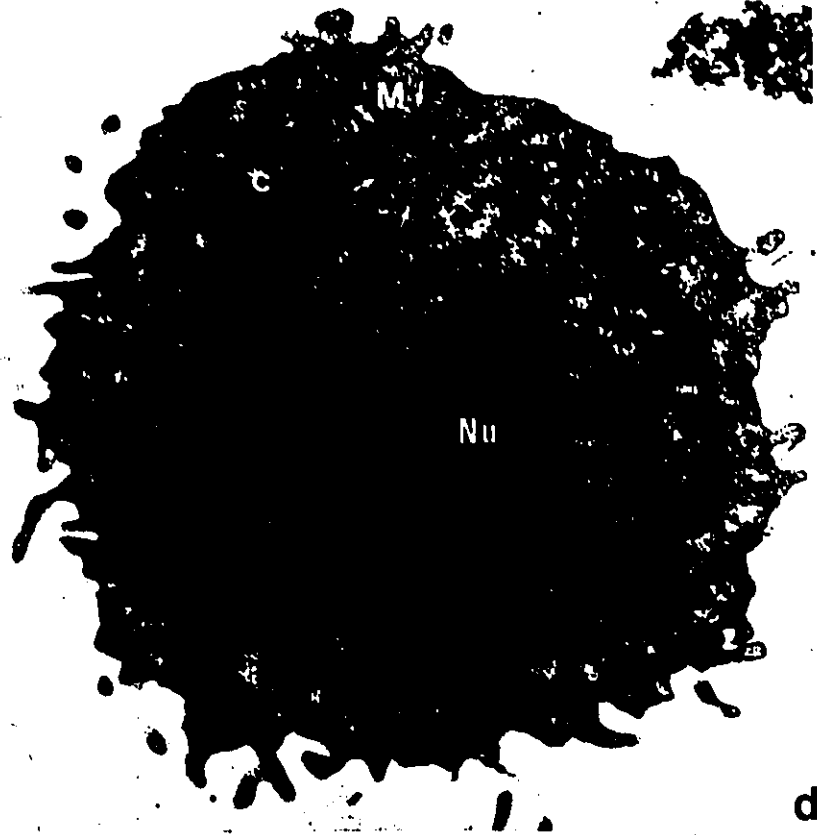
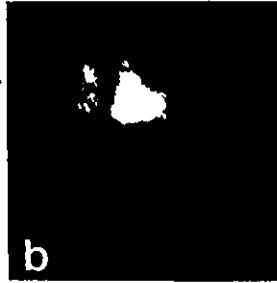
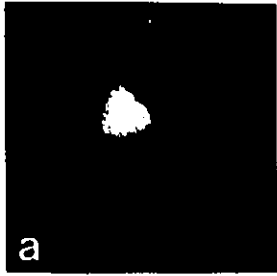


Figure 9 Electron microscopic observation of intermediate filaments in fully stimulated mouse lymphocytes

a. and b. Electron micrographs through the fibrogranular mass (M) observed in fully stimulated cells. Small arrays of intermediate filaments (arrowheads) are observed at the margin of the mass.

— 53 200 x

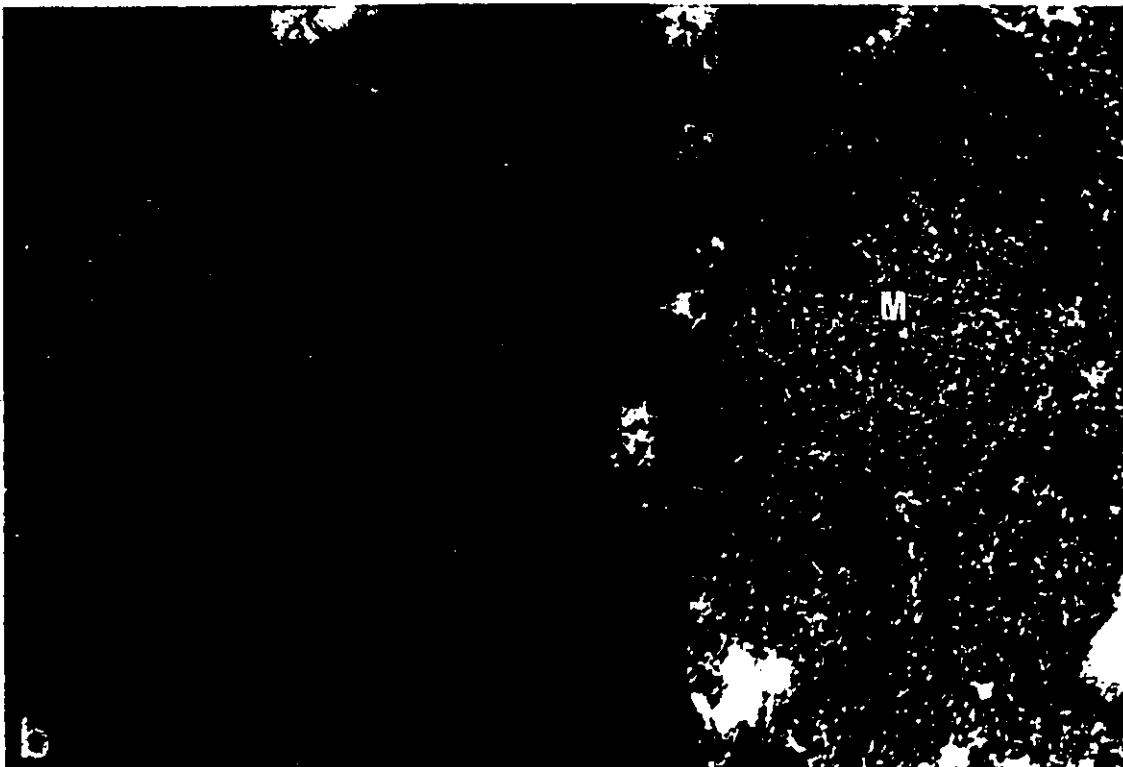


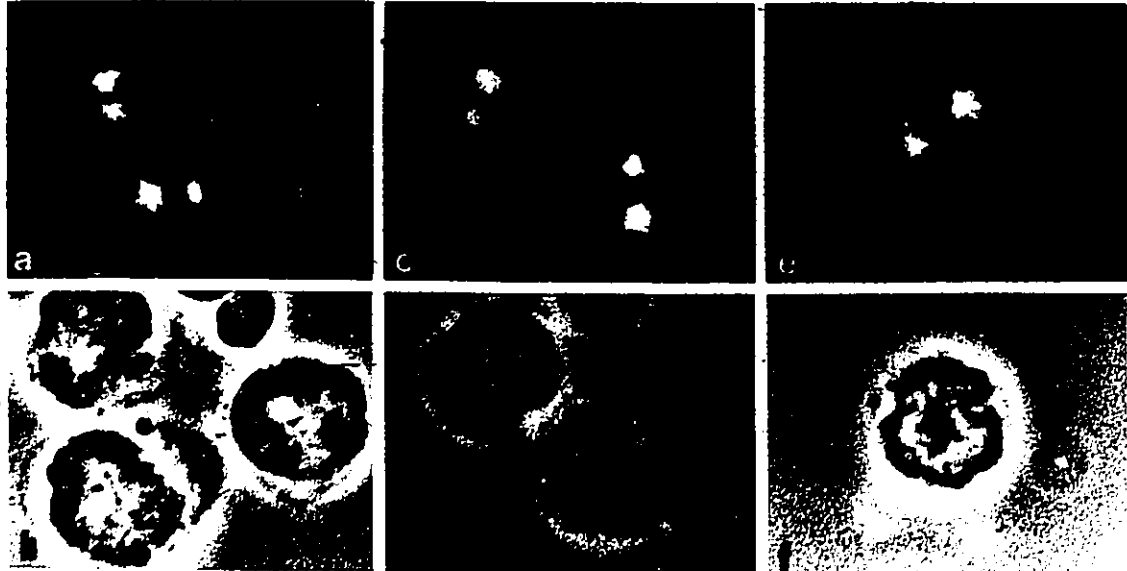
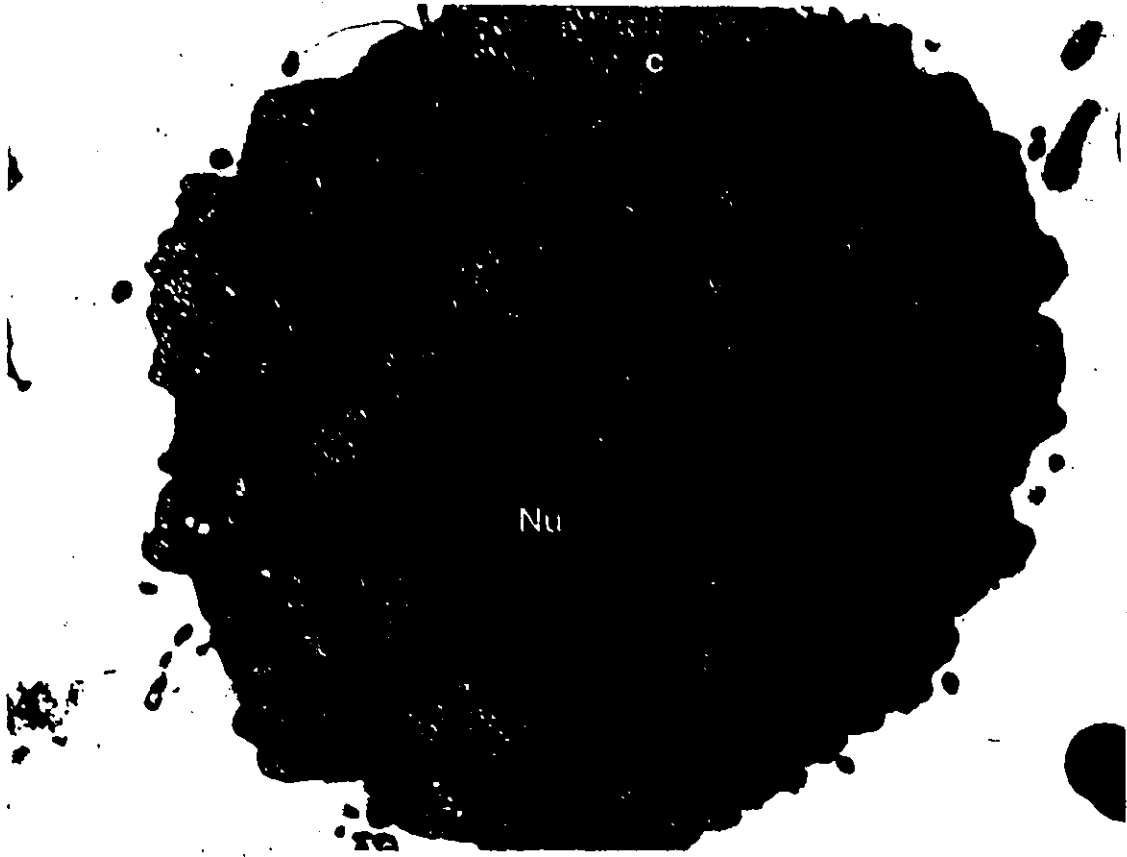
Figure 10 Typical aspect of a mouse lymphocyte during prophase by electron microscopy

The nuclear envelope is intact and condensed chromosomes are found at the periphery of the nucleus (N). The nucleolus (Nu) is still present. Microtubules can be seen in this section extending from one polar centrosome (C). — 13 300 x

Figure 11 Duplication/separation of the centrosome in mouse stimulated lymphocytes

Stimulated lymphocytes were stained with the rabbit antiserum to tubulin (a, c, e) and observed by phase contrast (b, d, f). Shortly after duplication (a, b), the two microtubule centers are located inside the same nuclear cleft. Microtubules are preferentially associated with the center proximal to the nucleus. During separation (c, d), only short tubulin fibers are detected on the two centers. In prophase (e, f), the centers are found in invaginations on either sides of the nucleus and spindle microtubules are organized.

— 2 000 x



associated with the center proximal to the nucleus. Apart from a few microtubules that appear to connect the two centers, no detectable tubulin fibers are observed in the vicinity of the distal center. During separation (Fig. 11c, d), most of the cytoplasmic microtubules are disassembled and only short tubulin fibers are detected on both centers. In prophase (Fig. 11e, f), the centers are found in invaginations on either sides of the nucleus and spindle microtubules are organized.

Distributions of vimentin and tubulin during the various stages of mitosis in lymphocytes were examined by double immunofluorescence (Fig. 12). Cells were identified as to mitotic stage according to the criteria proposed by Chaly *et al.* (1984), using primarily DNA staining with Hoechst 33258. Lymphocytes are round cells and no squashing was applied to preparations in order to preserve as much as possible the structural organization of both chromosomes and cytoskeletal components. For different mitotic stages, through-focus series of the cells were necessary to picture the whole patterns of vimentin or tubulin. The most informative optical sections were selected here to illustrate these patterns.

In cells that have entered prophase (Fig. 12a - c), the vimentin-positive aggregate observed in stimulated cells is no longer detected and vimentin now appears as filamentous arrays extending from the developing spindle poles around the nucleus. Following nuclear envelope breakdown in prometaphase (Fig. 12d - f; Fig. 13d), vimentin forms a filamentous cage-like structure enclosing the spindle apparatus and the chromosome mass. By electron microscopy, prominent arrays of intermediate filaments are found in the cytoplasm in close proximity to the prometaphase chromosomes (Fig. 13e). The cage-like

Figure 12 Mitotic fate of vimentin and tubulin during mitosis of mouse lymphocytes

Lymphocytes were double labelled with the rabbit antiserum to vimentin (a, d, d', g, j, j', m, p, p', s) and with the murine monoclonal anti-tubulin (b, b', e, h, k, n, q, q', t) and then counterstained with Hoechst 33258 (c, f, i, l, o, r, u). For some stages, two focal planes (eg. b/b', d/d', j/j', p/p', q/q') are shown. In prophase (a - c), vimentin appears as filaments extending from the developing pole. In prometaphase (d - f), a vimentin cage surrounds the mitotic spindle. The vimentin cage persists through metaphase (g - i) and early anaphase (j - l). In late anaphase (m - o), vimentin is detected in thick arrays extending the length of the cell. Vimentin staining is observed at the spindle poles and at the midbody during telophase (p - r). Vimentin is aggregated and the centrosomes are relocalized at a site near the midbody in each daughter cell during cytokinesis (s - u). — 1 300 x

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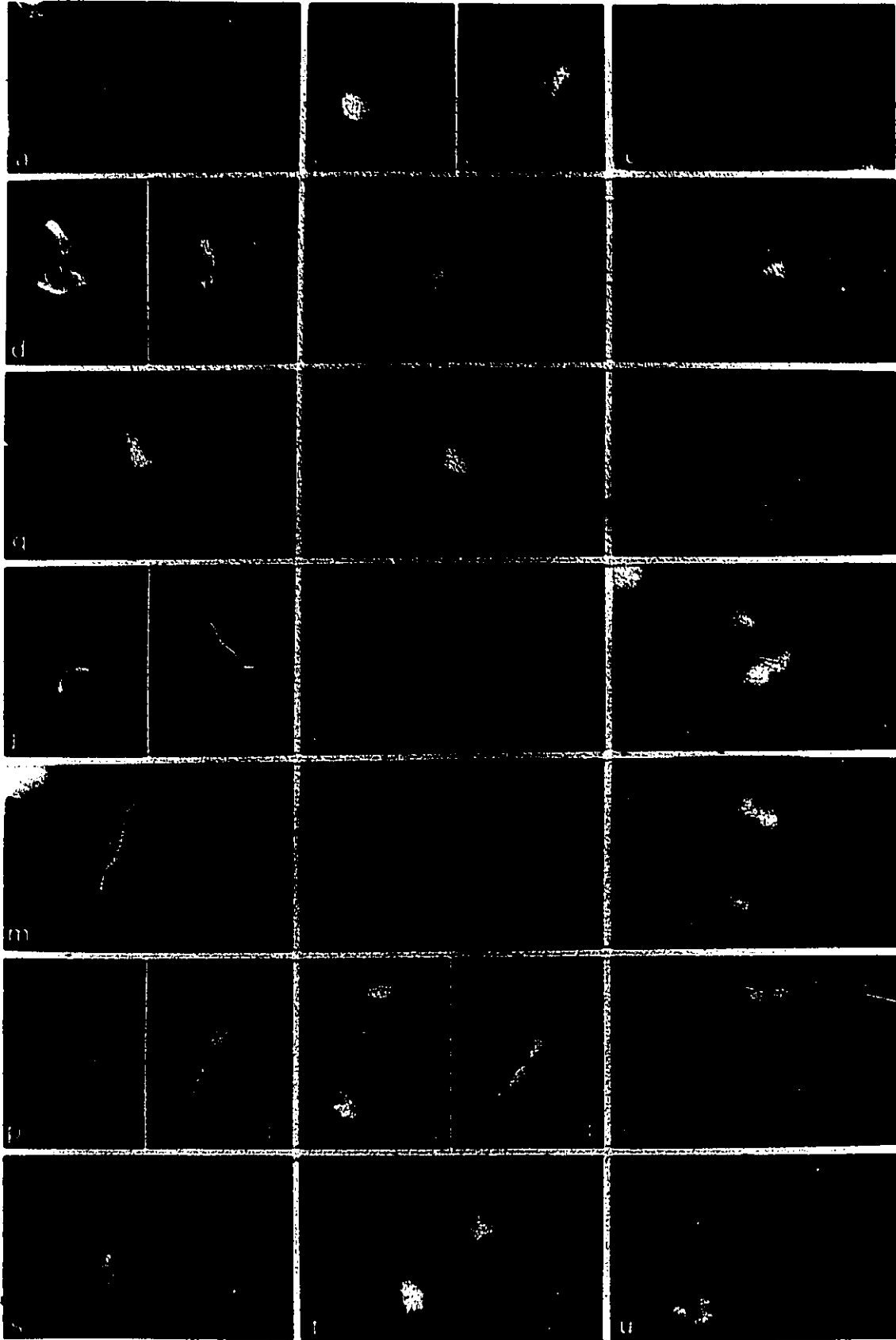
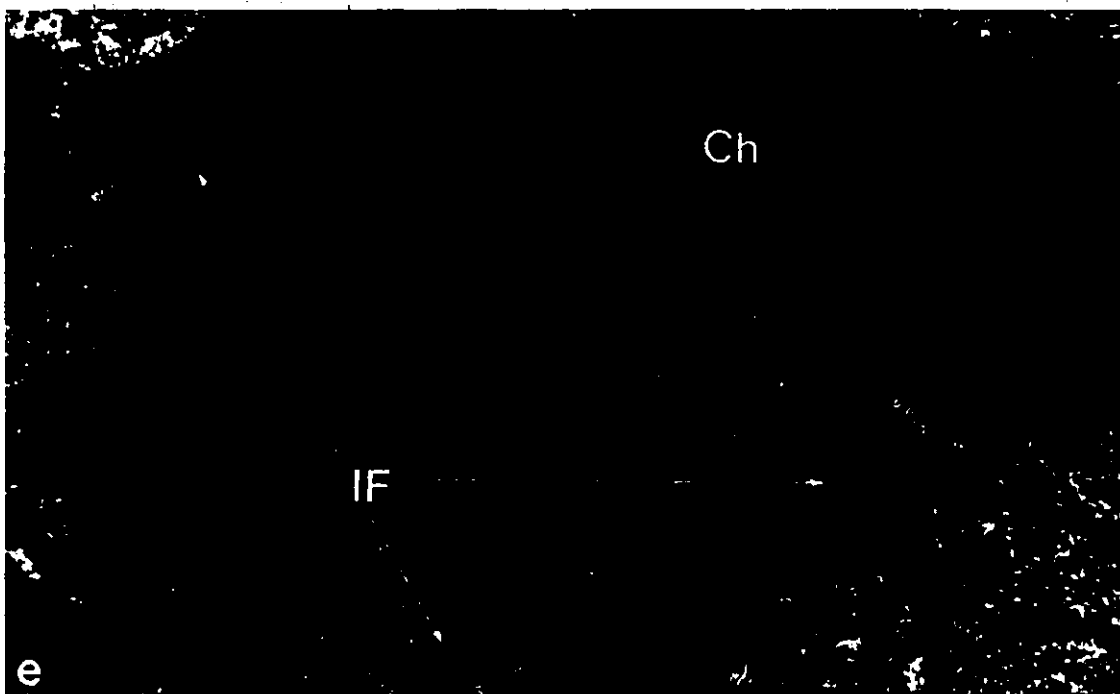
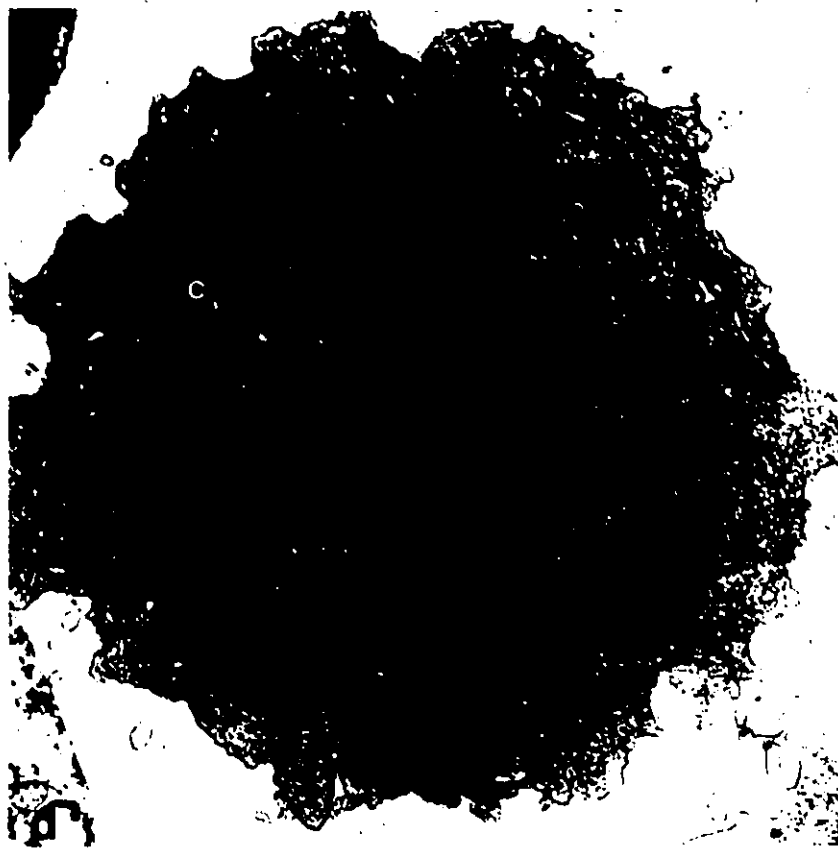
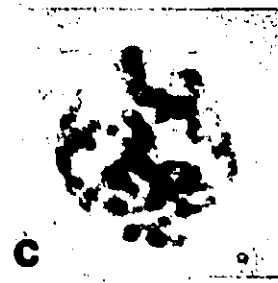


Figure 13 Microscopy of mouse lymphocytes in prometaphase

The lymphocyte observed by phase contrast in (c) was double labelled with the rabbit antiserum to vimentin (a) and the commercial murine monoclonal anti-vimentin (b). Identical patterns were detected with both anti-vimentins. During prometaphase, vimentin forms a filamentous cage enclosing the mitotic apparatus. — 2 000 x — d. Electron micrograph showing the typical aspect of a prometaphase cell. The nuclear envelope has been disrupted and condensed chromosomes occupy most of the cellular space. A polar centrosome (C) is seen on one side of the chromosome mass. — 15 800 x — e. High magnification view of a prometaphase cell showing the presence of intermediate filament arrays (arrows) in proximity to a chromosome (Ch). — 68 400 x



organization of vimentin filaments persists during the alignment of chromosomes at the metaphase plate (Fig. 12g - i) and the first steps of chromosome separation during early anaphase (Fig. 12j - l). During the formation of two chromosome sets at the spindle poles in late anaphase (Fig. 12m - o), a vimentin cage is no longer observed but rather vimentin filaments are present as one or two thick bundles extending between the spindle poles. Cytokinesis begins during telophase (Fig. 12p - r). The two chromosome sets still retain a cup-shape arrangement but individual chromosomes are difficult to distinguish (Fig. 12r). Vimentin staining is more conspicuous at the spindle poles and at the midbody but is also detected as granular specks over the whole cytoplasm (Fig. 12p and p'). At a later stage of cytokinesis/early G₁ (Fig. 12s - u), the nuclear envelope is reformed and the chromosomes are decondensed (Fig. 12u). The daughter centrosomes have been relocalized to a position near the midbody and interphase microtubule arrays have been reestablished (Fig. 12t). Vimentin appears as an aggregate, similar to that seen prior to mitosis, close to the centrosome in each daughter cell (Fig. 12s). Identical mitotic patterns of vimentin were detected by double immunofluorescence staining with the rabbit antiserum to vimentin and the commercial monoclonal anti-vimentin (Labsystems) (Fig. 13a - c).

The organizational fate of vimentin and tubulin during mitogenic stimulation of mouse splenic lymphocytes is schematically summarized in figure 14. The same sequence of rearrangements is observed during mitogenesis of bovine lymphocytes, including the aggregation of the vimentin system during blastogenesis (Fig. 15a - d; Fig. 15e - h), the formation of a filamentous cage-like structure during mitosis (Fig. 15i - l), as well as the relocalization of the daughter centrosomes and the accumulation of vimentin near the midbody during cytokinesis (Fig. 15m - p).

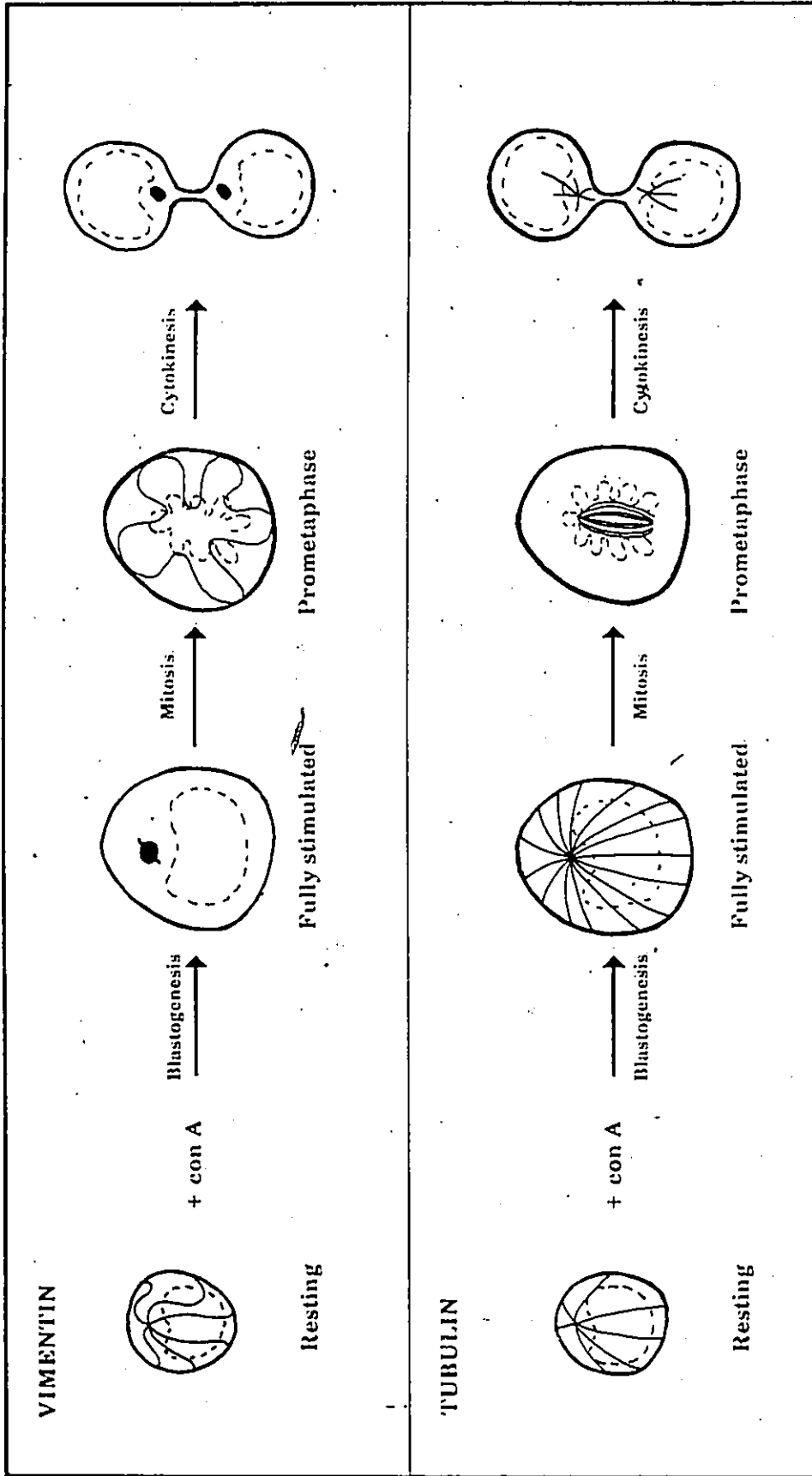
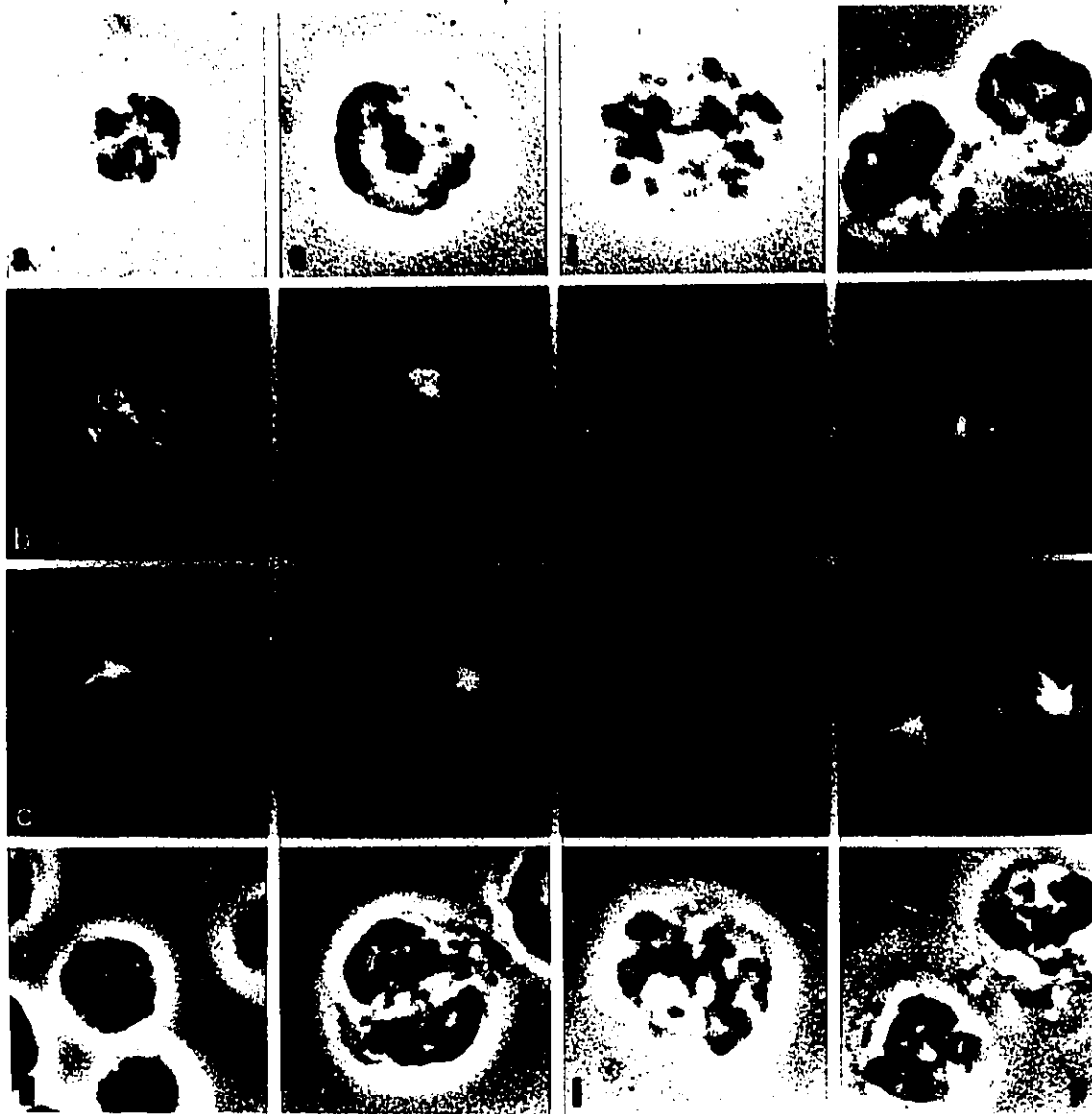


Figure 14 Organizational fate of vimentin and tubulin during mitogenic stimulation of lymphocytes.

Figure 15 Organizational fate of vimentin and tubulin during mitogenic stimulation of bovine lymphocytes

Bovine lymphocytes seen by phase contrast in (a), (e), (i), and (m) were stained with the rabbit antiserum to vimentin (b, f, j, n). Cells seen in (d), (h), (l), and (p) were labelled with the murine monoclonal anti-tubulin (c, g, k, o). In resting cells (a - d), vimentin forms a filamentous network and microtubules show a radial pattern. In stimulated cells (e - h), vimentin is detected as an aggregate adjacent to the nuclear cleft while microtubules still display a radial pattern. During metaphase (i - l), vimentin is observed as a filamentous cage enclosing the mitotic apparatus. During cytokinesis (m - p), vimentin is aggregated and the centrosomes are relocated at a site close to the midbody. — 2 000 x



SHORT-TERM EFFECTS OF MICROTUBULE SPECIFIC DRUGS

Throughout the different events of mitogenic stimulation in lymphocytes, there appears to be a spatial relationship between the cytoplasmic distribution of vimentin and the organization of the microtubule system. To investigate this relationship further, resting and con A-stimulated cells were treated over short-term periods with drugs that cause disassembly or reorganization of microtubules, and the effects of such treatments on the vimentin system were examined.

Exposure of resting and stimulated populations to 4 $\mu\text{g/ml}$ colchicine for 6 hours (Fig. 16) or to 1 $\mu\text{g/ml}$ colcemid for 4 hours (Fig. 17) consistently results in the complete disassembly of microtubules in approximately 90% of the cells as assessed by immunofluorescence. Only the centrosome or the mitotic poles can be detected in these cells using the rabbit antiserum against tubulin (Fig. 16c, g, k; Fig. 17c, g, k). The other 10% cells either show short residual microtubules attached on the centrosome (7%) or a few drug-resistant long tubulin fibers (3%). In contrast, the effects of nocodazole on lymphocytes are highly variable from one population to another. Stimulated populations are nevertheless consistently more sensitive to the drug than resting populations. Upon a 4-hour exposure to 10 $\mu\text{g/ml}$ of nocodazole, tubulin fibers can still be detected in 62% of resting cells (Fig. 18c, d) but are detected in only 21% of stimulated cells (Fig. 18g, h; Fig. 18k, l). However, the three microtubule-disrupting agents are equally potent in promoting the reorganization of the vimentin system (Fig. 16b, f, j; Fig. 17b, f, j; Fig. 18b, f, j) as previously described for other cell types (Bennett *et al.*, 1978; Hynes and Destree, 1978; Starger *et al.*, 1978; Celis *et al.*, 1984). In both resting and stimulated lymphocytes treated with drug, vimentin is

Figure 16 Short-term effects of colchicine on the organization of vimentin and tubulin in mouse lymphocytes

Lymphocytes were exposed to 4 $\mu\text{g/ml}$ colchicine for 6 hours. Cells seen by phase contrast in (a), (e), and (i) were then stained with the rabbit antiserum to vimentin (b, f, j) and cells in (d), (h), and (l) were labelled with the rabbit antiserum to tubulin (c, g, k).

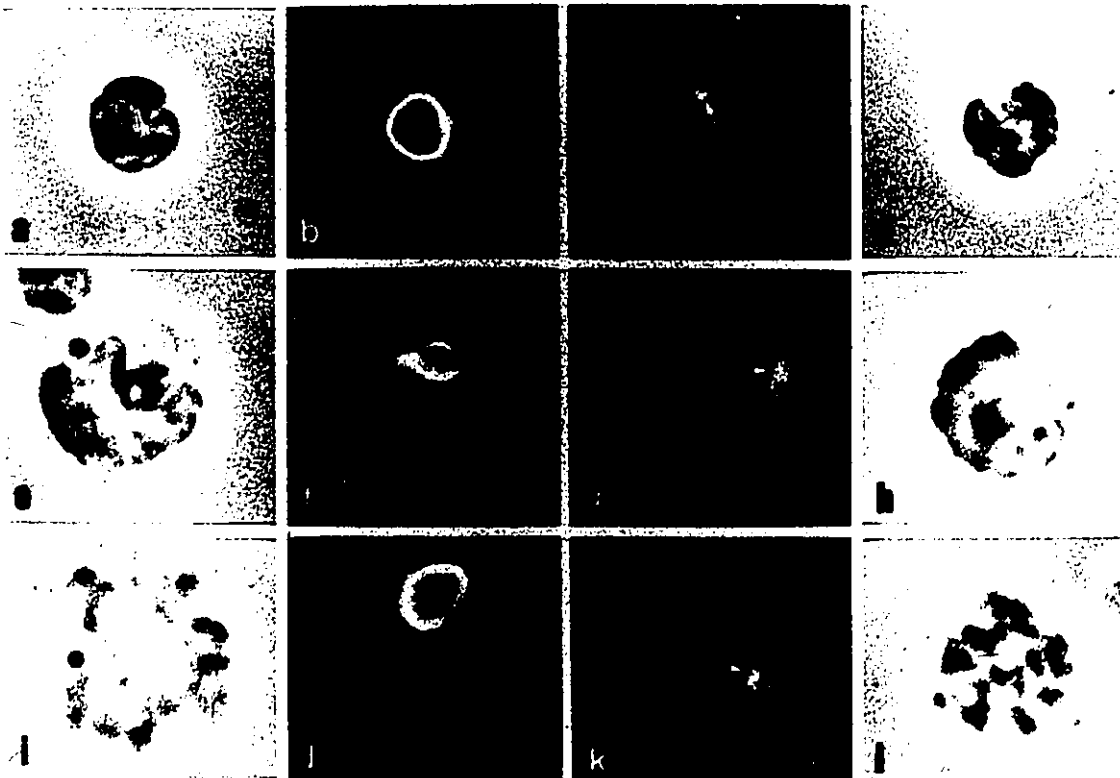
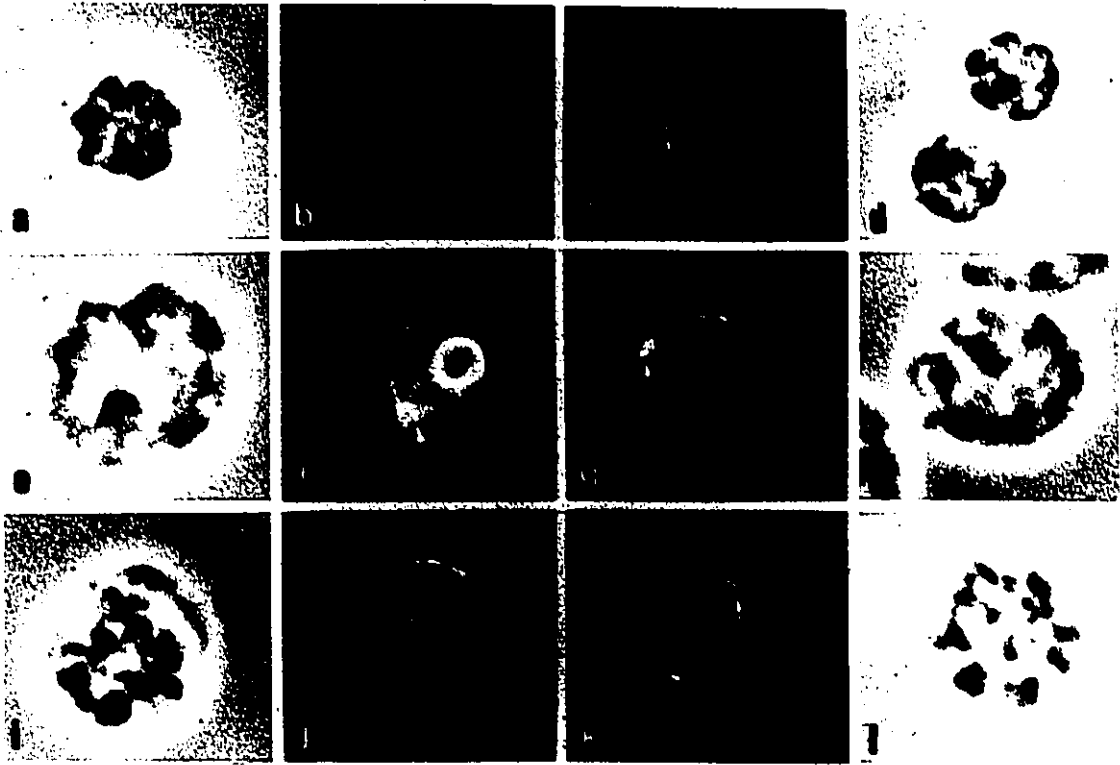
Microtubules have been disassembled and vimentin is reorganized into a ring-like structure in resting (a - d), stimulated (e - h) and mitotic (i - l) cells. In the colcemid-treated stimulated cell shown in (f), a vimentin aggregate similar to that observed in control stimulated cells is present (arrowhead). Arrows in (c), (g), and (k) indicate the position of the centrosome and the mitotic poles.

— 2 000 x

Figure 17 Short-term effects of colcemid on the organization of vimentin and tubulin in mouse lymphocytes

Lymphocytes were exposed to 1 $\mu\text{g/ml}$ colcemid for 4 hours. Cells seen by phase contrast in (a), (e), and (i) were then stained with the rabbit antiserum to vimentin (b, f, j) and cells in (d), (h), and (l) were labelled with the rabbit antiserum to tubulin (c, g, k).

Microtubules have been disassembled and vimentin is reorganized into a ring-like structure in resting (a - d), stimulated (e - h) and mitotic (i - l) cells. Arrows in (c), (g), and (k) indicate the position of the centrosome and the mitotic poles. — 2 000 x



f

Figure 18 Short-term effects of nocodazole on the organization of vimentin and tubulin in mouse lymphocytes.

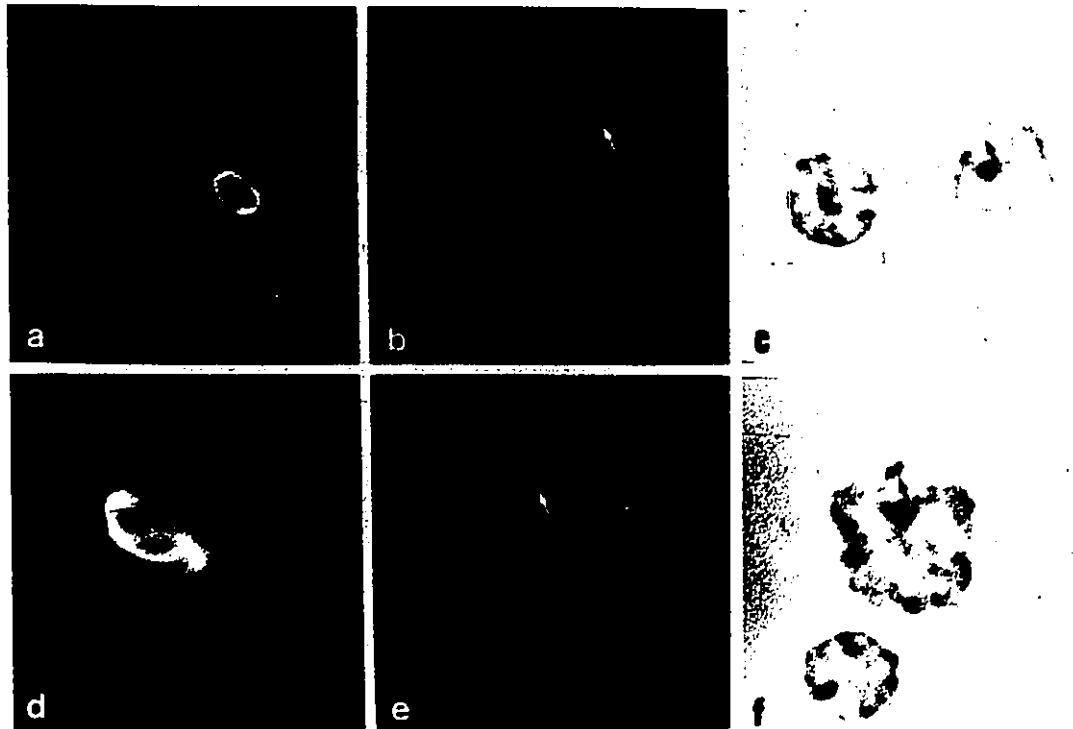
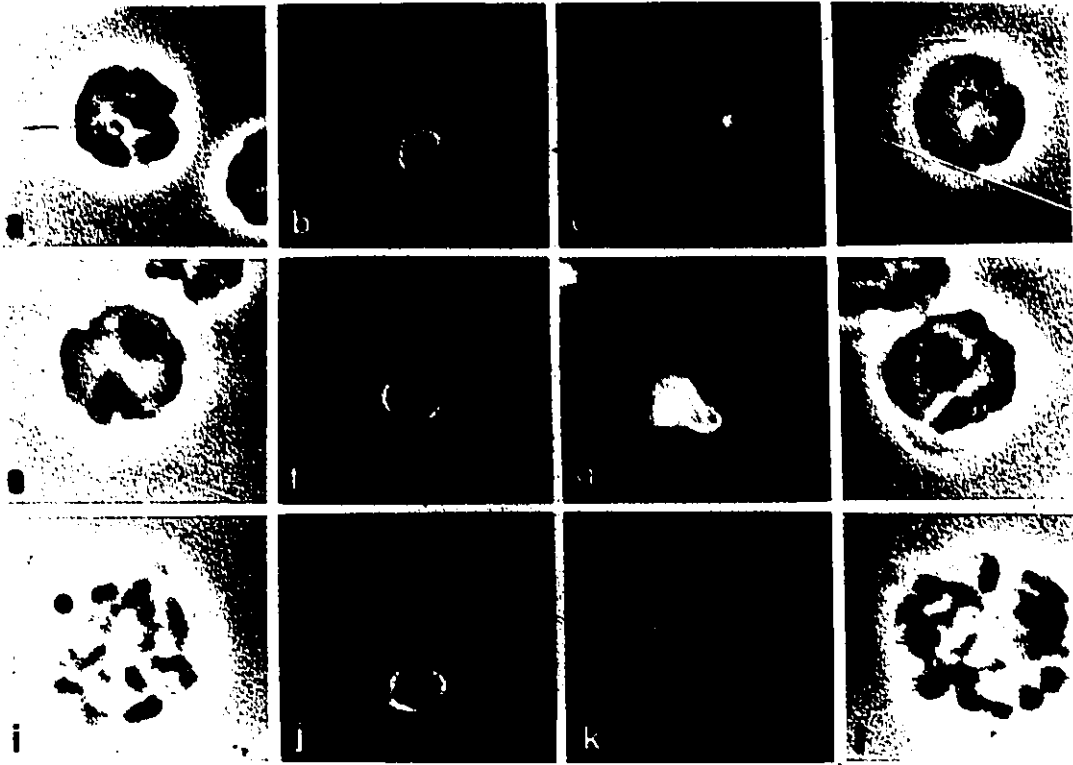
Lymphocytes were exposed to 10 $\mu\text{g/ml}$ nocodazole for 4 hours. Cells seen by phase contrast in (a), (e), and (i) were then stained with the rabbit antiserum to vimentin (b, f, j) and cells in (d), (h), and (l) were labelled with the rabbit antiserum to tubulin (c, g, k).

Vimentin is consistently detected as a ring-like structure although drug-resistant microtubules are still detected in 62% of resting cells (a - d), and in 21% of stimulated (e - f) or mitotic (i - l) cells.

— 2 000 x

Figure 19 Effects of colcemid on the spatial relationship between the vimentin system and the centrosome in mouse lymphocytes

Resting (a - c) and stimulated (d - f) lymphocytes were exposed to 1 $\mu\text{g/ml}$ colcemid for 4 hours. Cells seen by phase contrast in (c) and (f) were double labelled using the commercial murine monoclonal anti-vimentin (a, d) and the rabbit antiserum to tubulin (b, e). The location of the colcemid-induced vimentin ring is not related to the position of the centrosome (arrow). — 2 000 x



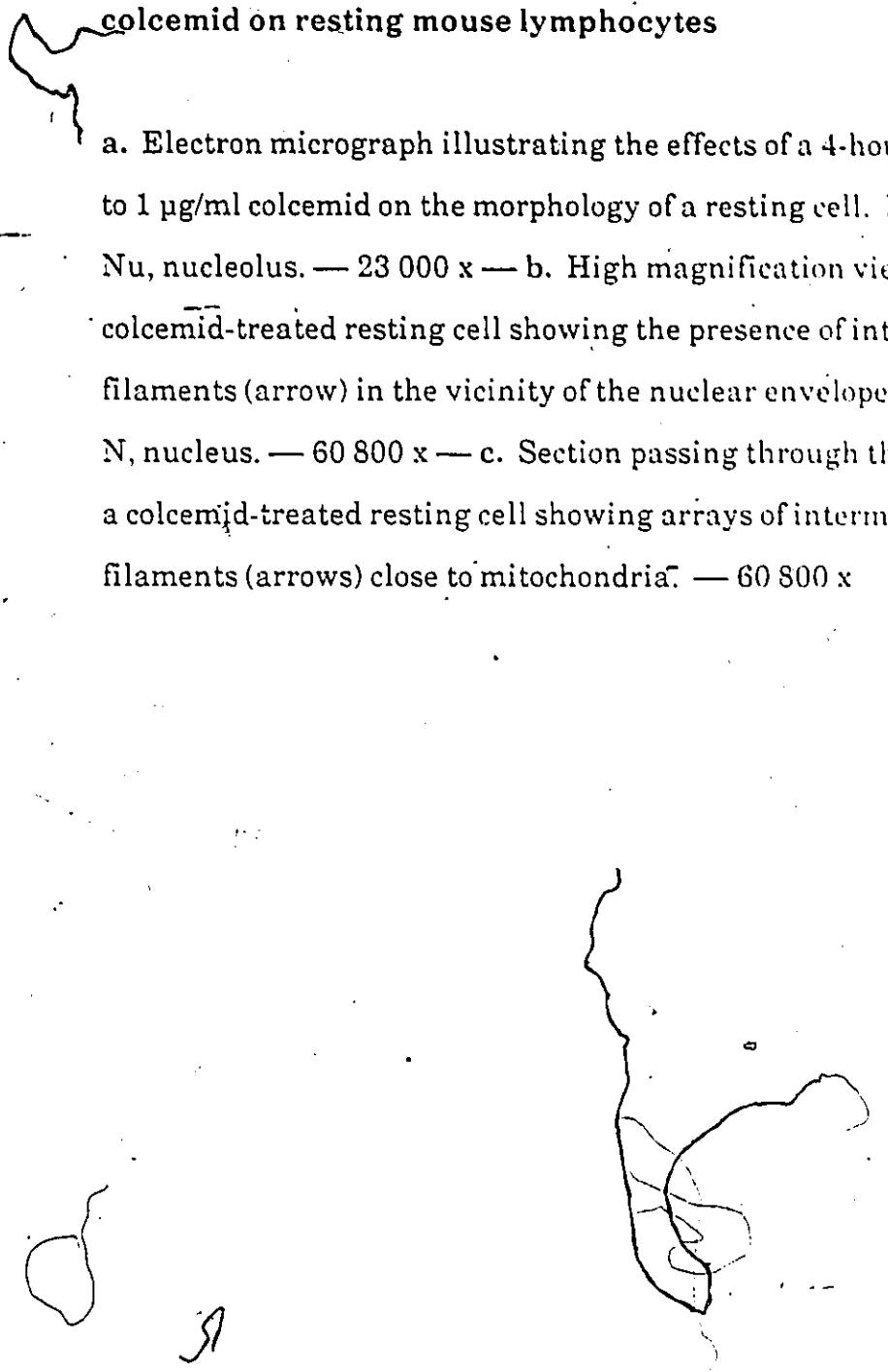
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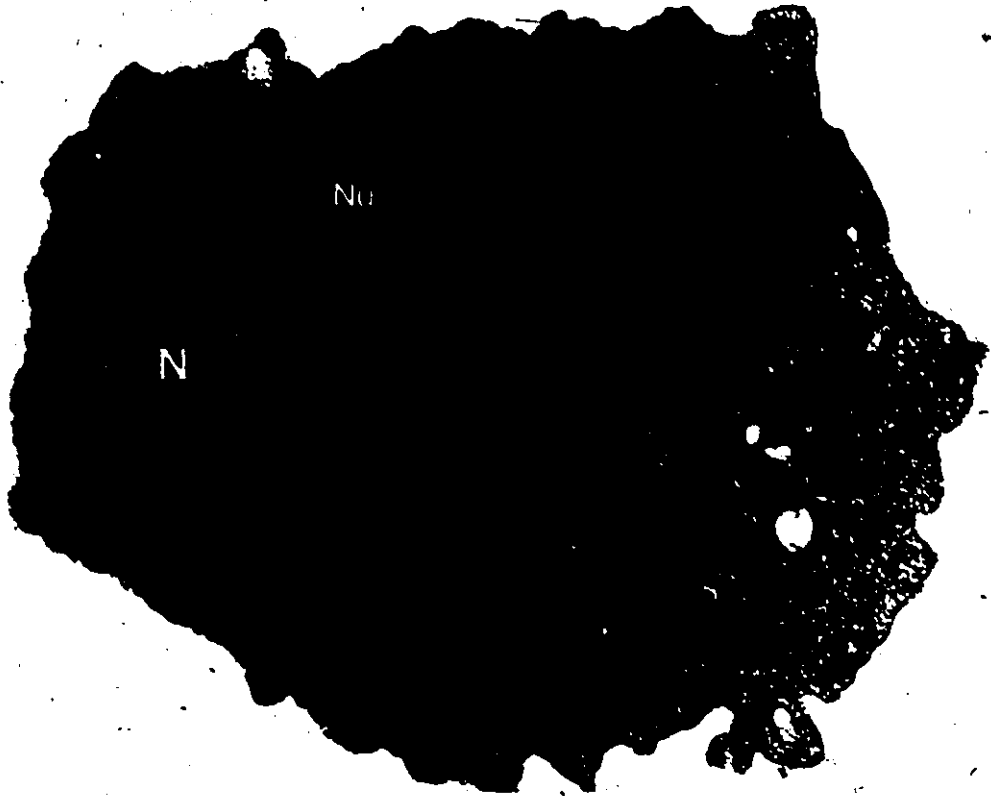
rearranged in a ring-like pattern. The vimentin-positive aggregate observed in control stimulated cells is also found in some drug-treated stimulated cells that show a vimentin ring (see for example figure 16f), but is not a consistent feature. Double immunostaining of resting and stimulated cells with the rabbit antiserum against tubulin and the commercial monoclonal anti-vimentin (Labsystems) (Fig. 19) further indicates that there is usually no correlation between the localization of the vimentin ring and the position of the centrosome.

By electron microscopy, resting and stimulated lymphocytes treated with 1 µg/ml of colcemid for 4 hours show important morphological changes (Fig. 20; Fig. 21). The normal spherical shape of these cells is altered by the formation of a large uropod, and the nuclear topography is modified by the appearance of convolutions (Fig. 20a; Fig. 21a). Structural changes are most evident in stimulated cells. The satellite bodies, which are normally collected in the region between the two centrioles in stimulated cells, are redistributed more uniformly in the pericentriolar material (Fig. 21b). The Golgi apparatus is usually fragmented. The association between the Golgi apparatus and the centrosome, however, is preserved and both organelles are still found as a complex located in a nuclear cleft. Microtubules are absent and arrays of intermediate filaments are observed close to the nuclear envelope as well as near mitochondria in the uropod of both resting and stimulated cells (Fig. 20b, c; Fig. 21c, d). No association between intermediate filaments and the centrosome is seen. In fact, the centrosomal region appears virtually free of any surrounding filamentous structures in such treated cells.

Cells blocked in mitosis show a prometaphase configuration (Fig. 22a). The mitotic poles as well as the cytoplasm surrounding the chromosomes are

Figure 20 Electron microscopic observation of short-term effects of colcemid on resting mouse lymphocytes

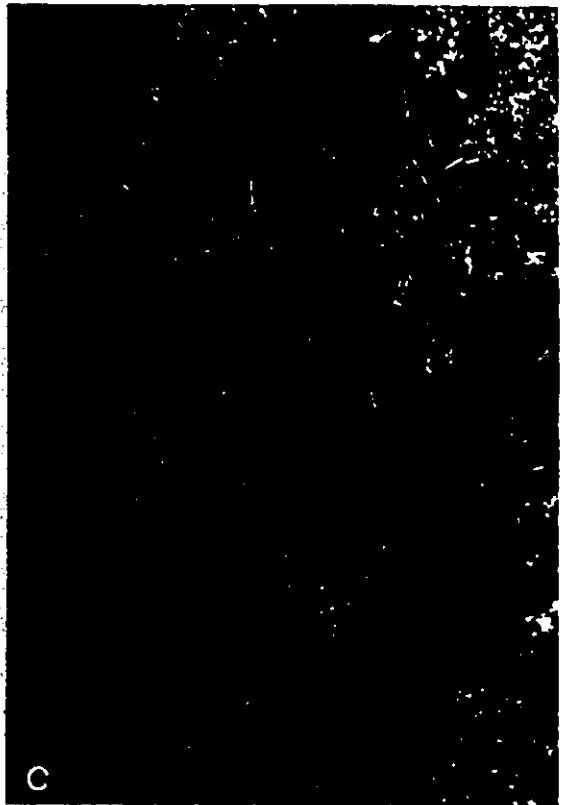
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- a. Electron micrograph illustrating the effects of a 4-hour exposure to 1 $\mu\text{g/ml}$ colcemid on the morphology of a resting cell. N, nucleus. Nu, nucleolus. — 23 000 x — b. High magnification view of a colcemid-treated resting cell showing the presence of intermediate filaments (arrow) in the vicinity of the nuclear envelope. N, nucleus. — 60 800 x — c. Section passing through the uropod of a colcemid-treated resting cell showing arrays of intermediate filaments (arrows) close to mitochondria. — 60 800 x
- The figure includes three hand-drawn sketches. Sketch 'a' is a simple outline of a cell with a large nucleus and a smaller nucleolus. Sketch 'b' shows a more detailed view of a cell with a nucleus and several thin, wavy lines representing intermediate filaments near the nuclear envelope. Sketch 'c' shows a cross-section of a cell with a nucleus, several mitochondria, and arrays of intermediate filaments near them. There are also some other faint sketches and a handwritten 'B' on the page.



a



b



c

Figure 21 Electron microscopic observation of short-term effects of colcemid on stimulated mouse lymphocytes

- a. Electron micrograph illustrating the effects of a 4-hour exposure to 1 $\mu\text{g/ml}$ colcemid on the morphology of stimulated cells. N, nucleus. C, centrosome. — 14 500 x — b. High magnification view of the centrosome of (a). Note that satellite bodies are distributed uniformly in the pericentriolar material. — 38 000 x — c. Micrograph showing the presence of intermediate filament arrays (arrows) in the vicinity of nuclear envelope and mitochondria in a colcemid-treated stimulated cell. — 60 800 x — d. Section passing through the uropod of a colcemid-treated stimulated cell showing an array of intermediate filaments (arrows) close to mitochondria. — 60 800 x

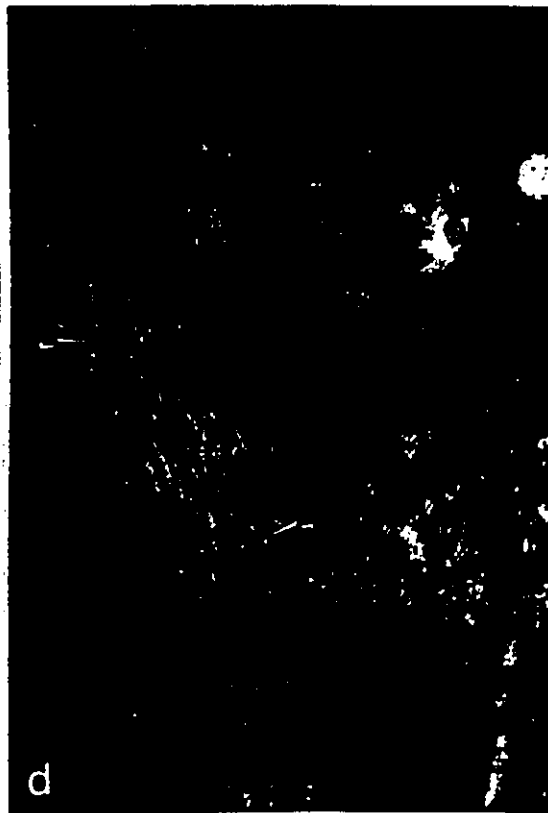
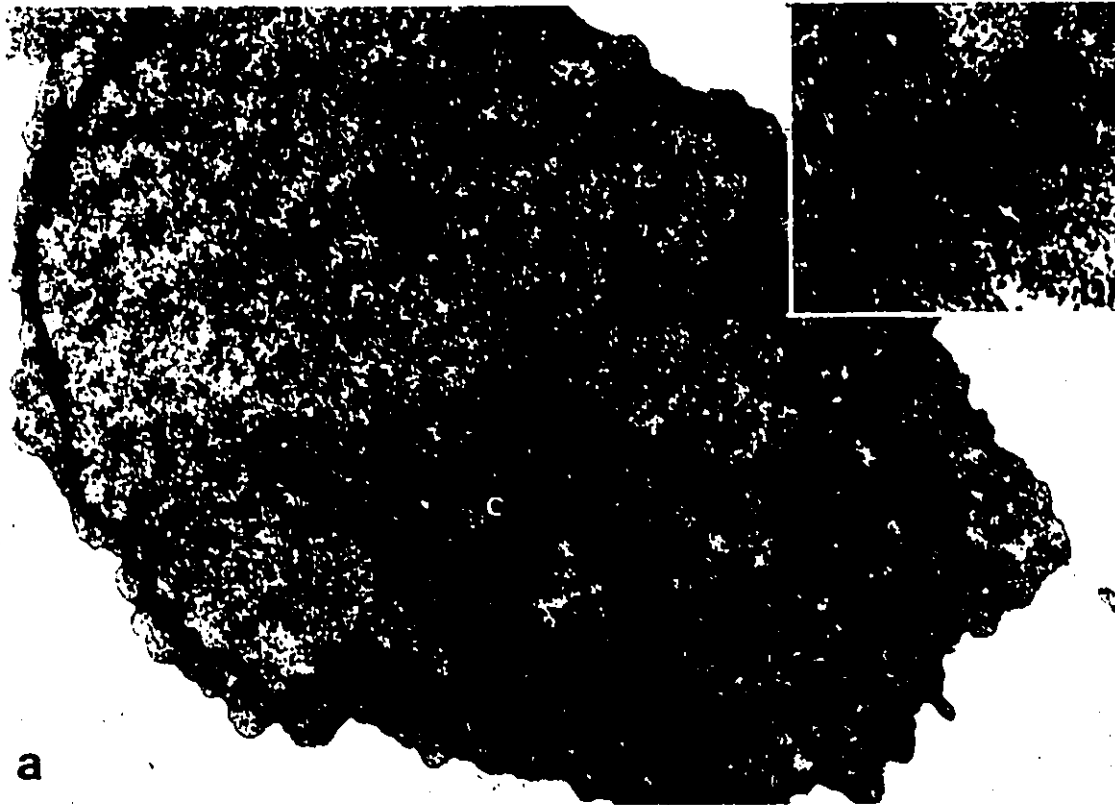


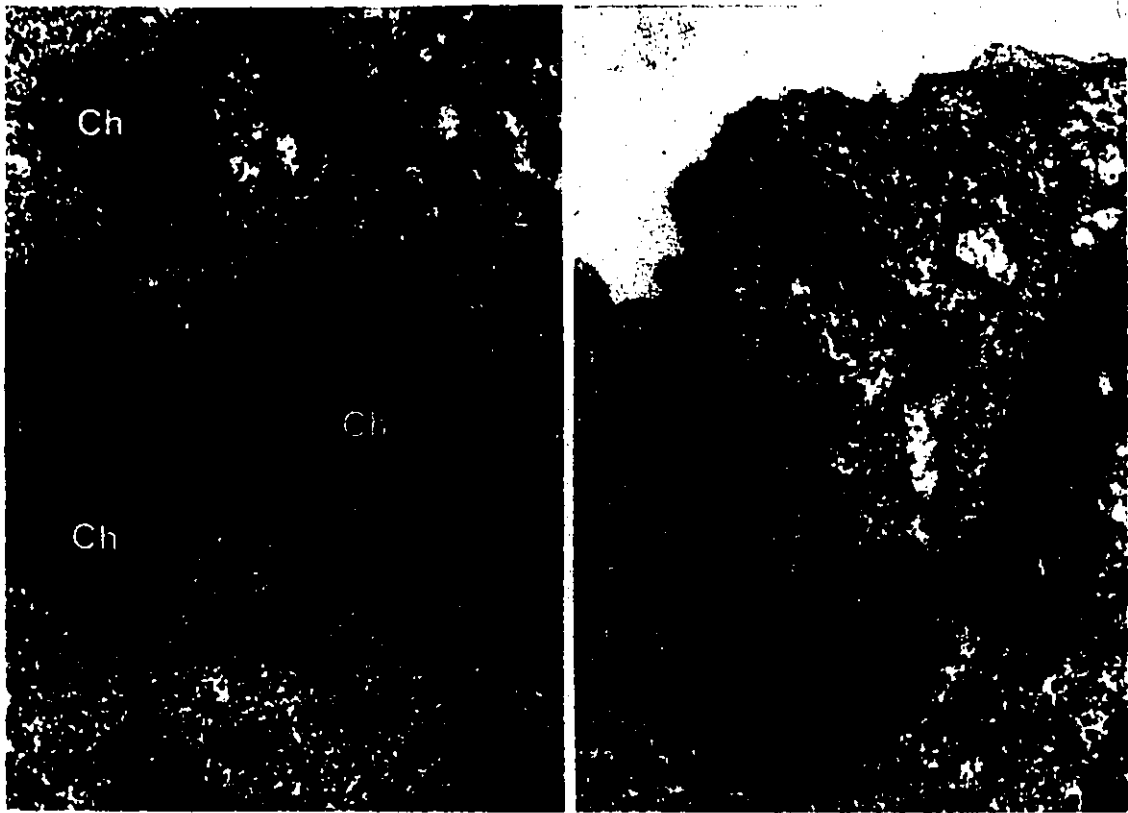
Figure 22 Electron microscopic observation of short-term effects of colcemid on mitotic mouse lymphocytes

a. Electron micrograph of a cell blocked in mitosis by a 4-hour exposure to 1 $\mu\text{g}/\text{ml}$ colcemid. The chromosomes display a prometaphase-like configuration. A polar centrosome (C) devoid of microtubules is seen on one side of the chromosome mass.

— 16 100 x — b. High-magnification view illustrating the absence of microtubules at the kinetochores and in the cytoplasm surrounding the chromosomes (Ch) in a colcemid-treated mitotic cell. — 60 800 x — c. Micrograph showing an intermediate filament array close to the plasma membrane of a colcemid-treated mitotic cell. — 60 800 x



a



completely devoid of microtubules (Fig. 22a, b). Arrays of intermediate filaments are seen at the periphery of the cells near the plasma membrane (Fig. 22c).

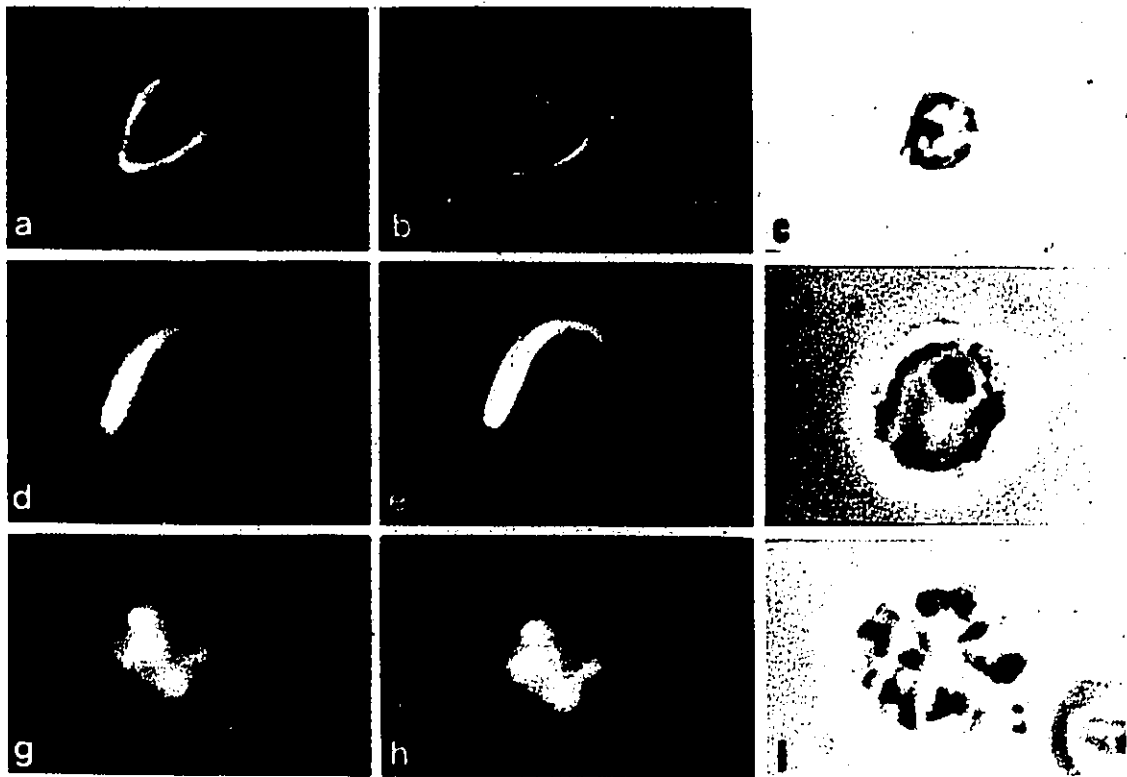
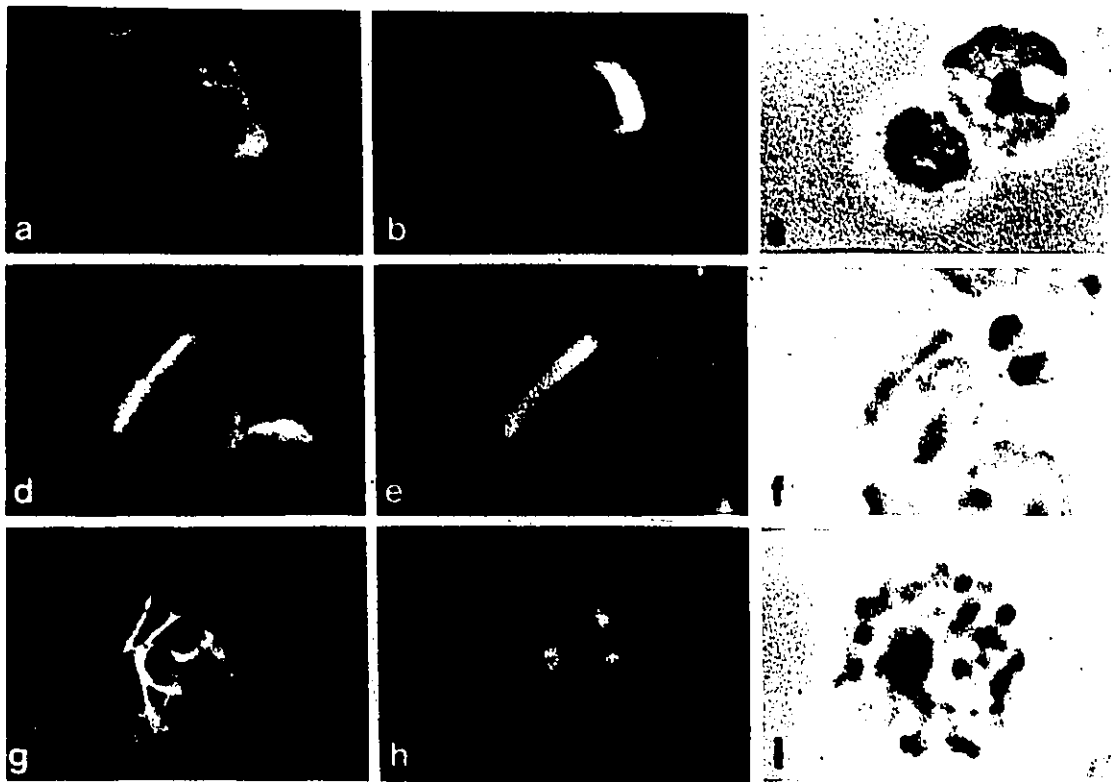
Effects of short-term exposures to the microtubule assembly-promoting drug taxol (10 $\mu\text{g/ml}$) on the tubulin and vimentin organizations in lymphocytes were observed by double immunofluorescence and are shown in figures 23 and 24. After 1-hour exposure to taxol (Fig. 23), microtubules have aggregated laterally to form small bundles extending from the centrosome in resting (Fig. 23b) and stimulated cells (Fig. 23e), and have formed multiple asters in mitotic cells (Fig. 23h). Bundling of microtubules is more striking in cells treated for 4 hours with the drug (Fig. 24). One or a few large bundles are visualized in resting cells (Fig. 24b), and in most stimulated cells (Fig. 24e) a single large bundle is detected extending the length of the cell. Multiple asters are still observed in mitotic cells (Fig. 24h). These reorganizations of microtubules in response to taxol are paralleled by rearrangements of the vimentin system. After 1-hour exposure, vimentin shows partial co-localization with microtubule bundles in resting and stimulated cells (Fig. 23a, d). In the mitotic cells (Fig. 23g), vimentin has not reorganized yet and appears in filamentous arrays separate from the microtubule asters. By 4-hour exposure, parallel reorganization of the two filamentous systems is more evident. Microtubules and vimentin filaments are co-localized into one or few large bundles extending from the centrosome in resting and stimulated cells (Fig. 24a, b; Fig. 24d, e). Vimentin staining is detected over the multiple microtubule asters in mitotic cells, usually arrested by the drug in prometaphase (Fig. 24g, h). The vimentin-positive aggregate present in control stimulated lymphocytes was still present in some cells treated for 1 hour with taxol but was not detected in cells exposed for 4 hours to the drug.

Figure 23 Organization of vimentin and tubulin in mouse lymphocytes after a 1-hour treatment with taxol

Lymphocytes were exposed to 10 $\mu\text{g/ml}$ taxol for 1 hour. Cells seen by phase contrast in (c), (f), and (i) were double labelled using the rabbit antiserum to vimentin (a, d, g) and the murine monoclonal anti-tubulin (b, e, h). The vimentin system shows partial co-localization with microtubules in resting (a - c) and stimulated (d - f) cells. In a mitotic cell (g - i), vimentin is detected as filamentous arrays while microtubules have formed multiple asters. — 2 000 x

Figure 24 Organization of vimentin and tubulin in mouse lymphocytes after a 4-hour treatment with taxol

Lymphocytes were exposed to 10 $\mu\text{g/ml}$ taxol for 4 hours. Cells seen by phase contrast in (c), (f), and (i) were double labelled using the rabbit antiserum to vimentin (a, d, g) and the murine monoclonal anti-tubulin (b, e, h). Vimentin filaments and microtubules are co-localized into one or few large bundles in resting (a - c) and stimulated (d - f) cells. Vimentin is detected over the multiple microtubule asters in mitotic cell. — 2 000 x



At the electron microscopic level (Fig. 25a, b), short-term exposure of lymphocytes to taxol does not severely affect the morphology of these cells and cellular shape is only slightly altered. The centrosome and the Golgi apparatus are displaced towards the plasma membrane as a whole complex without being disrupted. In both resting and stimulated cells, bundles of microtubules appear to terminate within the centrosome region with many individual microtubules ending on centriolar satellite bodies. Intermediate filaments were usually obscured in intact resting cells but were readily observed in digitonin-permeabilized resting cells or intact stimulated cells.

In 1-hour treated cells, arrays of intermediate filaments are observed in the perinuclear region and do not appear to be preferentially aligned with microtubule bundles (Fig. 26c, d). Parallel reorganization of intermediate filaments with microtubules is most evident in 4-hour treated cells. In these cells, intermediate filaments are found among microtubules near the centrosome (Fig. 27a, b) and in arrays of filaments running parallel to microtubule bundles (Fig. 27c, d). The fibrogranular mass observed in control stimulated cells was not found in sections of 4-hour treated stimulated cells but was still present in some cells exposed 1 hour to the drug. In 1-hour treated cells, the fibrogranular mass appears smaller than in control cells and is often located adjacent to the nuclear envelope (Fig. 26a, b). Mitotic cells display a prometaphase chromosomal arrangement (Fig. 28a). Some of the aster microtubules appear to end on pericentriolar material (Fig. 28c) while others seem to terminate within the interchromosomal region in a complex criss-crossed arrangement (Fig. 28b). Small arrays of intermediate filaments are observed at the cell periphery in 4-hour treated mitotic cells (Fig. 29b), and both at the cell periphery and in the cytoplasm surrounding the mitotic poles in 1-hour treated cells (Fig. 29a).

Figure 25 Morphology of resting and stimulated mouse lymphocytes after short-term treatment with taxol

a. Electron micrograph of a resting lymphocyte treated with 10 $\mu\text{g/ml}$ taxol for 4 hours. A thick bundle of microtubules is seen that extends from the centrosome (C) and passes near the surface of the morphotype I nucleus (N). — 25 500 x — b. Electron micrograph of a stimulated cell treated with 10 $\mu\text{g/ml}$ taxol for 1 hour. The centrosome (C) /Golgi complex has been displaced close to the plasma membrane. Microtubules are arranged in a thick bundle, with some terminating on satellite bodies (arrows). A small fibrogranular mass is seen close to the top left part of the nucleus (N). — 17 000 x

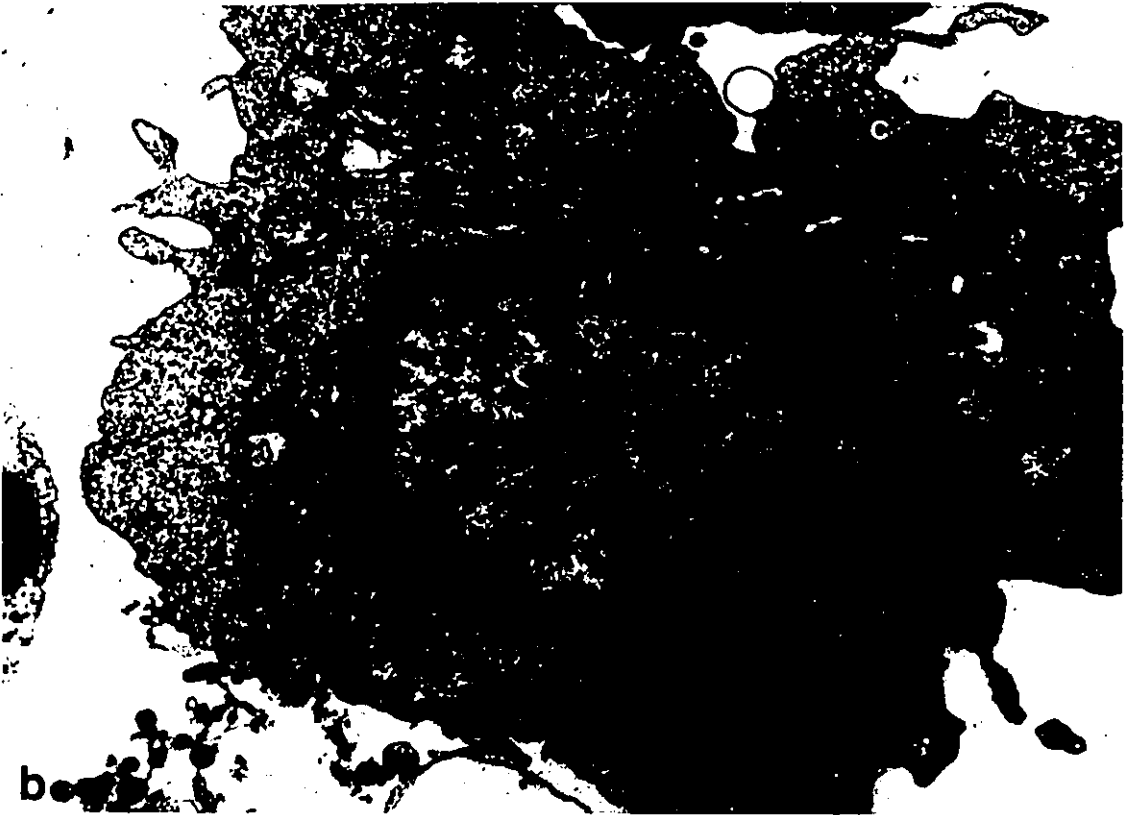
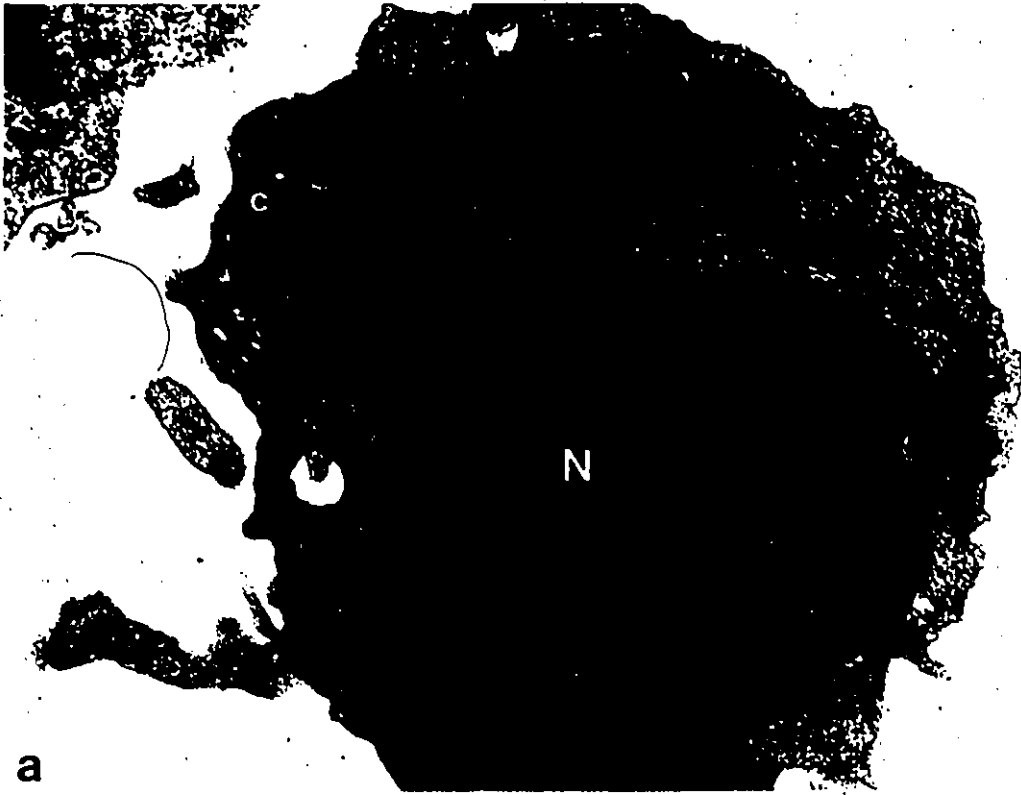


Figure 26 Electron microscopic observation of intermediate filaments in stimulated mouse lymphocytes after a 1-hour treatment with taxol

a, b. Electron micrographs illustrating the presence of a fibrogranular mass (arrows) close to the nuclear envelope in stimulated cells treated with 10 $\mu\text{g/ml}$ taxol for 1 hour. N, nucleus. — 32 000 x — c, d. Sections of stimulated cells exposed to taxol for 1 hour showing intermediate filament arrays (IF and arrows) in the perinuclear region. N, nucleus. — 32 000 x

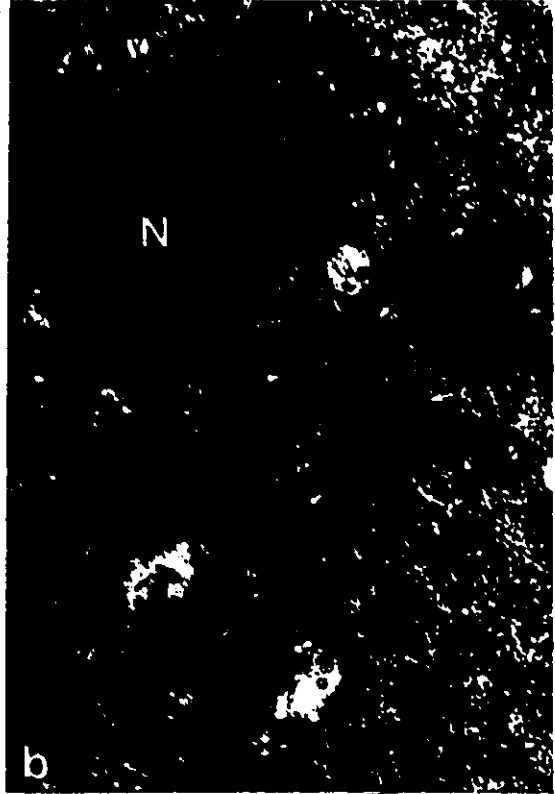


Figure 27 · Electron microscopic observation of intermediate filaments in stimulated mouse lymphocytes after a 4-hour treatment with taxol

a, b. Electron micrograph of stimulated cells exposed to 10 $\mu\text{g/ml}$ taxol for 4 hours showing intermediate filament arrays in longitudinal (IF) and cross-sectional views (arrows). Note the presence of intermediate filaments near the centrosome (C) in (b). N, nucleus. — 32 000 x —

c, d. Sections illustrating the parallel reorganization of intermediate filaments (arrows) with microtubule bundles in stimulated cells exposed to taxol for 4 hours. N, nucleus. — 30 000 x

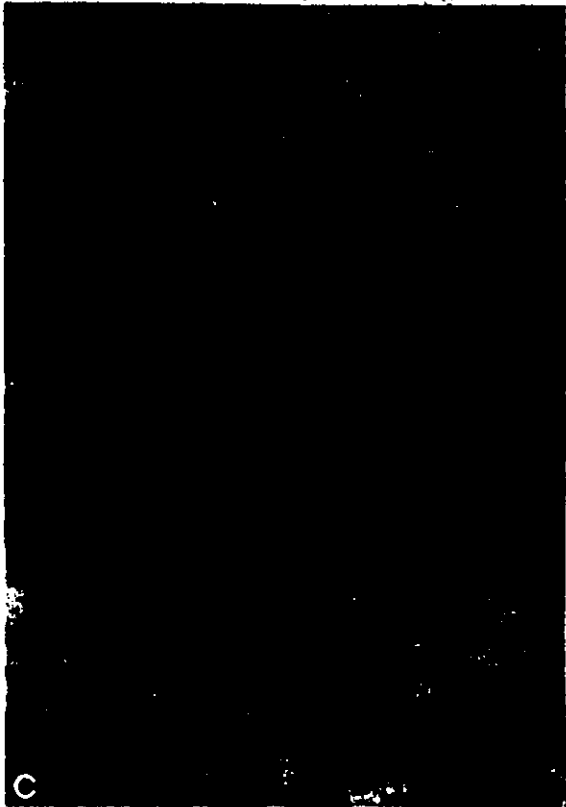
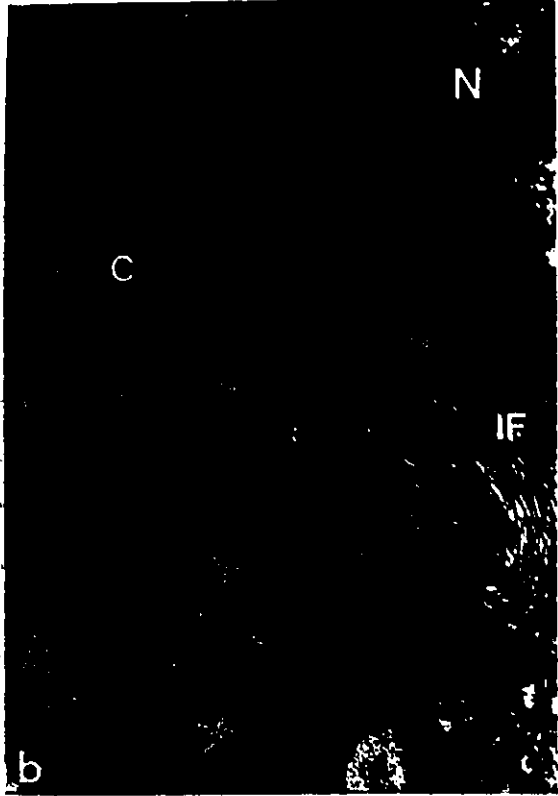


Figure 28 Morphology of mouse lymphocytes blocked in mitosis by a
4-hour exposure to taxol

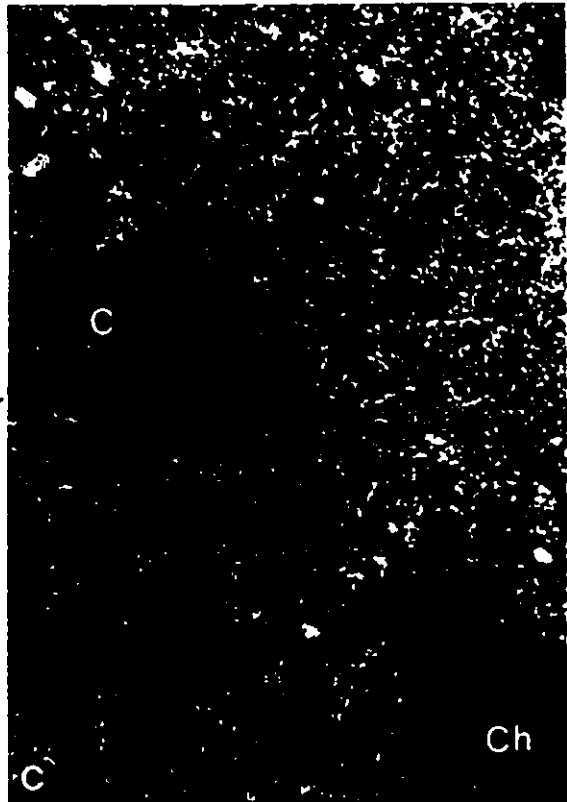
- a. Electron micrograph of a cell blocked in mitosis by taxol (10 $\mu\text{g/ml}$). The chromosomes display a prometaphase configuration. — 10 800 x —
- b. High magnification view of the interchromosomal region of the cell seen in (a) showing the complex criss-crossed arrangement of microtubules. — 35 600 x —
- c. Section of a taxol-treated mitotic cell showing microtubules extending from the centrosome(C) at various angles in the interchromosomal cytoplasm. Ch, chromosome. — 38 000 x



a



b



C

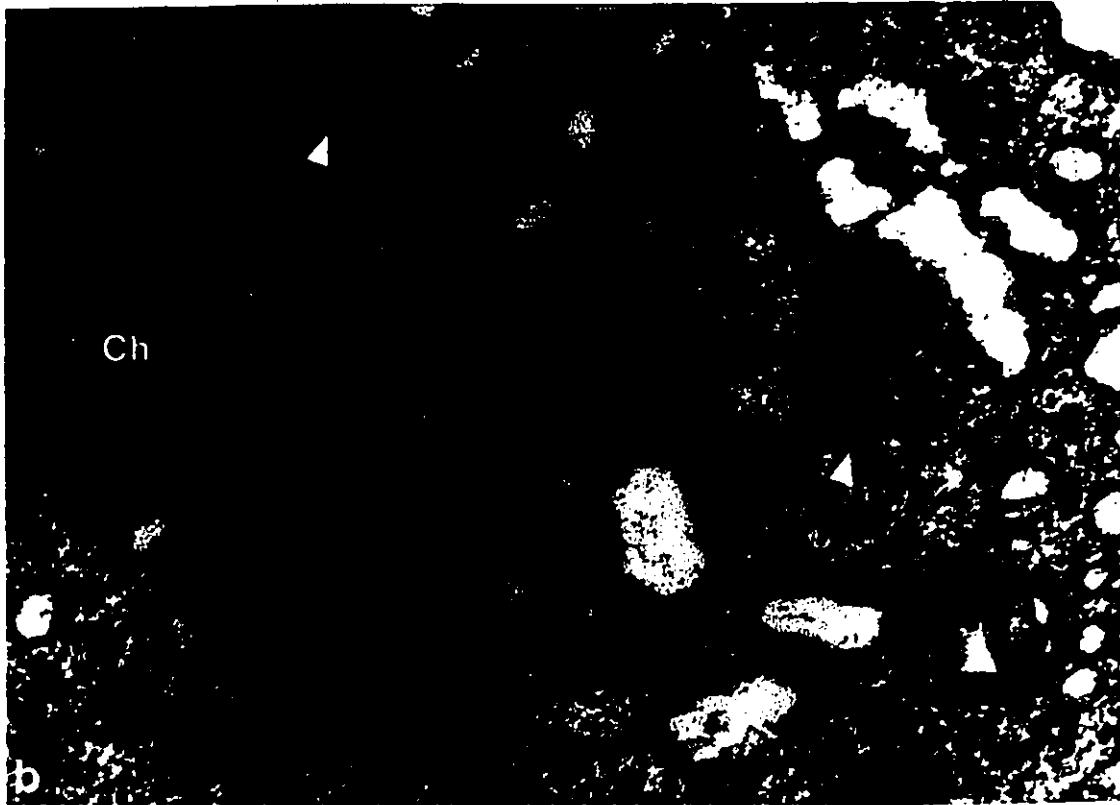
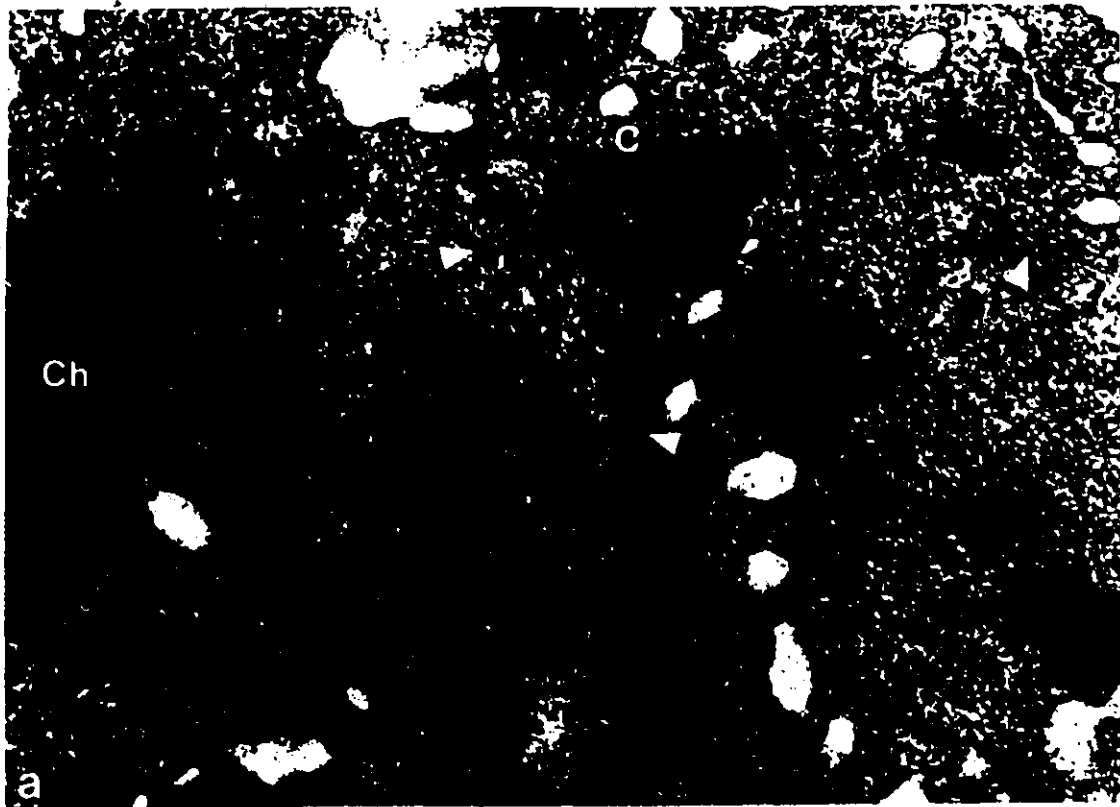
Ch

c

Figure 29 Electron microscopic observation of intermediate filaments in mitotic mouse lymphocytes after short-term treatment with taxol

a. Electron micrograph of a mitotic cell exposed to taxol for 1 hour. Small intermediate filament arrays (arrowheads) are observed in the vicinity of a polar centrosome (C) and at the cell periphery.

Ch, chromosome. — 36 000 x — b. Portion of a mitotic cell exposed to taxol for 4 hours. Small intermediate filament arrays (arrowheads) are found at the periphery of the cell and sometimes, as illustrated here, close to chromosomes (Ch). — 36 000 x



Short-term effects of microtubule specific drugs on vimentin and tubulin organizations in mouse splenic lymphocytes are schematically summarized in figures 30 and 31.

EFFECTS OF COLD TREATMENT

Populations of resting and stimulated lymphocytes were placed on ice for 1 hour prior fixation for immunofluorescence. The cells were briefly fixed and permeabilized on ice, and then further processed for immunofluorescence staining. This cold treatment results in the disassembly of most microtubules (Fig. 32c, d; Fig. 32g, h). Resting cells consistently appear to be more sensitive to cold treatment than stimulated cells. Using the monoclonal anti-tubulin, short residual microtubules can still be detected on the centrosome in only approximately 8% of resting cells, but are detected in at least 50% of stimulated cells. Cold-disassembly of microtubules does not appear to affect the organization of the vimentin system. Vimentin patterns in resting (Fig. 32a, b) and stimulated cells exposed to cold treatment (Fig. 32e, f) are identical to those observed in control cultures. Normal patterns of microtubules could be restored in both resting and stimulated cells exposed to cold by a post-incubation at 37°C for 10 minutes, also without affecting the vimentin system.

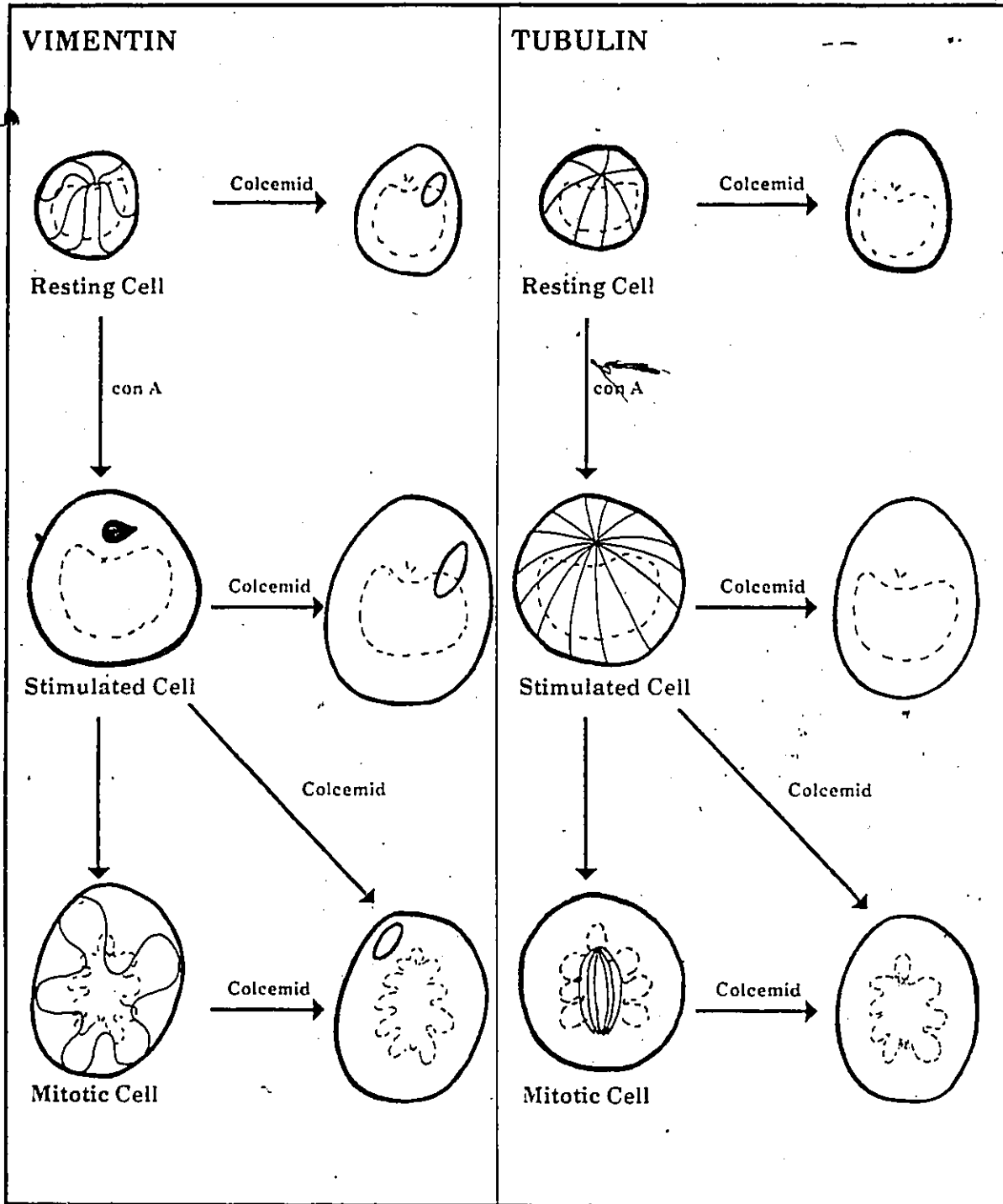


Figure 30 Short-term effects of colcemid on vimentin and tubulin organization in lymphocytes

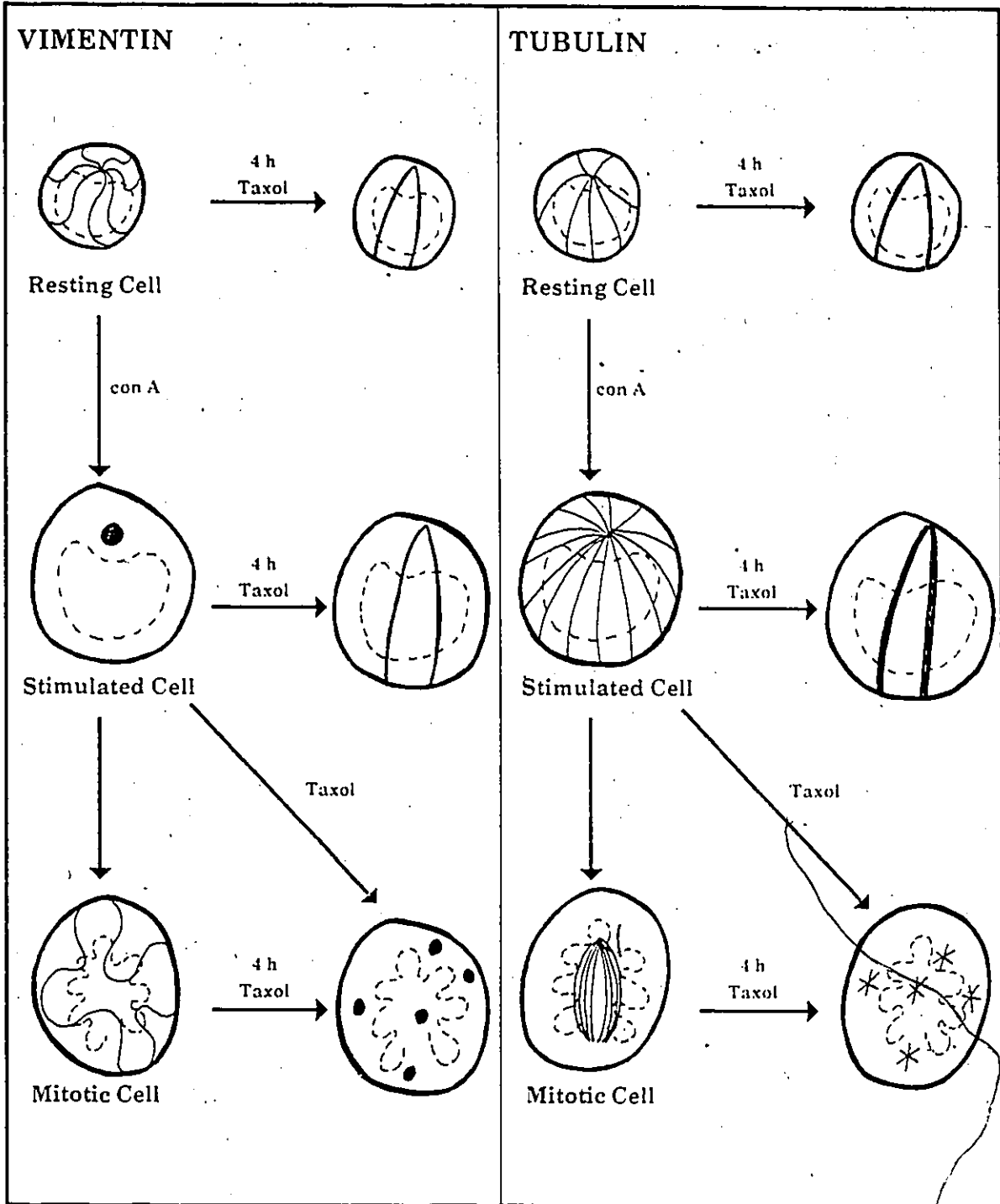
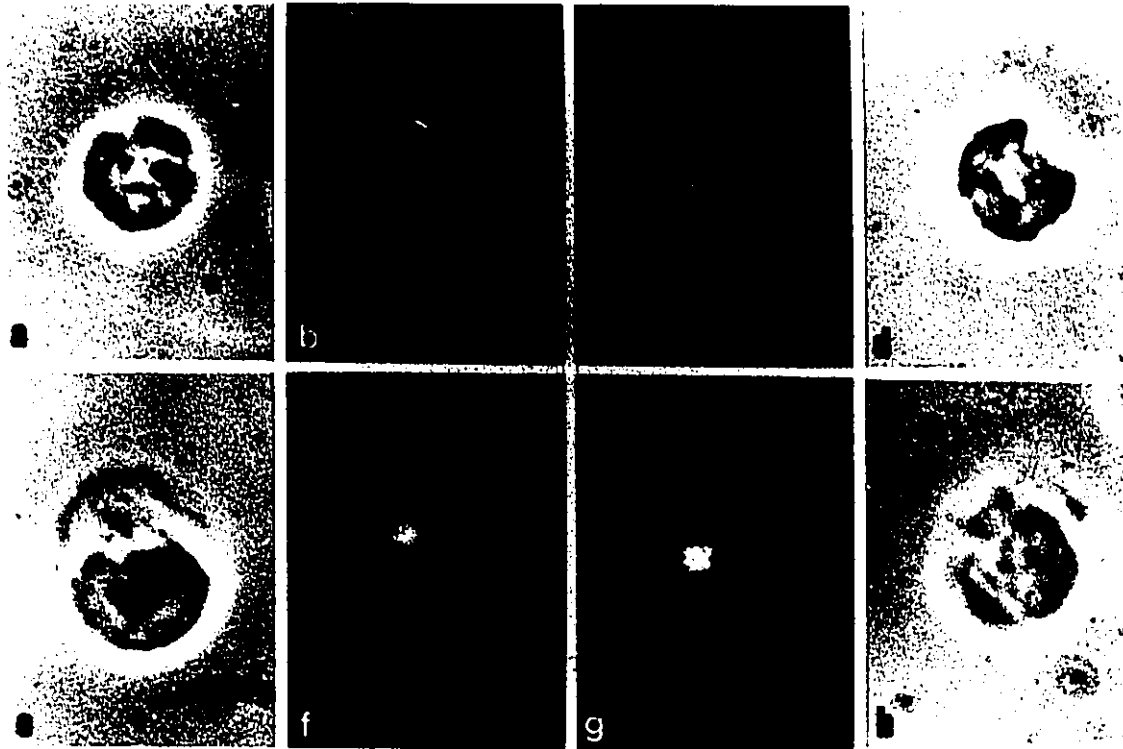


Figure 31 Short-term effects of taxol on vimentin and tubulin organization in lymphocytes

Figure 32 Effects of cold on the organization of vimentin and tubulin in mouse lymphocytes

Lymphocytes were exposed to cold for 1 hour. Cells seen by phase contrast in (a) and (e) were then stained with the rabbit antiserum to vimentin (b, f) and cells in (d) and (h) were labelled with the murine monoclonal anti-tubulin (c, g). In resting (a - d) and stimulated (e - h) cells, the patterns of vimentin are not affected by the disassembly of microtubules by cold. Short residual microtubules are detected on the centrosome of the cold-treated stimulated cell shown in (g). — 2 000 x



ORGANIZATIONAL FATE OF VIMENTIN DURING MITOGENIC STIMULATION OF LYMPHOCYTES IN THE PRESENCE OF MICROTUBULE SPECIFIC DRUGS

It has been reported previously that mitogenic stimulation of lymphocytes is strongly inhibited or delayed by microtubule-disrupting drugs such as colchicine or colcemid (for a review see Thyberg, 1984). In contrast, it has also been shown that taxol does not affect the major events of mitogenic stimulation up to the first mitosis (Cuthbert and Shay, 1983; Brown *et al*, 1985). In the past, the effects of these drugs on lymphocyte functions have been related to their specific action on the microtubule system. As the results described above demonstrate that microtubule specific drugs also induce extensive reorganization of vimentin in lymphocytes, it appeared of particular interest to examine the organizational fate of this cytoskeletal system during mitogenic stimulation in the presence of colcemid or taxol. Either colcemid or taxol was added at the same time as con A to lymphocyte populations which were then cultured for 48 hours in the continued presence of the drug. To evaluate the effects of long-term exposures of lymphocytes to these drugs, independently of the stimulation process, control resting populations were also cultured for 48 hours in the presence of colcemid or taxol.

The continued presence of colcemid (1 μ g/ml) during mitogenic stimulation of lymphocytes did not prevent all of the morphological changes that occur normally during blastogenesis. After 48 hours of stimulation, colcemid-treated cells have increased their size up to the control range (10-18 μ m in diameter) and display nuclear morphotypes characteristic of control stimulated populations (Fig. 33a). However, the cells also show structural alterations.

The Golgi apparatus is poorly developed and usually dispersed in the cytoplasm surrounding the centrosome, which has been displaced towards the plasma membrane (Fig. 33b). Colcemid also affects the entry of stimulated cells into S phase, as indicated by a markedly reduced incorporation of [³H]-thymidine at 48 hours (Table 1). Nevertheless, 5 to 10% of colcemid-treated stimulated cells does proceed through S phase and is arrested in mitosis. Such mitotic cells always exhibit highly aberrant chromosome arrangements and elongated cell shapes (Fig. 35g). By immunofluorescence, long-term effects of colcemid on both resting (Fig. 35a - c) and con A-stimulated cells (Fig. 35d - f) are indistinguishable from those observed after short-term treatment: microtubules are disassembled and the vimentin system is rearranged into a ring-like structure. In stimulated lymphocytes arrested in mitosis (Fig. 35g, h), vimentin has formed two ring structures, one at each extremity of these elongated cells. At the ultrastructural level, these cells display abnormal arrangements of chromosomes (Fig. 34a). Arrays of intermediate filaments are found close to the plasma membrane (Fig. 34b).

Table 1 EFFECT OF COLCEMID (1 µg/ml) AND TAXOL (10 µg/ml) ON [³H]-THYMIDINE INCORPORATION INTO Con A STIMULATED MOUSE SPLENIC LYMPHOCYTES.*

<u>Treatment</u>	<u>[³H]-thymidine incorporation (cpm)**</u>	<u>Percent of control</u>
Control resting (-conA)	5 473 ± 440	100
Stimulated (+ conA)	104 218 ± 10 676	1 904
conA + 1 µg/ml colcemid	20 902 ± 993	381
conA + 10 µg/ml taxol	78 712 ± 12 115	1 438

*Colcemid or taxol was added to cultures simultaneously with con A

** Values for [³H]-thymidine are means of triplicates ± SD

Figure 33 Morphology of a mouse lymphocyte stimulated in the continued presence of colcemid

a. Electron micrograph showing the morphological characteristics of a cell stimulated in presence of $1 \mu\text{g/ml}$ colcemid. The morphotype III nucleus (N) displays a structurally complex nucleolus (Nu). The centrosome (C) has been displaced near the plasma membrane. — 15 000 x —

b. High magnification view of the centrosome region of the section presented in (a). The centrosome is devoid of any microtubules. The Golgi apparatus has been dispersed and only a few fragments (arrows) of this organelle are still seen in proximity to the centrosome. N, nucleus. — 40 900 x



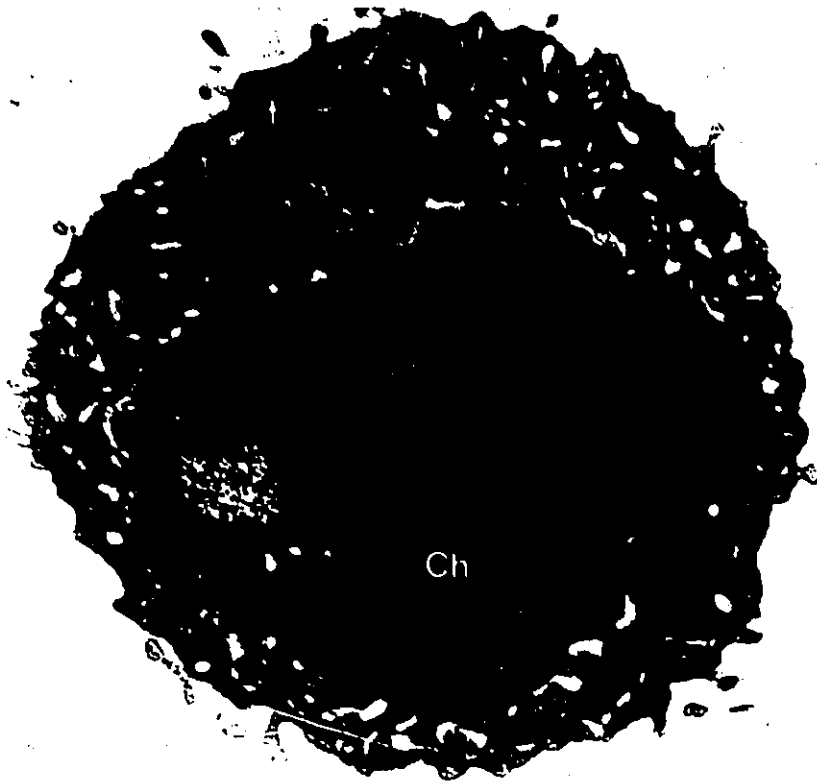
a



b

Figure 34. Electron microscopic observation of a mouse lymphocyte stimulated in the continued presence of colcemid and blocked in mitosis

a. Electron micrograph illustrating the aberrant arrangement of chromosomes (Ch) in a cell arrested in mitosis by exposure to 1 $\mu\text{g/ml}$ colcemid for the full stimulation period. Chromosomes appear to be linked to each other, forming a continuous arrangement. — 12 600 \bar{x} — b. High magnification view of the section presented in (a). Intermediate filament arrays (arrowheads) are seen close to the plasma membrane away from the chromosome mass. — 51 300 x



a

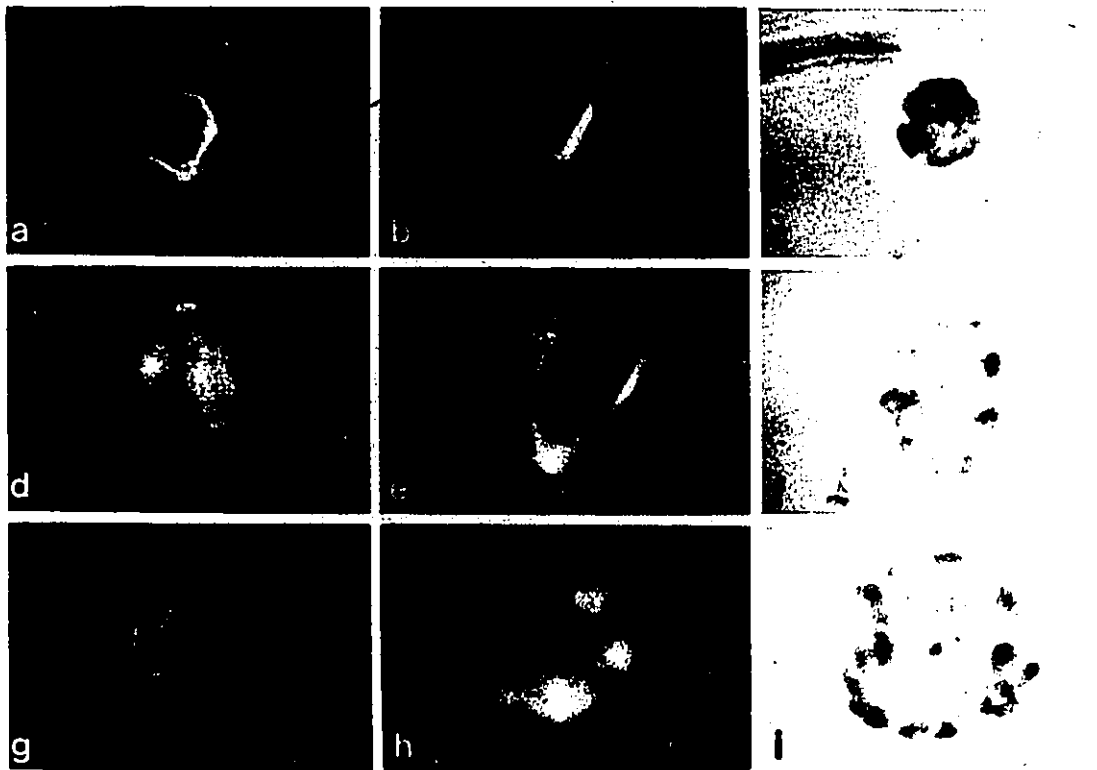
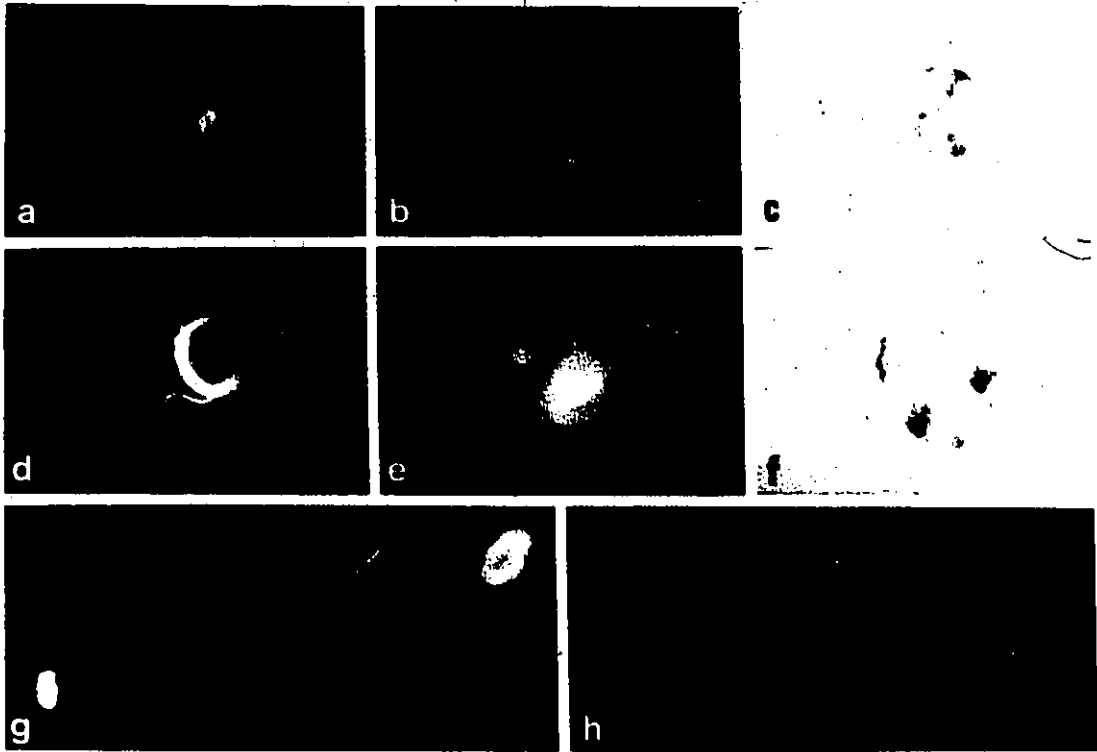


Figure 35 Long-term effects of colcemid on the organization of vimentin in mouse lymphocytes

Resting lymphocytes were either exposed to 1 $\mu\text{g/ml}$ colcemid (a - c) or to 1 $\mu\text{g/ml}$ colcemid and 4 $\mu\text{g/ml}$ con A (d - h) for 48 hours. Cells were then labelled with the rabbit antiserum to vimentin (a, d, g), counterstained with Hoechst 33258 (b, e, h) and observed by phase contrast as shown in (c) and (f). Vimentin is rearranged in a ring-like structure in resting (a - c) and stimulated (d - f) cells cultured in the continued presence of colcemid. In the elongated mitotic cell seen in (g), vimentin has formed two ring structures, one at each extremity, away from the aberrant chromosome mass (h). — 2 000 x

Figure 36 Long-term effects of taxol on the organization of vimentin and tubulin in mouse lymphocytes

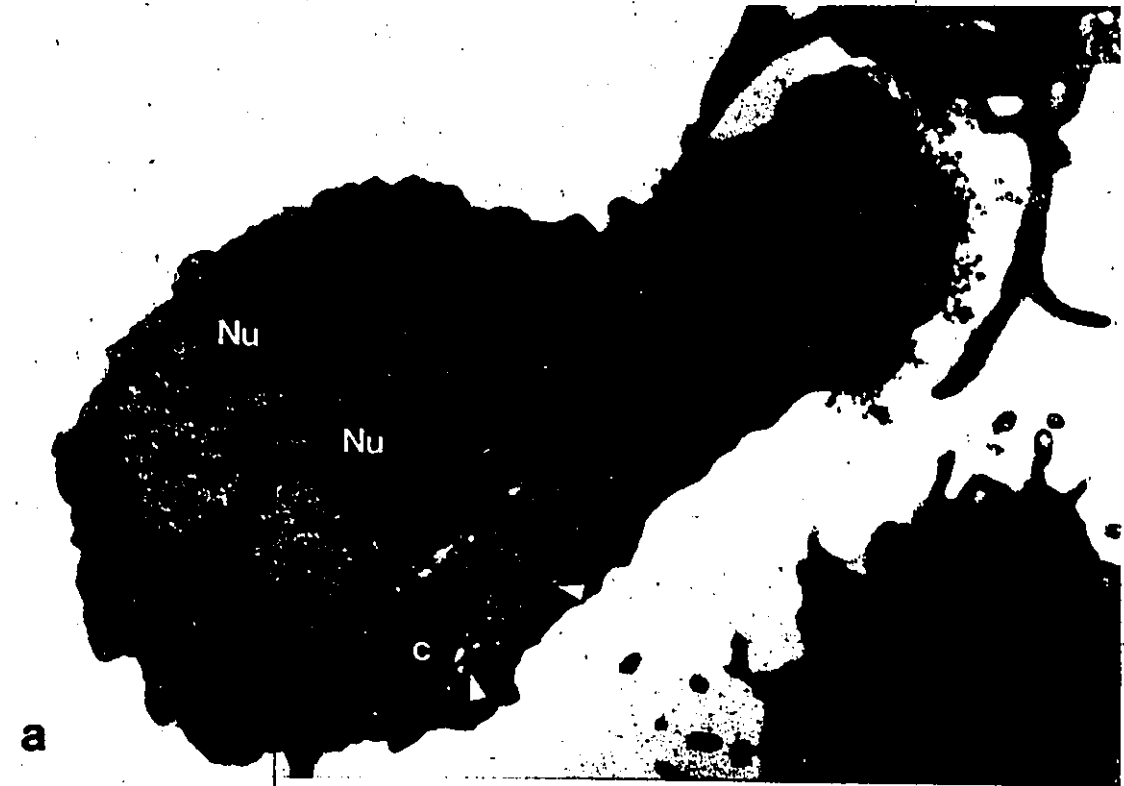
Resting lymphocytes were either exposed to 10 $\mu\text{g/ml}$ taxol (a - c) or to 10 $\mu\text{g/ml}$ taxol and 4 $\mu\text{g/ml}$ con A (d - i) for 48 hours. Cells were then double labelled with the rabbit antiserum to vimentin (a, d, g) and the murine monoclonal anti-tubulin (b, e, h), and observed by phase contrast (c, f, i). The parallel reorganization of vimentin filaments and microtubules is maintained in a resting cell cultured with taxol (a - c). Vimentin shows diffuse distribution and microtubule bundles are still present in a cell stimulated in the continued presence of taxol (d - f). When such a cell is arrested in mitosis (g - i), vimentin appears in filamentous arrays surrounding the multiple microtubule asters. — 2 000 x



Taxol (10 $\mu\text{g/ml}$) does not affect the major events of lymphocyte response to con A, including the increase in cell size, the structural changes of nucleus and cytoplasm (Fig. 37; Fig. 36f), and the DNA synthesis that ensues at 48 hours PS (Table 1). The drug-treated stimulated cells, however, do not proliferate but are arrested in mitosis (Fig. 36i). By immunofluorescence, the parallel bundling of vimentin filaments and microtubules observed in response to short exposures of lymphocytes to taxol is maintained in resting cells that were cultured in the presence of the drug for 48 hours (Fig. 36a - c). In contrast, in cells that were stimulated with con A in the continued presence of taxol (Fig. 36d - i), vimentin has reorganized independently of the microtubule system. Microtubule bundles are still present in cells that have progressed through stimulation (Fig. 36e; Fig. 37b). When such cells do enter mitosis, the microtubule bundles are apparently disassembled to form multiple asters (Fig. 36h). Vimentin does not co-localize with tubulin in stimulated cells nor in mitotic cells, but shows a sequence of reorganizations that resembles that observed in control cells. Vimentin is distributed diffusely in the cytoplasm of fully stimulated cells (Fig. 36d) and is arranged as filamentous arrays surrounding the aberrant spindle in the cells that have entered mitosis (Fig. 36h). By electron microscopy, a fibrogranular mass similar to that seen in control stimulated lymphocytes was not observed in large cells stimulated in presence of taxol. The only structural characteristics that distinguish stimulated taxol-treated cells from the control are the position of the centrosome/Golgi complex and the presence of the bundle of microtubules extending from the centrosome (Fig. 37a).

Figure 37 Electron microscopic observation of a mouse lymphocyte stimulated in the continued presence of taxol

- a. Electron micrograph illustrating the morphological characteristics of a cell stimulated in presence of 10 $\mu\text{g/ml}$ taxol. The centrosome(C) region and a microtubule bundle passing near the surface of the morphotype II nucleus (N) are seen. The Golgi apparatus (arrowheads) is not structurally altered and is still associated with the centrosome. Nu, nucleolus. — 15 400 x —
- b. High magnification of the section presented in (a) showing the centrosome/Golgi complex closely apposed to the plasma membrane. N, nucleus. Nu, nucleolus. — 41 800 x



Long-term effects of microtubule specific drugs on vimentin and tubulin organizations in mouse splenic lymphocytes are schematically summarized in figures 38 and 39.

ORGANIZATIONAL FATE OF VIMENTIN DURING REDISTRIBUTION OF SURFACE IMMUNOGLOBULIN

In numerous studies, the effects of colcemid and taxol on the short-term response of lymphocytes to the binding of a ligand, such as the anti-Ig-induced redistribution of sIg on B cells, have been causally related to the action of these drugs on the microtubule system. Yet, considering the effects of colcemid or taxol on the organization of the vimentin filaments in resting lymphocytes, it appears possible that the effects of these drugs on sIg redistribution may causally implicate not only microtubules but also the vimentin system. Possible involvement of vimentin filaments in the sIg capping process had been previously suggested (Zucker-Franklin *et al.*, 1979; Dellagi and Brouet, 1982). To further characterize the possible involvement of vimentin filaments during that process, the organizational fate of vimentin and its relation to both the site of sIg cap and the microtubule system (microtubules and centrosome) were examined in normal and drug-treated cells using immunofluorescence and electron microscopy.

At room temperature, exposure of lymphocyte populations to appropriate concentrations (see Materials and Methods) of rhodamine isothiocyanate- or ferritin-conjugated goat anti-mouse IgG results in the rapid cross-linking and redistribution of sIg on B cell subpopulation. As previously reported (Rogers *et al.*, 1981), the maximal percentage of sIg-positive cells

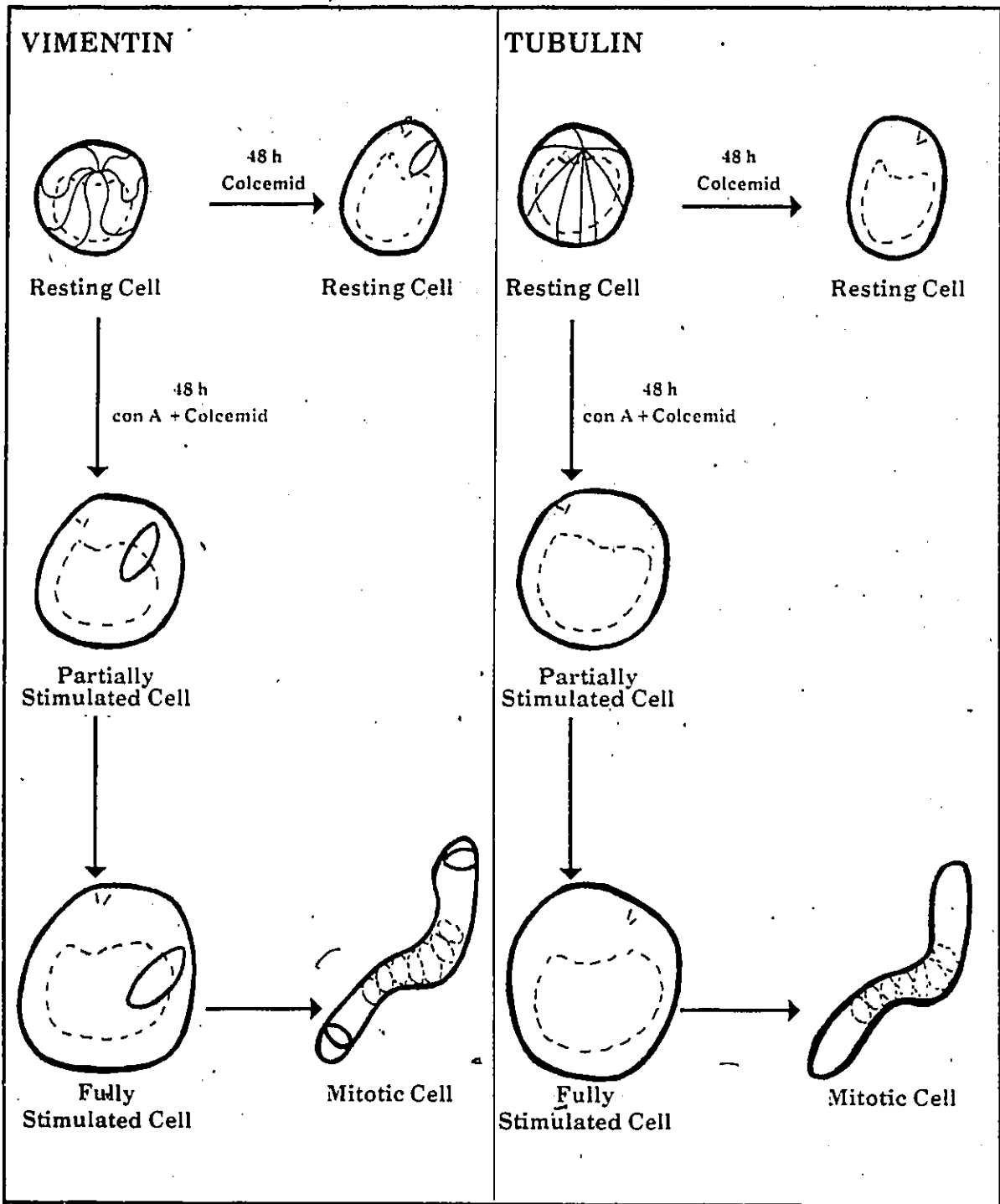


Figure 38 Organizational fate of vimentin and tubulin during mitogenic stimulation of lymphocytes in the continued presence of colcemid

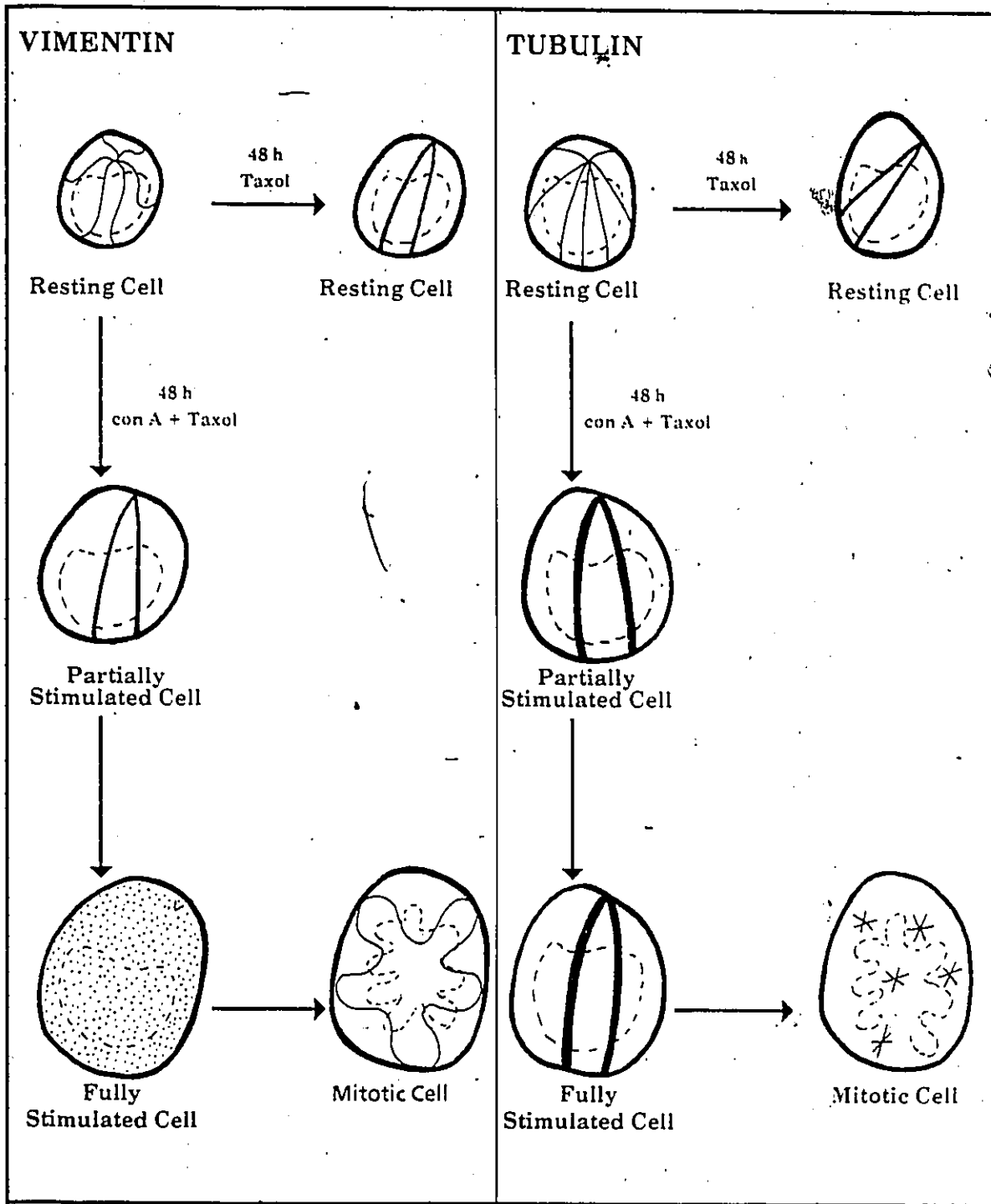


Figure 39 Organizational fate of vimentin and tubulin during mitogenic stimulation of lymphocytes in the continued presence of taxol

showing a cap is obtained after a 7-minute incubation with the ligand. This period of incubation was therefore used in all experiments.

The tubulin and vimentin patterns in labeled cells were examined by immunofluorescence at different stages during the redistribution of sIg. In B cells that show a uniform distribution of their receptors on the surface, networks of vimentin filaments (Fig. 40d - f) appear identical to those observed in the unlabelled T cells (Fig. 40a - c) and patterns of microtubules stay intact (Fig. 40g - i). During patch formation, patterns of vimentin filaments (see for example figure 41a, b) and microtubules are maintained. The migration of the patches is accompanied by the formation of an obvious uropod at one pole of the cells and is followed by the subsequent aggregation of receptors into a single cap over the newly formed uropod. The centrosome is always detected beneath the site of the cap and the radial pattern of microtubules is preserved during cap formation as previously described by Rogers *et al.* (1981) (Fig. 41d - f). Occasionally, diffuse tubulin staining is also seen at the site of the cap in cells displaying a well organized radial pattern of microtubules (Fig. 41g - i). In contrast, cap formation is accompanied by extensive changes in the organization of vimentin. The filamentous vimentin network of resting cells is dismantled during cap formation and a diffuse accumulation of vimentin is observed underneath the cap of sIg (Fig. 41a - c). By electron microscopy, the centrosome/Golgi complex is located underneath the site of the sIg cap (Fig. 42a). Arrays of intermediate filaments are not visualized in capped cells but rather a cytoplasmic mass of fibrogranular material is observed beneath the cap in sections passing through the centrosomal region (Fig. 42b).

Figure 40 Organization of vimentin and tubulin upon binding of anti-Ig on the surface of resting mouse lymphocytes

Resting lymphocytes seen by phase contrast in (c), (f), and (i) were exposed to 200 $\mu\text{g/ml}$ rhodamine goat anti-mouse IgG for 7 minutes (a, d, g) and then labelled with the rabbit antiserum to vimentin (b, e) or the rabbit antiserum to tubulin (h). In B cells that show a uniform distribution of receptor-ligand complexes, organization of vimentin (d - f) is identical to that observed in unlabelled T cells (a - c) and pattern of microtubules (g - i) is unchanged. — 2 000 x

Figure 41 Organization of vimentin and tubulin during redistribution of sIg on the surface of mouse lymphocytes

Resting lymphocytes seen by phase contrast in (c), (f), and (i) were exposed to 200 $\mu\text{g/ml}$ rhodamine goat anti-mouse IgG for 7 minutes (a, d, g) and then labelled with the rabbit antiserum to vimentin (b) or the rabbit antiserum to tubulin (e, h). Organization of vimentin at different stages during sIg redistribution is illustrated (a - c). Filamentous arrangements of vimentin are retained during patch formation (cell seen on the top middle) and, to some extent, in a cell that has not completed cap formation (cell on the left). In a capped B cell (cell on the right), vimentin is detected as a diffuse aggregate underneath the cap. The radial pattern of microtubules is maintained during cap formation (d - f; and g - h). A diffuse tubulin staining at the site of the cap is also observed in the cell shown in (h). Note that the centrosome is consistently found beneath the site of the cap. — 2 000 x

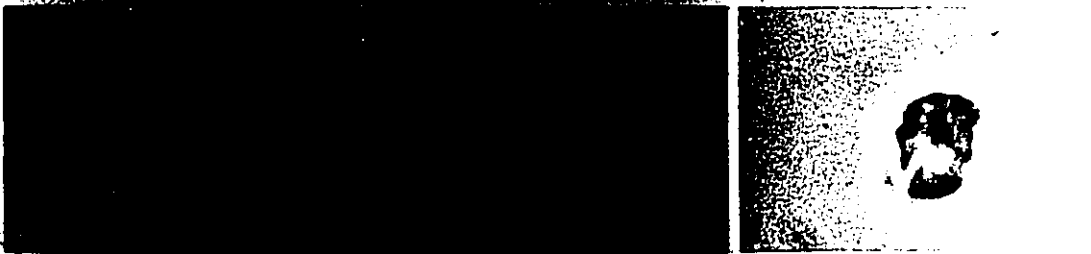
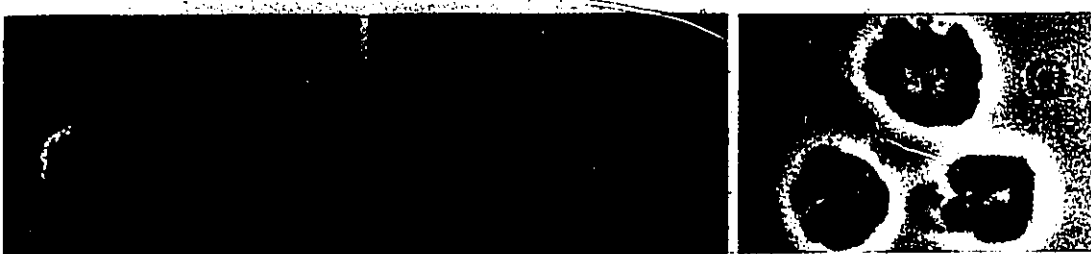
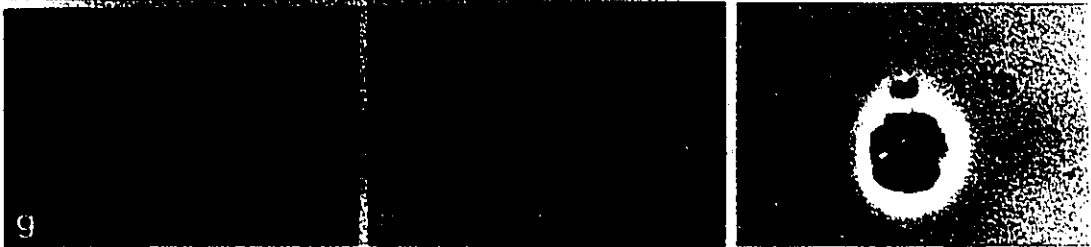
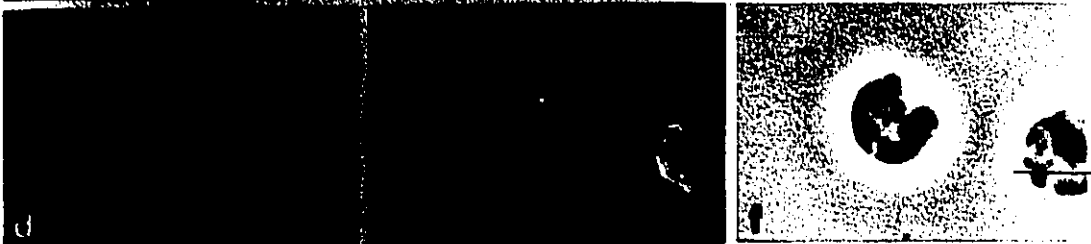
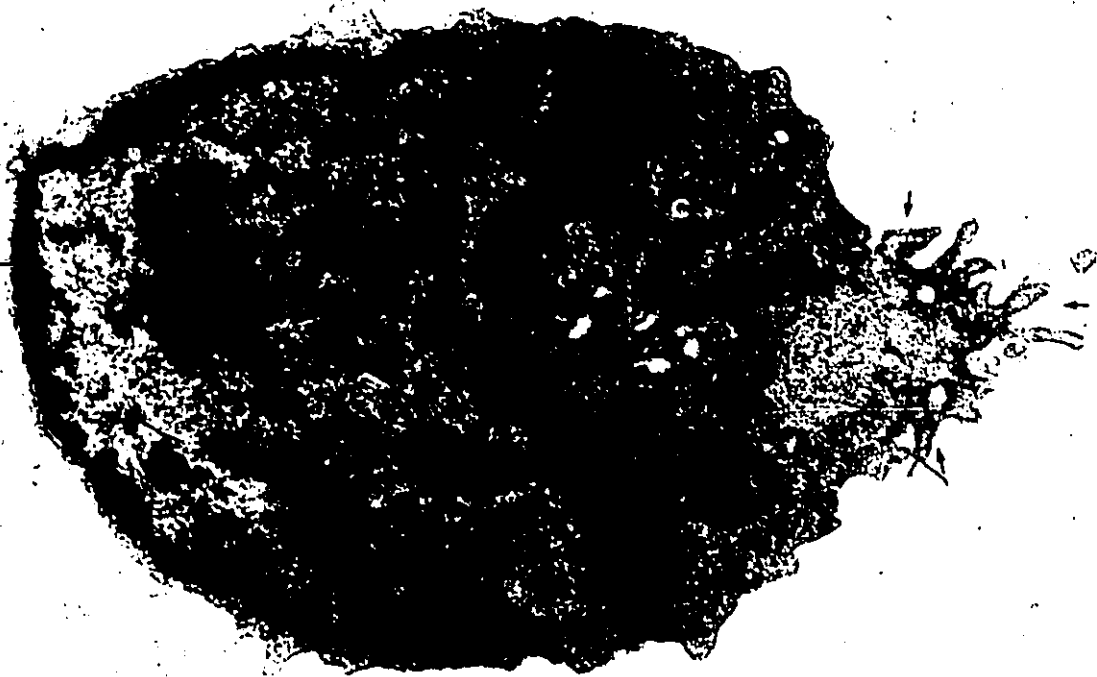


Figure 42 Electron microscopic observation of sIg capping on mouse lymphocytes

Resting lymphocytes were exposed to ferritin-conjugated goat anti-mouse IgG (see Materials and Methods) for 7 minutes and then processed for electron microscopy. a. Electron micrograph of a capped B cell. The ferritin label (arrows) is aggregated over the region of the cytoplasm containing the centrosome (C), the Golgi apparatus, and most of the mitochondria. A fibrogranular mass (M) is observed underneath the site of the cap. N, nucleus.

— 22 000 x — b. High magnification view of the section presented in (a) illustrating the structure of the fibrogranular mass observed underneath the site of the cap close to the centrosome/Golgi

complex. — 43 700 x



a



b

When resting cells pretreated for 4 hours with 1 $\mu\text{g/ml}$ colcemid or 10 $\mu\text{g/ml}$ taxol are exposed to anti-Ig, redistribution of sIg does proceed but the cap is formed anywhere on the cell surface without relation to the position of the centrosome (Figs. 43, 44). In capped cells pretreated with colcemid, microtubules are disassembled and only the centrosome can be detected with the polyclonal antiserum to tubulin (Fig. 43g - i). The vimentin ring observed in resting cells in response to this drug has disappeared. In approximately 20% of colcemid capped cells, vimentin appears as filamentous arrays that are particularly prominent in the cytoplasm underlying the site of the cap (Fig. 43a - c). In the other 80% of capped cells, vimentin is accumulated into a diffuse aggregate underneath the site of the cap (Fig. 43d - f). By electron microscopy, arrays of intermediate filaments were only occasionally seen in colcemid capped cells. However, the most striking feature of these cells is the presence of a cytoplasmic mass of fibrogranular material located at the site of the cap which usually does not show any relationship with the position of the centrosome (Fig. 45). No microtubules were observed in sections of colcemid capped cells.

In capped cells pretreated with taxol, microtubules are detected in one or few bundles running from the centrosome towards the site of the cap (Fig. 44d - f). The bundling of vimentin filaments, induced by the drug in resting cells, is maintained during cap formation. In capped cells, vimentin mainly appears as one or two large arrays of filaments extending from a focus near the plasma membrane and usually ending at the site of the cap (Fig. 44a - c). By electron microscopy, the cytoplasmic mass of fibrogranular material observed in normal and colcemid-capped cells is less prominent and shows a more granular structure in taxol capped cells. Bundles of microtubules are organized from the centrosome which is displaced to a position close to the plasma membrane

Figure 43 Organization of vimentin and tubulin during sIg capping on colcemid-treated mouse lymphocytes

Resting lymphocytes seen by phase contrast in (c), (f), and (i) were treated with 1 $\mu\text{g/ml}$ colcemid for 4 hours prior to exposure to 200 $\mu\text{g/ml}$ rhodamine goat anti-mouse IgG for 7 minutes (a, d, g). Cells were then labelled with the rabbit antiserum to vimentin (b, e) or the rabbit antiserum to tubulin (h). In colcemid capped cells, vimentin is detected either as filamentous arrays that are most prominent underneath the sIg cap (a - c) or as a diffuse aggregate underneath the sIg cap (d - f). Microtubules have been disassembled and only the centrosome is detected by the anti-tubulin in colcemid capped cells (g - h). Note that there is no correlation between the position of the centrosome and the site of the cap. — 2 000 x

Figure 44 Organization of vimentin and tubulin during sIg capping on taxol-treated mouse lymphocytes

Resting lymphocytes seen by phase contrast in (c) and (f) were treated with 10 $\mu\text{g/ml}$ taxol for 4 hours prior to exposure to 200 $\mu\text{g/ml}$ rhodamine goat anti-mouse IgG for 7 minutes (a, d). Cells were then labelled with the rabbit antiserum to vimentin (b) or the rabbit antiserum to tubulin (e). In the taxol capped cell shown in (a - c), vimentin appears as a large filamentous array extending the length of the cell. Microtubules (d - f) are also arranged in a few thick bundles running from a site on the plasma membrane towards the site of the cap. — 2 000 x

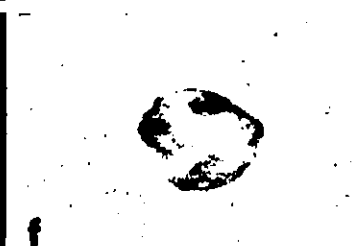
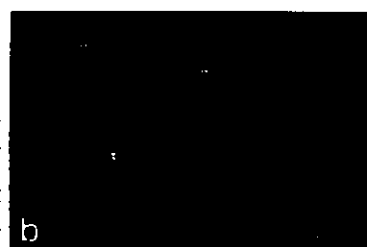
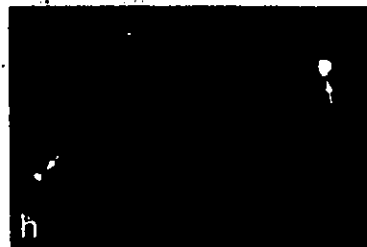
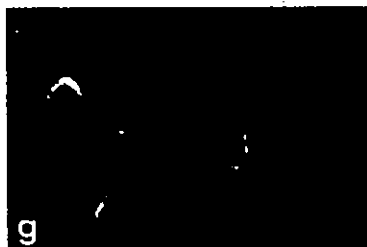
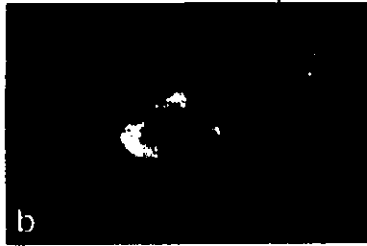
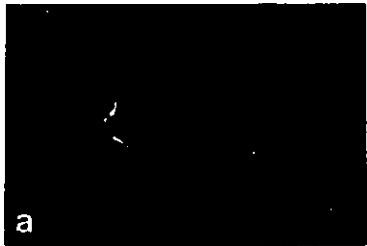


Figure 45. Electron microscopic observation of sIg capping on colcemid-treated mouse lymphocytes

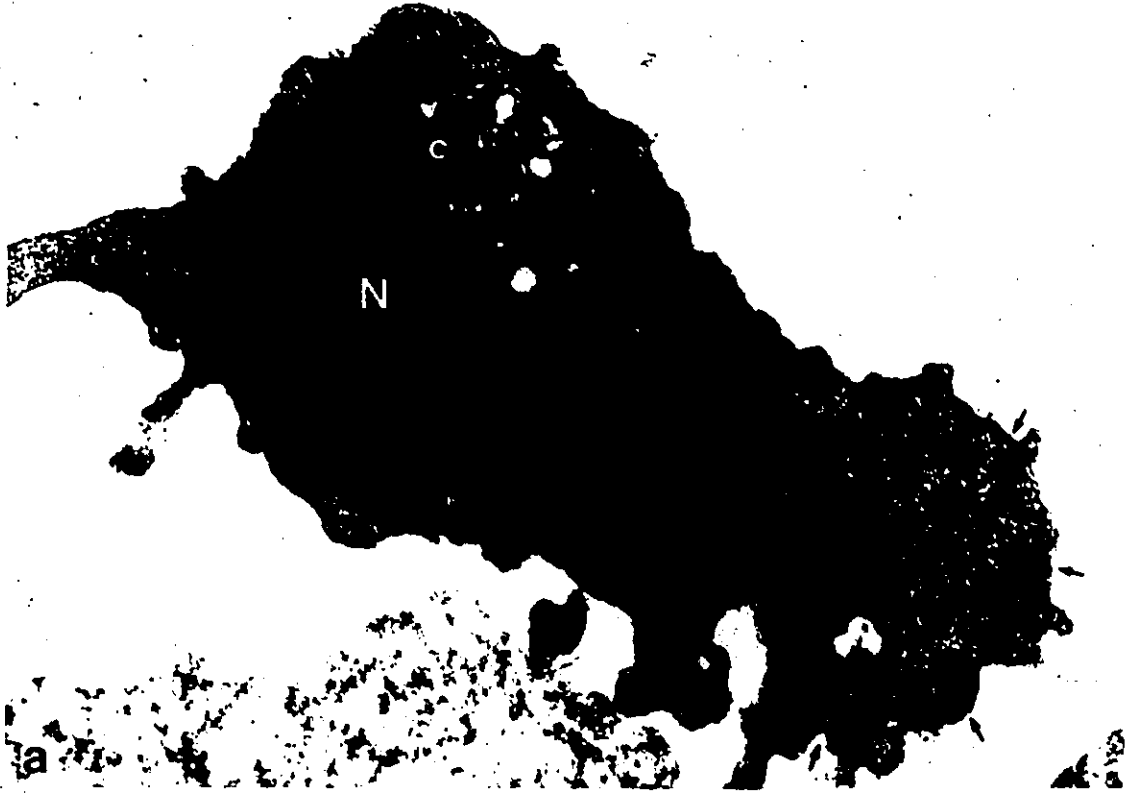
Resting lymphocytes were treated with 1 $\mu\text{g/ml}$ colcemid for 4 hours prior to exposure to ferritin-conjugated goat anti-mouse IgG for 7 minutes. Cells were then processed for electron microscopy.

a. Electron micrograph of a colcemid capped B cell. The ferritin-label (arrows) is aggregated over the uropod. The cytoplasmic organelles are excluded from the region of the cap, the Golgi apparatus is fragmented and the position of the centrosome (C) does not show any spatial relationship to the site of the cap. A

fibrogranular mass (M) is found underneath the cap. N, nucleus.

— 22 000 x — b. High magnification view of the centrosome region in the section presented in (a). The centrosome has been displaced close to the plasma membrane and is devoid of any microtubules.

— 43 700 x — c. High magnification view of the section (a) showing the structure of the fibrogranular mass observed underneath the cap. — 43 700 x



without any relationship to the site of the cap (Fig. 46). Some microtubules can be observed that terminate on the plasma membrane at the site of the cap (Fig. 46c). Intermediate filament arrays were not evident in these cells but may have been obscured by the presence of microtubules.

Organizational fate of vimentin and tubulin during sIg capping in normal and drug-treated mouse splenic lymphocytes is summarized schematically in figure 47.

ORGANIZATIONAL FATE OF VIMENTIN AND ITS SPATIAL RELATIONSHIP TO THE CENTROSOME DURING DIVISION IN CELLS THAT GROW ATTACHED AND IN SUSPENSION

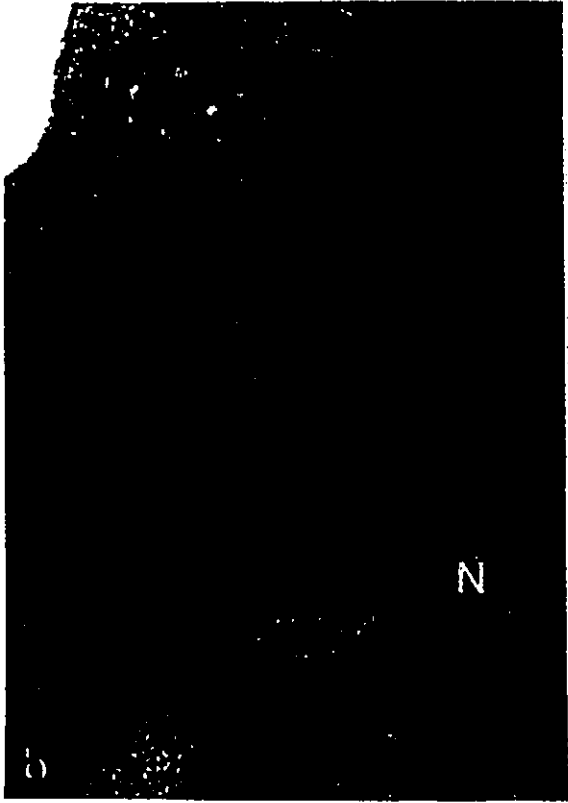
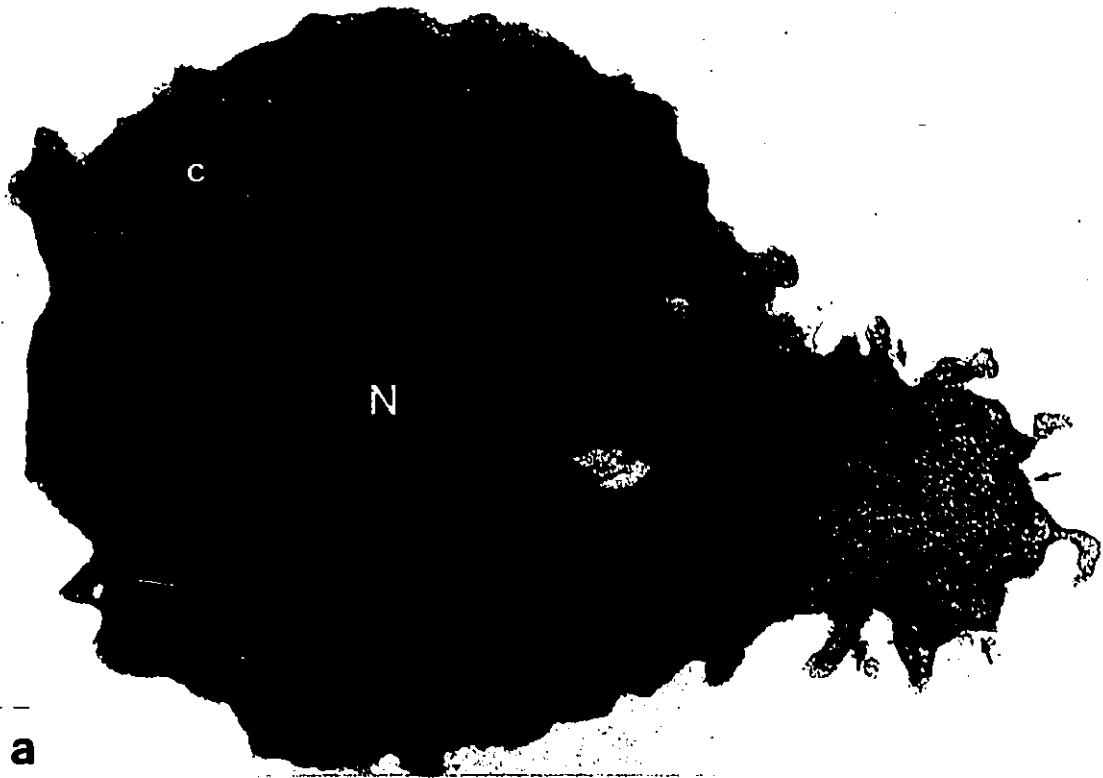
The mitotic fate of intermediate filaments had been previously examined only in flat cells that grow attached to a substrate. In these cells, interphase cytoplasmic networks of intermediate filaments were transiently either rearranged or disintegrated during mitosis but, in both cases, were reestablished during cytokinesis in early G₁ daughter cells. The organizational fate of vimentin during mitosis and cytokinesis of lymphocytes presented above in this thesis constitutes the first description of cytoplasmic rearrangements of intermediate filaments in suspension cells. In lymphocytes, the vimentin system is rearranged from a diffuse aggregate located near the centrosome into a transient filamentous cage-like structure during mitosis and is reaggregated at a site close to the midbody in each daughter cell during cytokinesis. Double immunofluorescence has further demonstrated that the spatial relationship between the vimentin system and the centrosome observed prior to mitosis in

Figure 46 Electron microscopic observation of sIg^c capping on taxol-treated mouse lymphocytes

Resting lymphocytes were treated with 10 $\mu\text{g/ml}$ taxol for 4 hours prior to exposure to ferritin-conjugated goat anti-mouse IgG for 7 minutes. Cells were then processed for electron microscopy.

a. Electron micrograph of a taxol capped B cell. The ferritin-label (arrows) is aggregated over the uropod. The centrosome (C)/Golgi complex has been displaced towards the plasma membrane without any relationship to the site of the cap. A microtubule bundle is seen extending near the surface of the nucleus (N). — 22 000 x —

b. High magnification view of the centrosome region in the section presented in (a). N, nucleus. — 43 700 x — c. High magnification view of the section (a) showing some microtubules (arrows) that terminate on the plasma membrane at the site of the cap. — 43 700 x



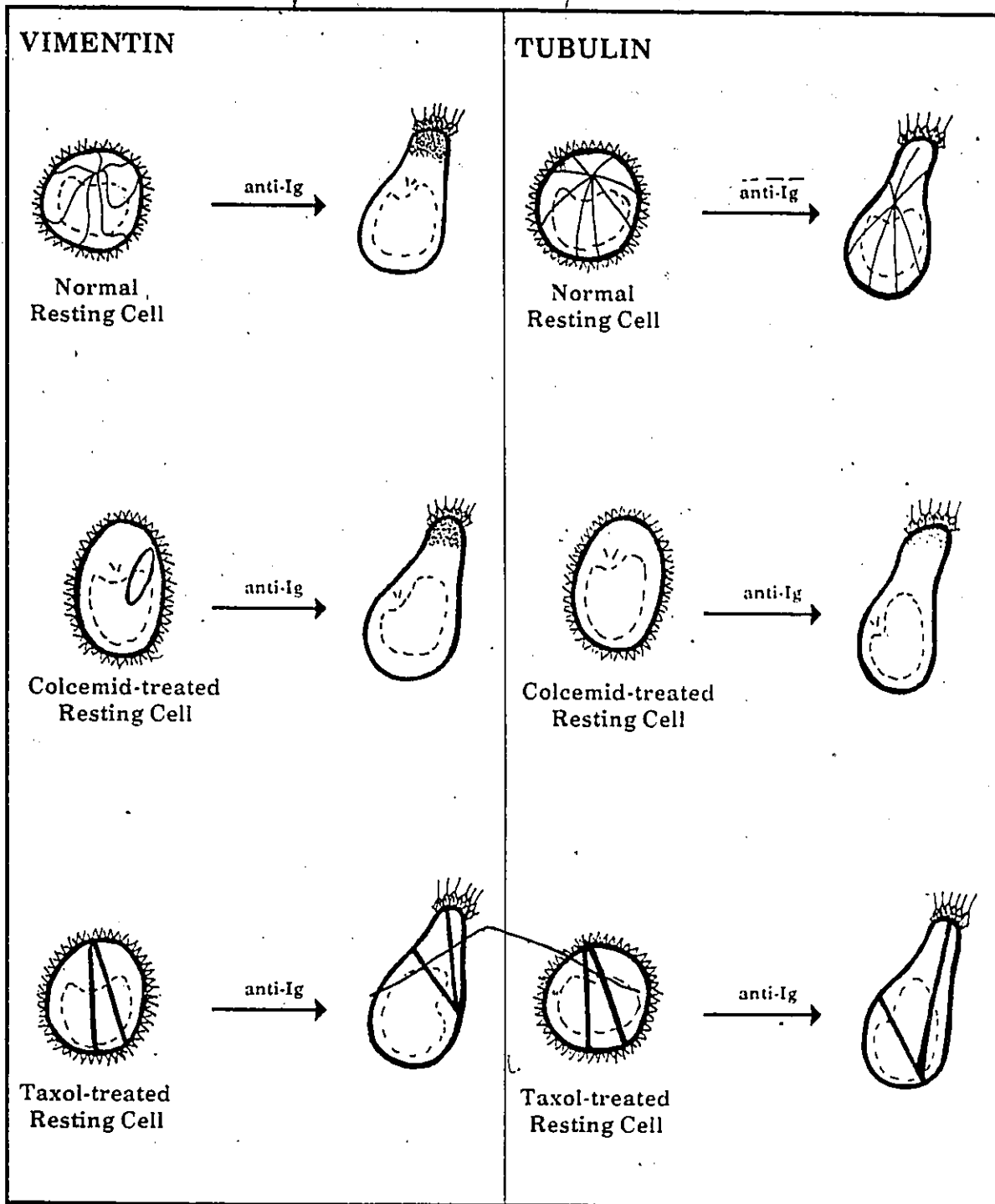


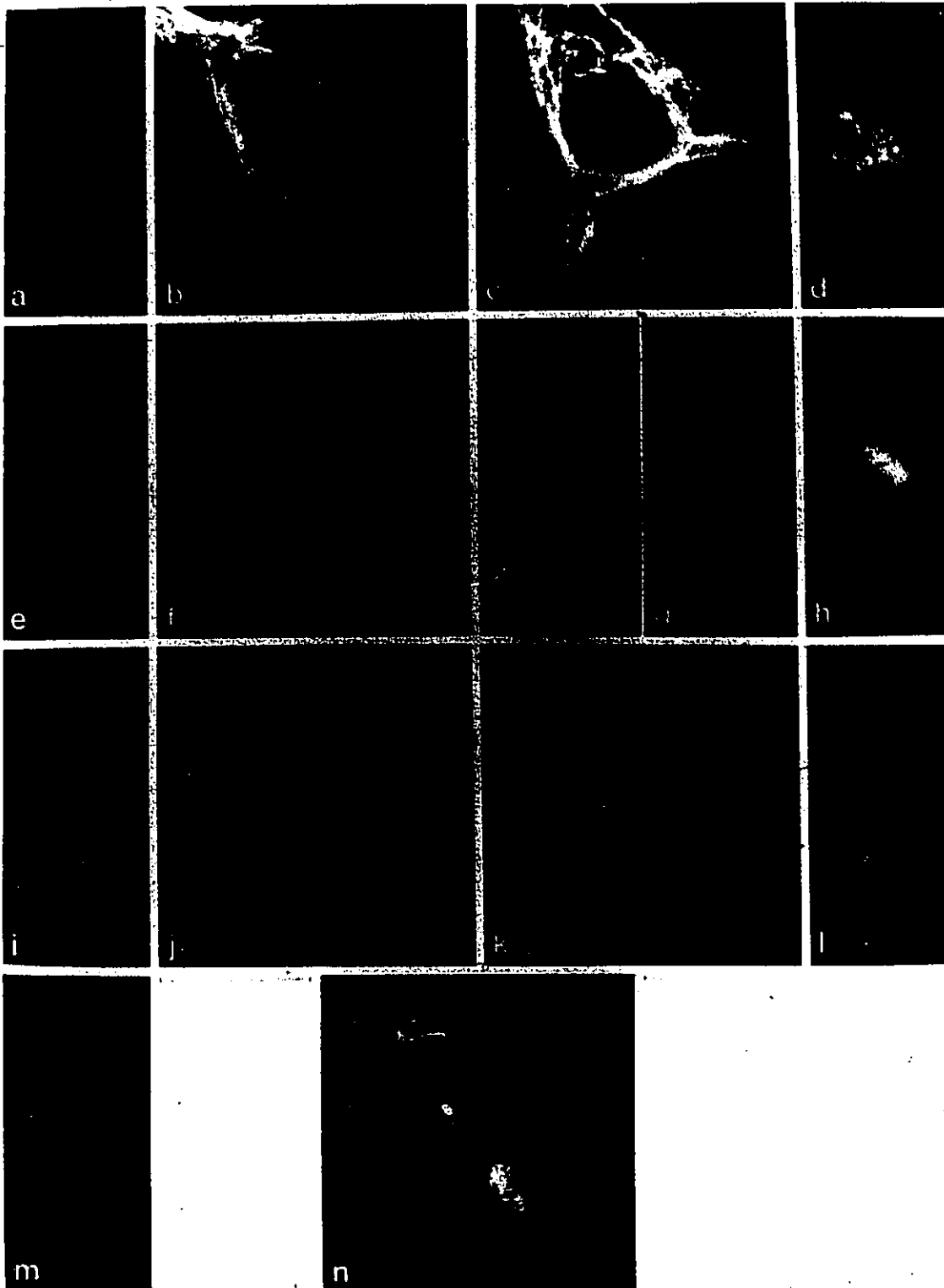
Figure 47 Organizational fate of vimentin and tubulin during capping of sIg in normal and drug-treated mouse splenic B cells

lymphocytes is reestablished in early G₁ daughter cells, as the aggregation of vimentin is paralleled by the relocalization of the centrosomes from the poles to a position close to the midbody during cytokinesis. Because such features in the behavior of intermediate filaments and centrosomes had not been previously observed in other cells, it appeared of interest to determine if they were peculiar to the lymphocyte system or could be of more general occurrence. Hence, a comparative study of the organizational fate of vimentin and its relationship to the microtubule system during mitosis and cytokinesis was performed by immunofluorescence on attached and suspension cell lines as well as on mouse L cells that were grown attached or in suspension.

Observations were carried on two attached cell lines, mouse 3T3 fibroblasts which express only vimentin-type intermediate filaments and epithelial PtK2 cells which contain both cytokeratin and vimentin filaments. Figure 48 illustrates the major features of the mitotic fate of vimentin in 3T3 cells as revealed with the rabbit antiserum to vimentin and the monoclonal anti-vimentin (from Dr. Marceau, Université Laval). The cytoplasmic network of vimentin filaments observed during interphase (Fig. 48a - d) in these cells is retained during prophase. As the cells round up and the nuclear membrane is disrupted during prometaphase, vimentin filaments are reorganized into a cage-like structure that surrounds the mitotic apparatus through metaphase (Fig. 48e - h) and anaphase. The cage is then constricted at the midbody during telophase/cytokinesis (Fig. 48i - l) to form two separate filamentous entities enclosing the chromosome sets and from which cytoplasmic interphase networks of vimentin are reestablished during G₁. In these cells, the centrosomes remain distant from the midbody during cytokinesis (Fig. 48m, n). As previously documented by Aubin *et al.* (1980) and confirmed here using the rabbit antiserum

Figure 48 Organizational fate of vimentin during mitosis and its spatial relationship to the centrosome during cytokinesis in mouse 3T3 cells

Mouse 3T3 cells were labelled either with the rabbit antiserum to vimentin (b, f, j), the murine monoclonal anti-vimentin (Dr. Marceau, Université Laval) (c, g, g', k) or the murine monoclonal anti-tubulin (n), and then counterstained with Hoechst 33258 (a, d, e, h, i, l, m). Similar staining patterns were detected with both anti-vimentins. During interphase (a - d), vimentin forms a complex network extending from the nucleus to the cell periphery. During metaphase (e - h), vimentin is detected as a cage-like structure. (g) and (g') are two focal planes of the same cell. During cytokinesis, vimentin is observed as two separate entities which enclose the daughter cell nuclei (i - l). At that stage, cytoplasmic microtubules start to reorganize and the centrosomes remain distant from the midbody (m, n). — 1 200 x



to vimentin and the monoclonal anti-vimentin (from Dr. Marceau, Université Laval), very similar rearrangements are detected during mitosis of PtK2 cells (Fig. 49). In these cells, which remain flat during mitosis, cytoplasmic arrays of vimentin filaments reminiscent of the interphase network (Fig. 49a - d) are however retained through the whole process of division as revealed with both the polyclonal and monoclonal antibodies to vimentin (Fig. 49e - l). The centrosomes of PtK2 cells do not relocalize during cytokinesis and are detected at the poles distant from the midbody (Fig. 49m, n).

Vimentin and tubulin patterns of two suspension lymphoid cell lines, mouse thymoma EL4 (Fig. 50) and mouse lymphoma SP2/0 (Fig. 51), were also examined. During interphase in both cell lines (Fig. 50c; Fig. 51c, d), the microtubule system shows a radial arrangement similar to that observed in stimulated lymphocytes. In EL4 cultures, vimentin appears during interphase as either a filamentous or diffuse aggregate (Fig. 50a, b). In SP2/0 populations, vimentin is consistently detected as a diffuse aggregate in interphase cells (Fig. 51a, b). During division of EL4 cells, the vimentin and tubulin systems follow the same sequence of rearrangements as those described in lymphocytes. During prophase of EL4 (Fig. 50d - f), vimentin forms a filamentous cage that encloses the mitotic apparatus through prometaphase (Fig. 50g - i), metaphase (Fig. 50j - l) and the first steps of anaphase. The cage has disappeared in late anaphase and, at this stage, vimentin is detected as thick arrays running through the length of the cell (Fig. 50m - o). Vimentin is observed at the mitotic poles and at the midbody during telophase (Fig. 50p - r). The centrosomes are relocalized and vimentin is aggregated close to the midbody in each daughter cell during cytokinesis (Fig. 50s - u). In SP2/0, vimentin exhibits a diffuse distribution through the process of mitosis while tubulin shows the usual sequence of mitotic

Figure 49 Organizational fate of vimentin during mitosis and its spatial relationship to the centrosome during cytokinesis in PtK2 cells

PtK2 cells were labelled either with the rabbit antiserum to vimentin (b, f, j), the murine monoclonal anti-vimentin (Dr. Marceau, Université Laval) (c, g, k, k') or the murine monoclonal anti-tubulin (n), and then counterstained with Hoechst 33258 (a, d, e, h, i, l, m). Similar staining patterns were detected with both anti-vimentins. During interphase (a - d), vimentin forms a complex network extending from the nucleus to the cell periphery. During metaphase (e - h), cytoplasmic arrays of vimentin filaments reminiscent of the interphase network are still detected and form a cage-like structure enclosing the chromosomes. During cytokinesis, vimentin arrays which have been constricted at the midbody surround the chromosome sets in daughter cells (i - l). (k) and (k') are two focal planes of the same cell. At that stage cytoplasmic microtubules start to assemble on the centrosomes which remain distant from the midbody (m, n). — 1 200-x

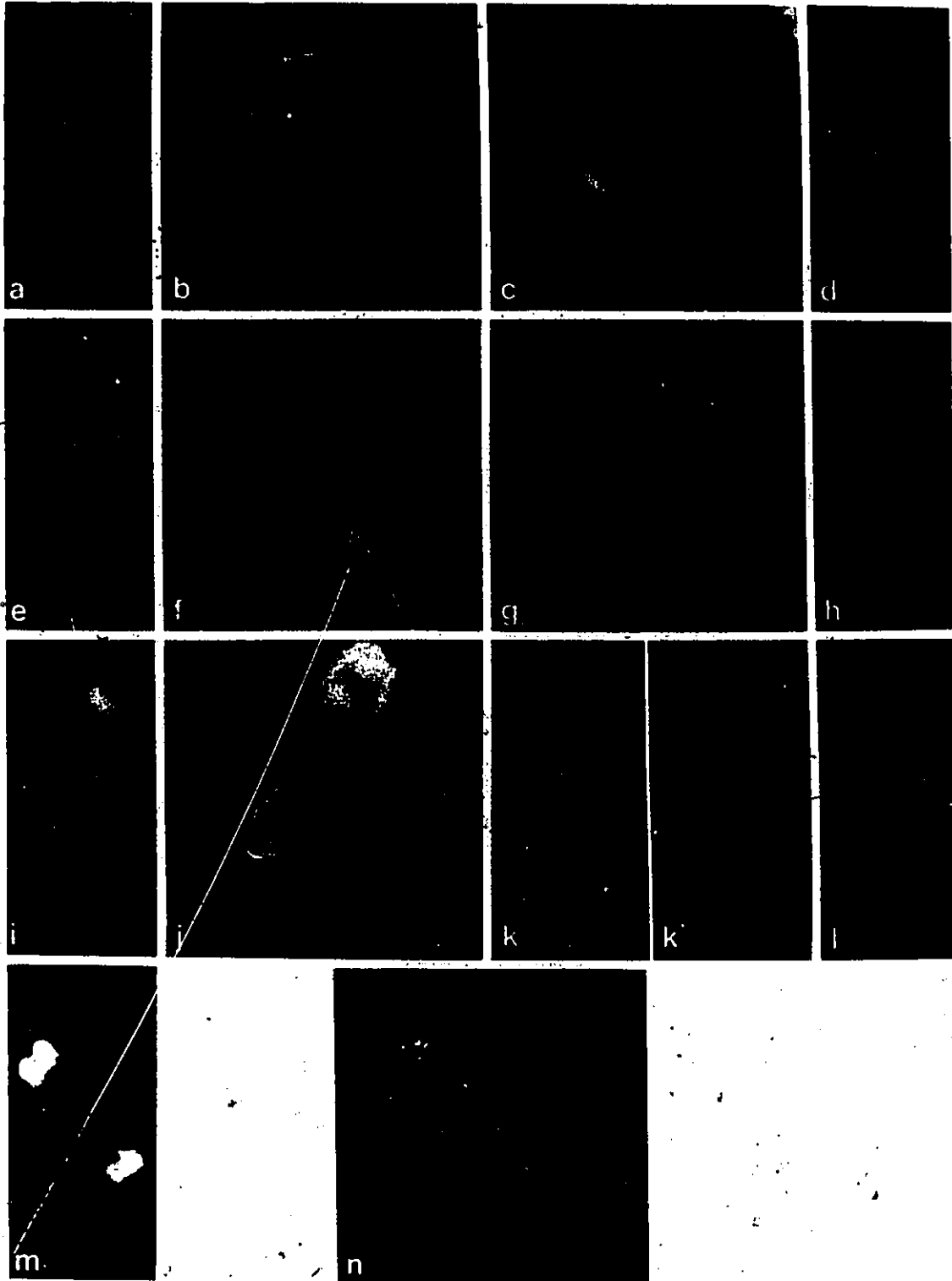
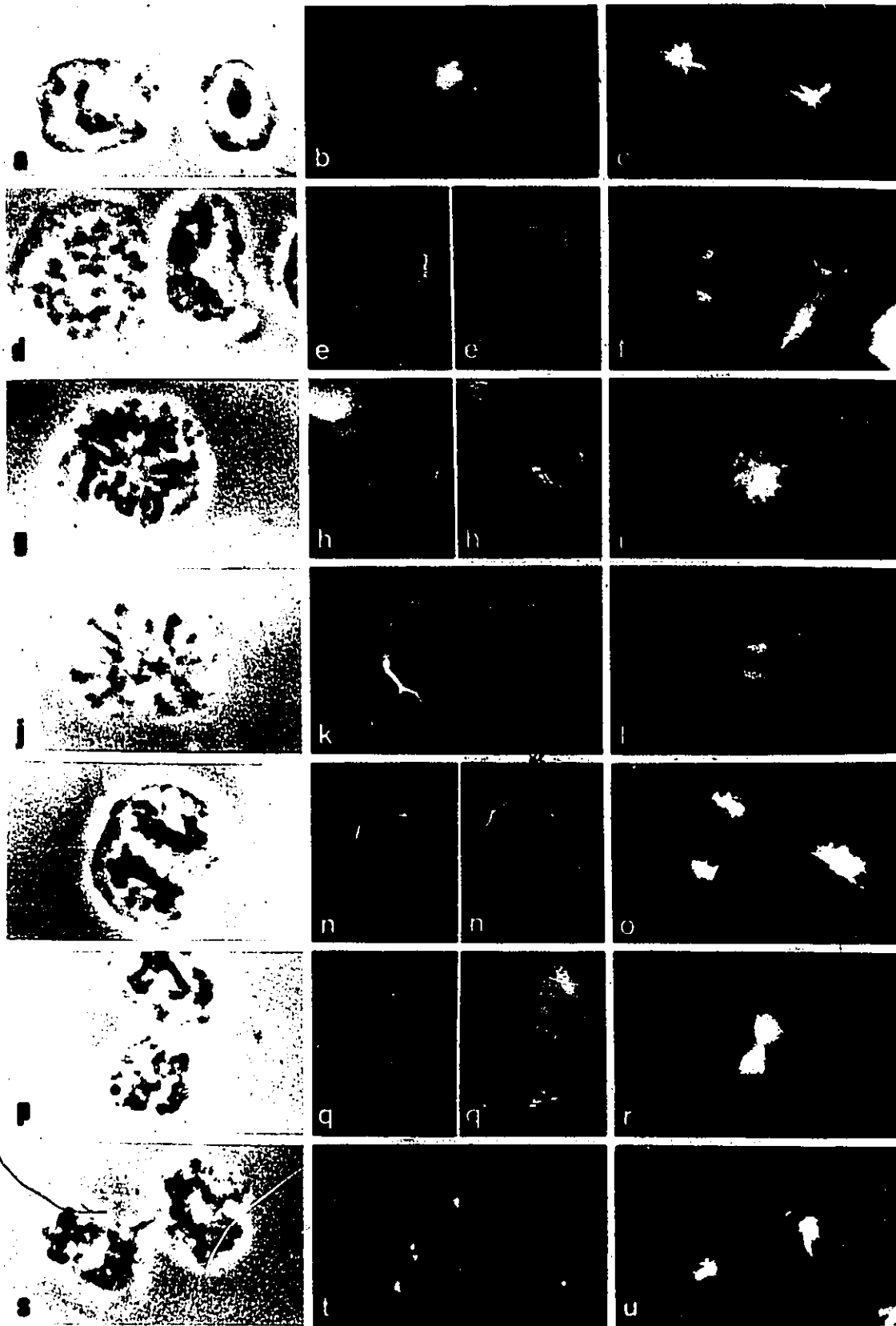


Figure 50 Organizational fate of vimentin and tubulin during division of mouse thymoma EL4 cells

EL4 cells were labelled with the rabbit antiserum to vimentin (b, e, e', h, h', k, n, n', q, q', t) or the murine monoclonal anti-tubulin (c, f, i, l, o, r, u), and observed by phase contrast (a, d, g, j, m, p, s). For some stages, two focal planes (eg. e/e', h/h', n/n', q/q') are shown. During interphase (a - c), vimentin is detected as an aggregate adjacent to a nuclear cleft and microtubules show a radial pattern. As the cells enter prophase (d - f), vimentin appears as filamentous arrays which form a cage-like structure surrounding the mitotic apparatus during prometaphase (f - i) and metaphase (j - l). In late anaphase (m - o), vimentin appears as thick bundles extending from pole to pole. Vimentin is observed at the midbody and at the mitotic poles during telophase (p - r). Vimentin is aggregated and the centrosomes are relocalized on both sites of the midbody during cytokinesis (s - u). — 1 200 x



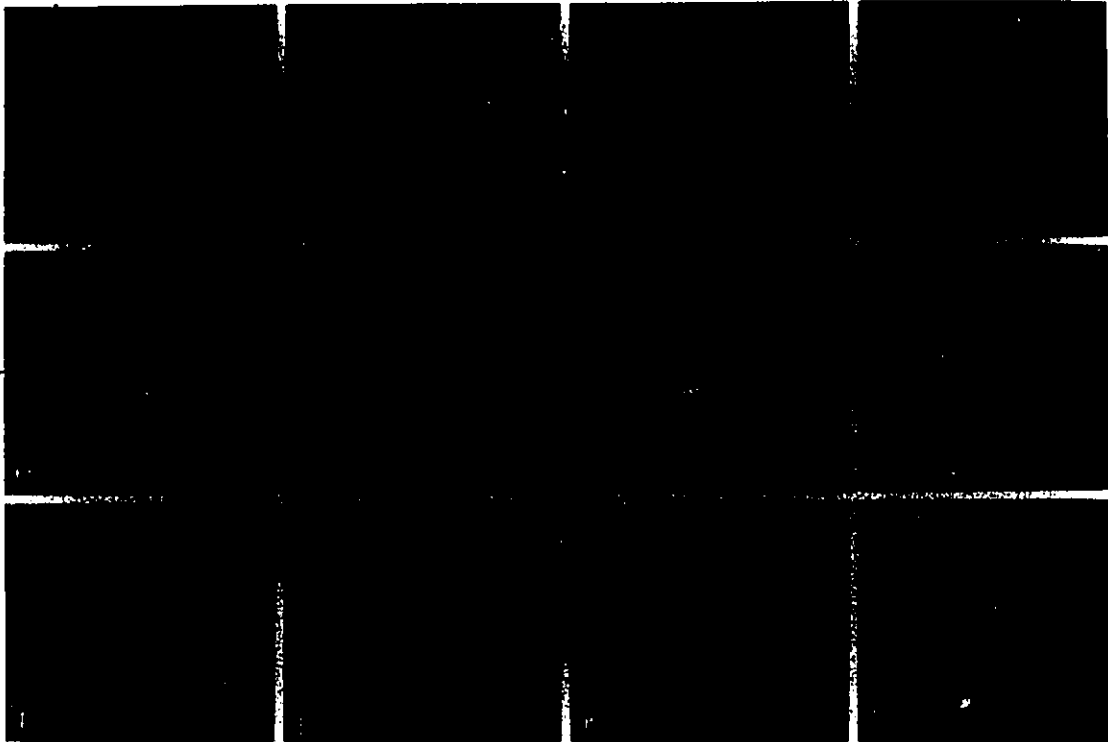
rearrangements (Fig. 51e - h). During cytokinesis, diffuse vimentin staining is more conspicuous and the centrosomes are relocalized on both sides of the midbody, in each daughter cell (Fig. 51i - l).

Finally, we have used immunofluorescence with antibodies against vimentin, tubulin, centriole and centromeres, and compared the localization of these respective antigens during division of mouse L cells grown either attached or in suspension. When cultured as monolayers, L cells display the flat elongate shape typical of fibroblastic cells. In these cells, the vimentin and tubulin systems form two complex networks of fibers extending from the perinuclear region to the plasma membrane (Fig. 52a - c). The centrioles are located adjacent to the nucleus (Fig. 52d), and the centromeres show a random nuclear distribution (Fig. 52e). During prophase, vimentin forms a filamentous cage-like structure that surrounds the nucleus and the developing spindle (Fig. 52f - h). Mitotic poles are detected in invaginations on opposite sides of the nucleus (Fig. 52i), and the centromeres are still randomly distributed in the nucleus (Fig. 52j). The vimentin cage persists through the subsequent stages of mitosis, enclosing the chromosomes and the mitotic spindle from prometaphase till telophase (Fig. 52k - n and p - s; Fig. 53a - d and f - i). Centromeres appear to be orderly positioned as soon as prometaphase (Fig. 52o) and then aligned at the metaphase plate (Fig. 52t). During chromosome separation in anaphase and early telophase, centromeres are detected at the poles (Fig. 53e, j). The vimentin cage structure is constricted during late telophase to form two filamentous structures enveloping the chromosome sets (Fig. 53k, l) and from which cytoplasmic networks of vimentin are reestablished during cytokinesis (Fig. 53p, q). During cytokinesis, the centrosomes remain at the poles distant from the midbody, as revealed both with the anti-tubulin (Fig. 53m, r) and the

Figure 51 Organizational fate of vimentin and tubulin during division of mouse lymphoma SP2/0 cells

SP2/0 cells were labelled with the rabbit antiserum to vimentin (b, f, j) or the murine monoclonal anti-tubulin (c, g, k), and counterstained with Hoechst 33258 (a, d, e, h, i, l). During interphase (a - d), vimentin is detected as a diffuse aggregate adjacent to the nucleus and microtubules show a radial pattern. During metaphase (e - h), vimentin shows a diffuse distribution and the mitotic spindle has been established. During cytokinesis (i - l), diffuse vimentin staining is more conspicuous and the centrosomes are relocalized on both sides of the midbody in each daughter cell.

— 1 200 x



C

Handwritten scribble consisting of a loop and a tail.

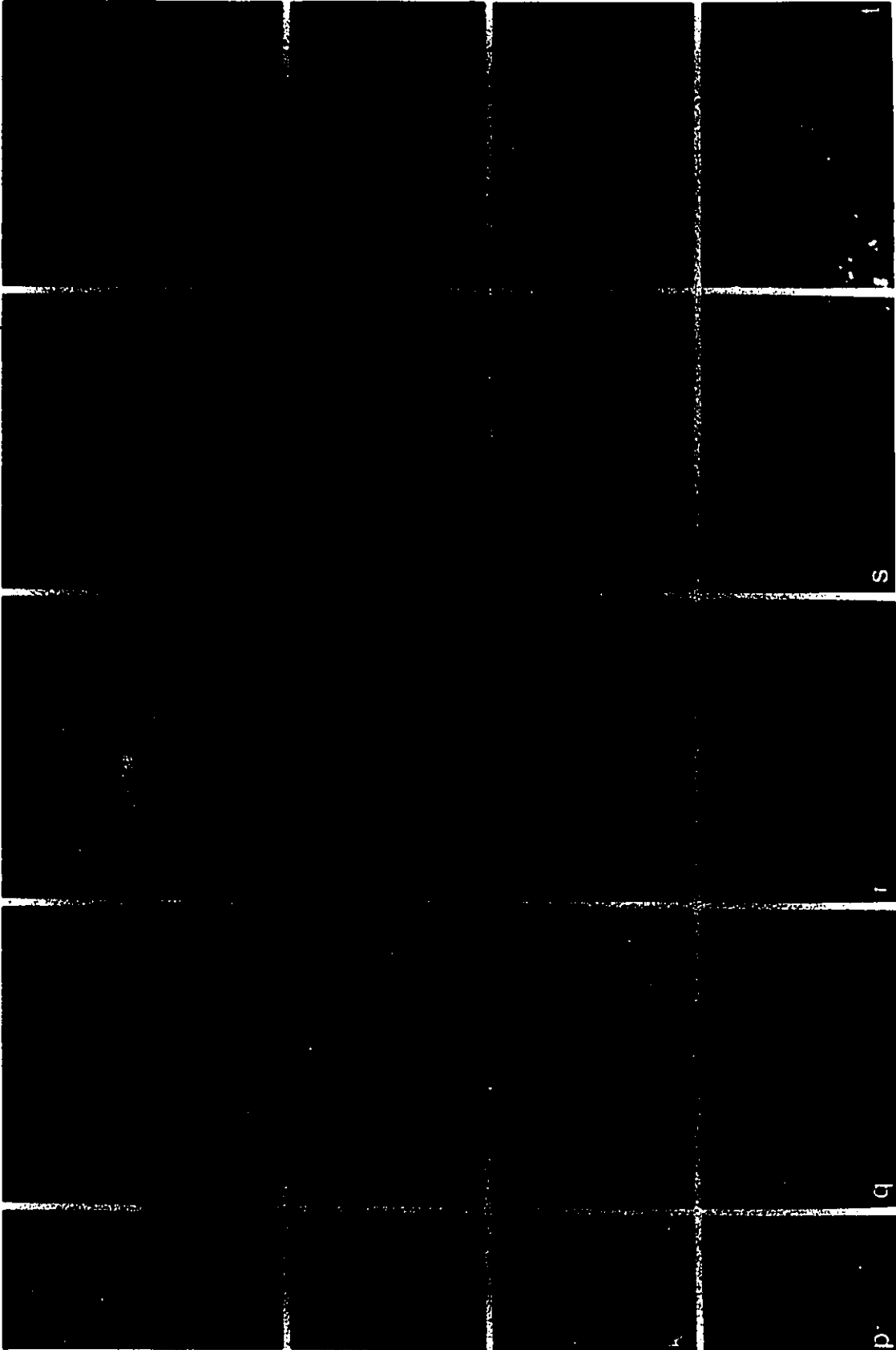
Handwritten vertical line with a small mark at the bottom.

Handwritten mark resembling a stylized 'Y' or a similar symbol.

Handwritten mark resembling a stylized 'L' or a similar symbol.

⁹ Figure 52 Organizational fate of vimentin, tubulin, centrioles and centromeres during division of mouse L cells grown attached (I)

Attached mouse L cells, processed for immunofluorescence according to the aldehyde fixation, were labelled with the rabbit antiserum to vimentin (b, g, g', I, I', q) or the murine monoclonal anti-tubulin (c, h, m, r). Immunolabelling of centrioles (d, i, n, s) or centromeres (e, j, o, t) was performed on attached cells using alcohol fixation. Mitotic stages were identified based on DNA staining with Hoechst 33258 (a, f, k, p). (a - e), interphase cells. (f - j), cells in prophase. (k - o), cells in prometaphase. (p - t), cells in metaphase. For some stages, two focal planes (eg. g/g', I/I') are shown. Refer to the text for description. --- 1 200 x



1

S

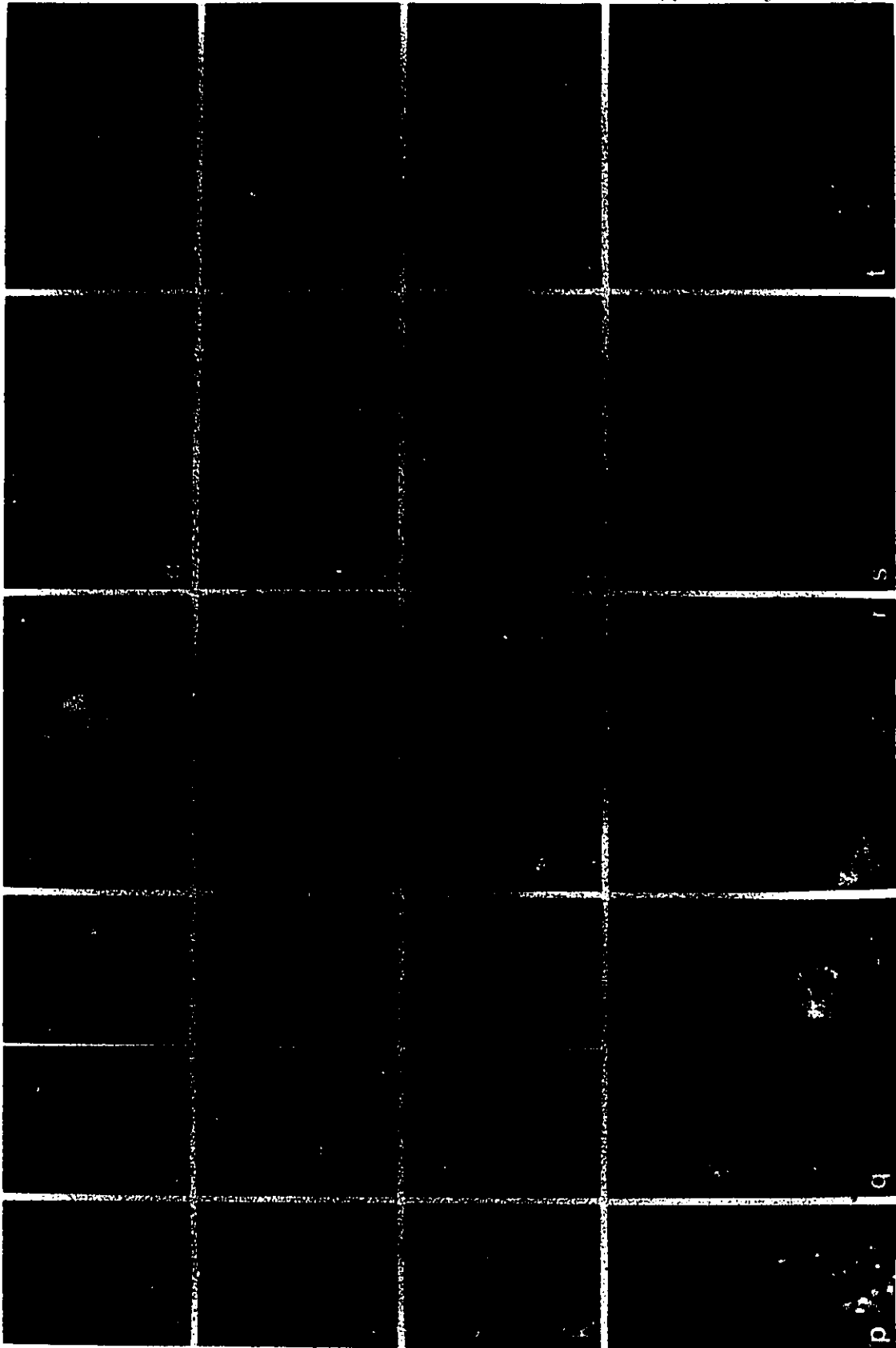
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p

p

Figure 53 Organizational fate of vimentin, tubulin, centrioles and centromeres during division of mouse L cells grown attached (II)

Attached mouse L cells, processed for immunofluorescence according to the aldehyde fixation, were labelled with the rabbit antiserum to vimentin (b, b', g, g', l, l', q) or the murine monoclonal anti-tubulin (c, h, m, r). Immunolabelling of centrioles (d, i, n, s) or centromeres (e, j, o, t) was performed on attached cells using alcohol fixation. Mitotic stages were identified based on DNA staining with Hoechst 33258 (a, f, k, p). (a - e), cells in anaphase. (f - j), cells in early telophase. (k - o), cells in late telophase. (p - t), cells in early G₁. For some stages, two focal planes (eg. b/b', g/g', l/l') are shown. Refer to the text for description. --- 1 200 x



t

s

l

p

p

anti-centriole (Fig. 53n, s). Centromeres are detected at the poles during late telophase (Fig. 53o) and are randomly distributed in the nucleus of daughter cells in early G_1 (Fig. 53t).

L cells grown in suspension display a rounded shape. During interphase in these cells, the vimentin system is accumulated into an aggregate adjacent to the nucleus (Fig. 54a, b) and microtubules are arranged in a radial pattern (Fig. 54c). The centrioles are located adjacent to the nucleus (Fig. 54d) and the centromeres show a random distribution (Fig. 54e). From that distinct interphase organization, the vimentin and tubulin systems follow a sequence of rearrangements during mitosis in suspension cells identical to those observed in attached cells (Fig. 54f - i, k - n, p - s; Fig. 55a - d, f - i). Positioning of centromeres through the different stages of mitosis also proceeds in suspension cells (Fig. 54j, o, t; Fig. 55e, j) as in attached cells. During cytokinesis, vimentin is detected as two filamentous structures surrounding the daughter nuclei (Fig. 55k, l and p, q). In contrast to attached cells, no cytoplasmic extensions of vimentin are observed in early G_1 daughter cells. The centrosomes are relocated near the midbody during cytokinesis of suspension cells as detected with the anti-tubulin (Fig. 55m, r) and the anti-centriole (Fig. 55n, s). Centromeres, which are detected at the poles during late telophase (Fig. 55o), have been redistributed randomly at the nuclear surface (Fig. 55t).

Figure 54 Organizational fate of vimentin, tubulin, centrioles and centromeres during division of mouse L cells grown in suspension (I)

Suspension mouse L cells, processed for immunofluorescence according to the aldehyde fixation, were labelled with the rabbit antiserum to vimentin (b, g, g', l, l', q, q') or the murine monoclonal anti-tubulin (c, h, m, r). Immunolabelling of centrioles (d, i, n, s) or centromeres (e, j, o, t) was performed on suspension cells using alcohol fixation. Mitotic stages were identified based on DNA staining with Hoechst 33258 (a, f, k, p). (a - e), interphase cells. (f - j), cells in prophase. (k - o), cells in prometaphase. (p - t), cells in metaphase. For some stages, two focal planes (eg. g/g', l/l', q/q') are shown. Refer to the text for description. — 1 200 x

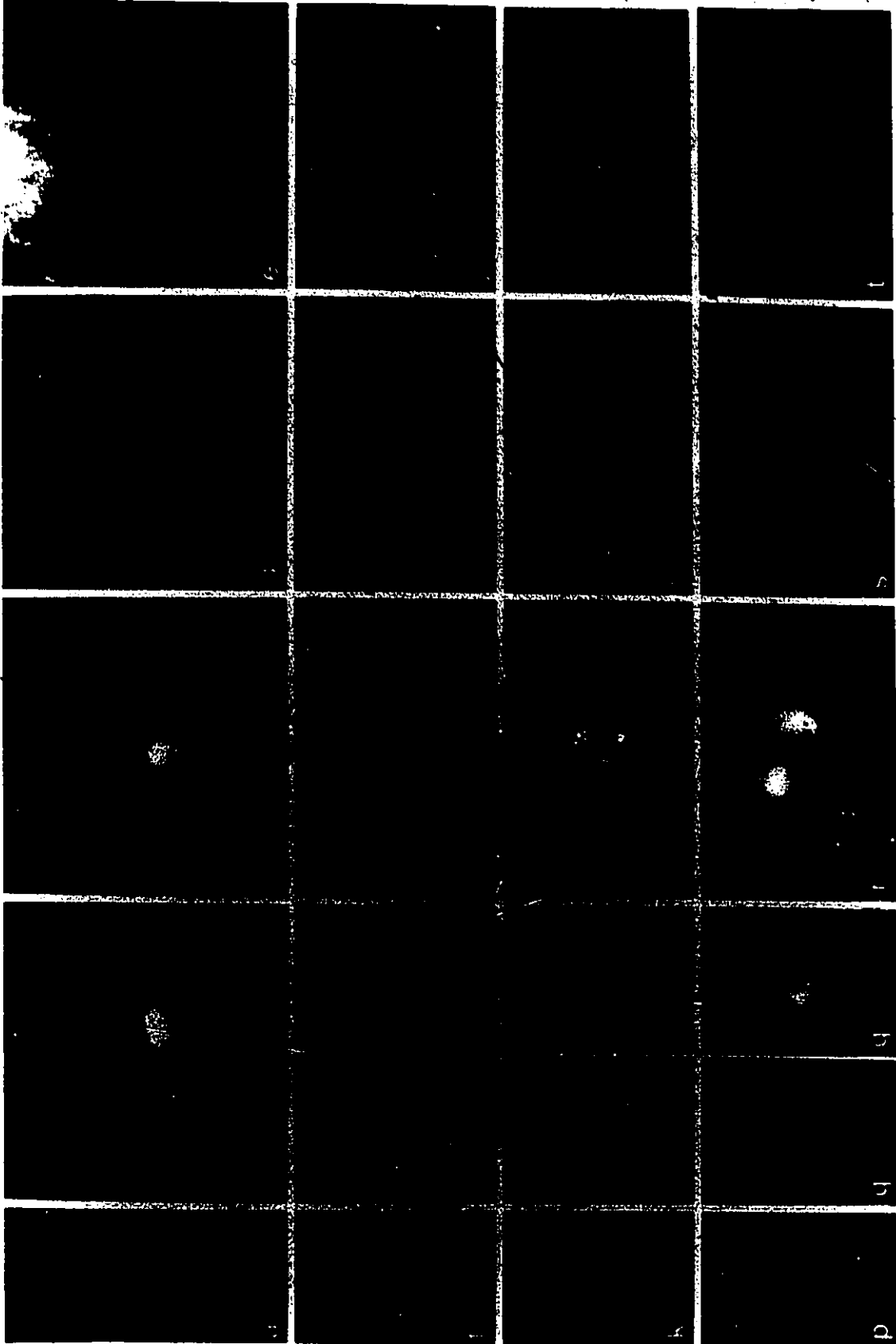


Figure 55 Organizational fate of vimentin, tubulin, centrioles and centromeres during division of mouse L cells grown in suspension (II)

Suspension mouse L cells, processed for immunofluorescence according to the aldehyde fixation, were labelled with the rabbit antiserum to vimentin (b, b', g, g', l, l', q, q') or the murine monoclonal anti-tubulin (c, h, m, r). Immunolabelling of centrioles (d, i, n, s) or centromeres (e, j, o, t) was performed on suspension cells using alcohol fixation. Mitotic stages were identified based on DNA staining with Hoechst 33258 (a, f, k, p). (a - e), cells in anaphase. (f - j), cells in early telophase. (k - o), cells in late telophase. (p - t), cells in early G₁. For some stages, two focal planes (eg. b/b', g/g', l/l', q/q') are shown. Refer to the text for description. — 1 200 x

			1
			5
			1
			q
			q
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DISCUSSION

ORGANIZATIONAL FATE OF VIMENTIN DURING MITOGENIC STIMULATION

Using immunofluorescence, with polyclonal and monoclonal antibodies to vimentin, and electron microscopy, it has been shown here that intermediate filaments are dynamic structures which undergo extensive cytoplasmic rearrangements during the mitogenic stimulation of lymphocytes. In resting B and T lymphocytes, vimentin is organized as a complex filamentous network which appears spatially related to both the microtubule pattern and the position of the centrosome. During mitogenic stimulation of these cells by con A, the vimentin is aggregated in the vicinity of the centrosome/Golgi complex while microtubules still extend radially from the centrosome to the cell periphery. Intermediate filaments were rarely visualized by electron microscopy in sections of stimulated cells indicating that the aggregate detected by immunofluorescence is probably not composed of vimentin in a filamentous form. On the other hand, the frequent observation in these cells of a fibrogranular mass in the vicinity of the centrosome/Golgi complex, which also corresponds to the position of the vimentin-positive aggregate seen by immunofluorescence, strongly suggests the inclusion of vimentin into an alternative non-filamentous structure similar to those described in different cultured cells (Horwitz *et al.*, 1981; Franke *et al.*, 1982; Jones *et al.*, 1985). This possibility was further supported by the presence of intermediate filaments at the margins of the fibrogranular mass in some sections.

In a previous study on human peripheral blood lymphocytes, Dellagi *et al.* (1983) reported that vimentin would undergo distinct organizational fates during mitogenic stimulation of B and T cells. According to these authors, the filamentous network present in both cell types prior to addition of the mitogen would be retained in stimulated T cells but replaced by a perinuclear aggregate of vimentin in stimulated B cells. From these observations, it has been concluded that B cells would tend to lose expression of vimentin during their maturation into plasma cells. In contrast, I have not observed distinct behaviors of vimentin in B and T cell subpopulations during con A stimulation of murine or bovine mixed lymphocyte populations. A perinuclear vimentin aggregate was consistently detected in fully stimulated cells (with a diameter $> 10 \mu\text{m}$) comprising both B and T cells. Furthermore it has been demonstrated here that filamentous arrays of vimentin are rapidly reextended in stimulated cells upon exposure to anti-microtubule drugs. These results do not support a loss of vimentin expression in stimulated cells but rather indicate that the vimentin positive aggregate most likely corresponds to a reorganization of the vimentin system during lymphocyte stimulation.

In the absence of mitogen, resting lymphocytes retained an unchanged cytoplasmic network of vimentin filaments over long term culture periods. This observation excludes the possibility that aggregation of vimentin may simply result from culturing conditions and indeed provides evidence that reorganization of vimentin represents a specific response of lymphocytes to the mitogen. It has been previously demonstrated that the number of microtubules, the cellular tubulin content and the microtubule initiation capacity of the centrosome progressively increase up to 4-5 fold during blastogenesis of murine lymphocytes (Rudd *et al.*, 1979; Waterhouse *et al.*, 1983; Schweitzer and Brown,

1984). Campos and Brown (1986) have recently reported that increased microtubule assembly during blastogenesis was accompanied by relocalization of calmodulin from a diffuse distribution in resting cells to an accumulation at the centrosome in fully stimulated cells, and have suggested that calmodulin-centrosome interactions may regulate the assembly capacity of the centrosome. In the present study, I have shown that vimentin reorganization does not temporally parallel the changes in tubulin expression but rather occurs as a late event during blastogenesis. For instance, cytoplasmic networks of vimentin filaments indistinguishable from those of resting cells could be observed in partially stimulated cells which already exhibited increases in cell size and in numbers of assembled microtubules. This asynchrony in the behavior of the two systems makes it unlikely that the vimentin network aggregates during blastogenesis to accommodate the assembly of new microtubules.

From my observations, on the other hand, it appears possible that reorganization of the vimentin system may be related to some cell cycle-dependent regulatory process of disassembly/assembly required for the entry of lymphocytes into S phase and/or for the extensive rearrangements of vimentin occurring with the onset of mitosis. It has been previously demonstrated (for a review, see Thyberg, 1984) and confirmed in this study that, in the continued presence of the microtubule depolymerizing drug colcemid, lymphocytes still respond to the mitogen by increasing their size and undergoing most of the structural cytoplasmic and nuclear changes characteristic of blastogenesis, but do not proceed normally into S phase. In contrast, it has also been shown here and in previous studies (Cuthbert and Shay, 1983; Brown *et al.*, 1985) that the microtubule-assembly promoting drug taxol does not affect the major events of lymphocyte stimulation by mitogen up to the first mitosis.

Early kinetic analyses of the inhibitory effects of microtubule depolymerizing drugs on the mitogenic response of lymphocytes have led to the proposal by Edelman's group (Edelman *et al.*, 1973; Yahara and Edelman, 1973; Edelman, 1976) that intact submembranous arrays of microtubules are required for transmembrane communication of the mitogenic signal and commitment of resting cells to undergo mitogenesis. This proposal has been questioned by more recent kinetic studies of the inhibitory effects of such drugs (Rudd *et al.*, 1979) and by the taxol experiments which demonstrate that the major events of lymphocyte stimulation, including the increase in cell size, the structural changes of nucleus and cytoplasm as well as DNA synthesis, occur normally in the presence of an extensively reorganized microtubule system (Cuthbert and Shay, 1983; Brown *et al.*, 1985). The causal relationship between removal of microtubules and inhibition of lymphocyte stimulation, therefore, has remained elusive. Considering side effects of microtubule depolymerizing drugs on the cytoplasmic distribution of organelles, Thyberg (1984) has recently proposed that microtubules could play an indirect role in mitogenic responses by ensuring adequate cellular organization for efficient cell growth. For instance, the centrosome/Golgi apparatus association normally observed in lymphocytes may be important in the lymphocyte responses to mitogen. This association is disrupted in cells stimulated in the continued presence of colcemid but is maintained in taxol-treated cells.

The effects of microtubule drugs on the organizational fate of vimentin during mitogenic stimulation had not been considered previously. From my results, it is clear that the early structural events of blastogenesis are not dependent on an intact cytoplasmic organization of the vimentin system since they proceed normally in lymphocytes stimulated in the continued presence of

colcemid or taxol which exhibit altered patterns of vimentin. There appears, however, to exist a correlation between entry of stimulated lymphocytes into S phase and disintegration of vimentin filaments in late G₁. DNA synthesis proceeds normally in taxol-treated cells in which vimentin filaments only transiently parallel the drug-induced microtubule bundles and are disintegrated during late G₁. In contrast, DNA synthesis is inhibited in colcemid-treated cells that retain filamentous arrays of vimentin through stimulation. These observations strongly suggest that disintegration of vimentin filaments may be important for the progression of blast cells into the proliferative phase of the mitogenic stimulation. The mechanisms regulating such rearrangements of the vimentin system remain to be determined. From observation on lymphocytes stimulated in presence of taxol, it is evident that disintegration of vimentin filaments during mitogenic stimulation in late G₁ does not depend on the normal organization of the microtubule system. Furthermore, the inability of lymphocytes stimulated in presence of colcemid to resume disintegration of the drug-induced rings of vimentin filaments in late G₁, while rapid reorganization of such structures occurs during sIg redistribution in resting B cells pre-treated with colcemid, indicates that the dynamics of the vimentin system may be controlled via alternative mechanisms depending on the nature of signals generated at the plasma membrane and/or the functional state of the cells. It cannot be concluded, however, that microtubules are not involved in or necessary for the disintegration of vimentin filaments during mitogenic stimulation. The possibility that an extensively reorganized microtubule system still maintains important cellular functions for the reorganization of the vimentin system in late G₁ cannot be excluded either.

Cell cycle-dependent factors modulating the state of intermediate filament organization have been previously invoked by a few investigators (Horwitz *et al.*, 1981; Lehto *et al.*, 1982; Savion *et al.*, 1982; Celis *et al.*, 1983). Unfortunately, unlike the monomer-polymer transitions of tubulin and actin for which much physical chemical data are available, nothing is known about the molecular processes involved in the *in vivo* reorganization of intermediate filaments. There is some evidence that phosphorylation of intermediate filament proteins by endogenous cAMP-dependent and -independent kinases may regulate the interaction of intermediate filaments with other cytoskeletal components or cellular structures (Celis *et al.*, 1983; Wang, 1985; Fey *et al.*, 1983). Both *in vitro* (Steinert *et al.*, 1982b) and *in vivo* (Bravo *et al.*, 1982a; Bravo *et al.*, 1982b; Celis *et al.*, 1983; Celis *et al.*, 1985) investigations have failed, nevertheless, to demonstrate an influence of the phosphorylated state of these proteins on their properties to assemble into polymers. On the other hand, it is well known that intermediate filaments and their subunit proteins are highly susceptible to degradation by endogenous Ca^{2+} -dependent thiol proteases (Schlaepfer and Micko, 1978; Gard *et al.*, 1979). Based on the observation that vimentin proteins show high affinity for nucleic acids (Traub *et al.*, 1983; Traub and Nelson, 1983), Traub (1985) has recently proposed that proteolytic degradation of vimentin filaments in response to signals which cause increases in intracellular free Ca^{2+} may be used by the cells to transmit information from the plasma membrane to the nucleus. According to this proposal, partial digestion of vimentin filaments by Ca^{2+} -activated proteases would release vimentin substructures no longer competent to form intermediate filaments but active in signal transmission as nucleic acid binding proteins. That indeed intermediate filament proteins may fulfill a nuclear function *in vivo* has received support from the demonstration by

Ward *et al.* (1983) that cytokeratin proteins are within cross-linking distance of nuclear DNA in intact Novicoff ascites hepatoma cells.

Like the cytoplasm, the interphase nucleus possesses structural elements determining its form and internal organization. These elements have collectively been termed "the nuclear matrix" and mainly consist of a peripheral porelamina complex, an intranuclear matrix, and a nucleolar matrix (Bouteille *et al.*, 1983). Several experimental approaches have indicated that the chromatin fibers of interphase nucleus are organized into repeating supercoiled domains or loops whose topological arrangement would be constrained by the attachment of chromatin to the peripheral lamina (Hancock, 1982). There is some *in vitro* evidence that lamins A, B, and C, the major polypeptide components of the lamina, may bind directly to DNA and influence the folding order of chromosome domains (Comings and Wallack, 1978; Lebkowski and Laemmli, 1982; Bouvier *et al.*, 1985; Boulikas, 1986; Bureau *et al.*, 1986). In regard to a possible nuclear function for intermediate filament proteins, it is relevant here to point out the recent data indicating that lamins constitute a previously unrecognized class of intermediate filament proteins. It has been demonstrated that lamins A and C display extensive regions of sequence and structural homology to all five classes of cytoplasmic intermediate filament proteins (McKeon *et al.*, 1986) and that these proteins are competent to assemble spontaneously into a meshwork of 10 nm filaments under near-physiological conditions (Aebi *et al.*, 1986). These new findings raise the theoretically attractive possibility that cytoplasmic intermediate filament proteins could be involved in nuclear processes either directly as nucleic acid binding proteins or indirectly through protein-protein interactions with their nuclear structural homologues in the peripheral lamina. In the light of these data, disassembly-reassembly of the lamina occurring during

mitosis and related to cell-cycle controlled phosphorylation-dephosphorylation of lamina proteins (Gerace *et al.*, 1984) may account as the first evidence that the phosphorylated state of intermediate filament proteins influences their properties to form polymers.

During lymphocyte stimulation, there exists a transition point in late G₁ at which a second external signal, the binding of IL-2 to its receptors, is required for the cells to progress further into the proliferative phase (for a review see: Hume and Weidemann, 1980). It has been suggested that the cellular events generated by this second signal may be regulated by the intracellular levels of cGMP. This suggestion has been based on the observations that the addition of IL-2 to macrophage-depleted lymphocyte cultures results in increased intracellular levels of cGMP and that the signal generated by the binding of IL-2 can be mimicked by agents which promote increase in intracellular levels of cGMP, such as carbamoylcholine, imidazole or dibutyryl-cGMP (Katz *et al.*, 1978). Most cGMP-dependent events in lymphocytes are known to occur in the nucleus and to require the presence of Ca²⁺ (Coffey *et al.*, 1977; Coffey *et al.*, 1978). It has been proposed that, through a specific kinase, cGMP would activate the synthesis, phosphorylation and DNA binding of nuclear proteins, and would increase the synthesis and activity of different enzymes such as phosphoribosylpyrophosphate synthetase, ornithine decarboxylase and DNA-dependent RNA polymerases I and II. Together with the demonstration of an increased traffic of proteins between the cytoplasmic and nuclear compartments—and an enlarged numbers of nuclear pores in con A stimulated cells (Maul *et al.*, 1971; Maul *et al.*, 1972; Scott and Marchesi, 1972), these observations emphasize the value of lymphocyte stimulation as a model system to test the hypothesis of a nuclear function for intermediate filament proteins (Traub, 1985).

In cultured cells, intermediate filaments appear as a complex filamentous network extending between the nucleus and the cell surface. Based on this organization, it has been proposed that intermediate filaments may function as integrators of the cellular space (Lazarides, 1980; Lazarides, 1982) and ensure maintenance of nuclear morphology and positioning (Letho *et al.*, 1978; Virtanen *et al.*, 1979; Woodcock, 1980; Staufenbiel and Deppert, 1982). During stimulation, the total cell size of lymphocytes enlarges up to three fold and the number and activity of cellular organelles increase. Extended cytoplasmic arrays of intermediate filaments are not observed in fully stimulated cells by electron microscopy and vimentin is detected as an aggregate located in the centrosomal region by double immunofluorescence. More numerous microtubules are assembled on the centrosome in these cells and some of them appear to terminate on nuclear pores. These observations argue against the sole involvement of intermediate filaments in the integration of cellular space, and moreover suggest a possible contribution of the microtubule system to such a structural function in lymphocytes. Furthermore, electron microscopic observations performed on lymphocytes treated with colcemid or taxol provide support to the suggestion previously postulated by Franke (1971; 1974) that nuclear morphology may be dependent on interactions of microtubules with the nuclear envelope. Removal of microtubules by colcemid, which necessarily disrupts interactions between the microtubule system and the nuclear envelope, has been shown to result in dramatic alteration of nuclear morphology. On the other hand, the shape of the nucleus is not altered by treatment with taxol which promotes extensive reorganization of microtubules but does not disrupt the association of microtubules with nuclear envelope, as illustrated here in lymphocytes and documented previously in cultured ovarian granulosa cells by Herman *et al.* (1983).

Reextension of filamentous arrays of vimentin from the developing spindle poles with the onset of mitosis in lymphocytes supports a direct involvement of this cytoskeletal system in mitotic events. The general features of vimentin rearrangements during the different mitotic stages in lymphocytes are similar to those observed in different flattened culture cells (see Results and : Hynes and Destree, 1978; Aubin *et al.*, 1980; Zieve *et al.*, 1980; Blose and Buschnell, 1982). In almost all cases, vimentin forms a filamentous cage-like structure during prophase that encloses the mitotic apparatus till anaphase. The cage usually disappears at this stage, and vimentin filaments are found in thick arrays extending the length of the cells in late anaphase. In flattened culture cells, filamentous arrays of vimentin are retained in telophase and are cleaved at the midbody during cytokinesis to form two separate filamentous entities which surround the daughter nuclei and from which cytoplasmic intermediate filament networks are reestablished in early G₁ daughter cells. A most striking and different reorganization of vimentin occurs during telophase in lymphocytes. At this stage, vimentin is aggregated in each daughter cell and, by the time the cells reach cytokinesis, vimentin is detected as an aggregate located at a site on either sides of the midbody which also corresponds to the position of the centrosomes. No cytoplasmic extension of vimentin was observed in daughter cells.

The reorganization of vimentin during cytokinesis in lymphocytes resembles that observed during blastogenesis in late G₁. This observation and the recent report (Campos and Brown, 1986) that calmodulin remains associated with the centrosome in daughter lymphocytes during cytokinesis are consistent with the suggestion that lymphocytes return after an initial round of cell division to a non-cycling state which may correspond to a pre-stimulated state distinct from that of G₀ resting cells. This hypothesis has been previously drawn from the

experimental evidences that the proliferative response of lymphocytes to a second mitogenic signal is much more rapid (Ling and Holt, 1967) and that the RNA profile of these cells markedly differs from that of quiescent (G_0) cells (McCairns *et al.*, 1984). Considering these data, it appears still more likely that vimentin aggregation may be related to the functional state of lymphocytes. It would be interesting to examine the possibility that the aggregation of vimentin during blastogenesis and that occurring during cytokinesis of lymphocytes may involve the same regulatory mechanisms.

RELATIONSHIP TO THE MICROTUBULE SYSTEM

Double immunofluorescence with antibodies to vimentin and tubulin has demonstrated that through the entire cell cycle of lymphocytes, the distribution of vimentin is spatially related either to the microtubule patterns or the position of the centrosome. The use of microtubule specific drugs has furthermore provided evidence for a close spatial relationship between the vimentin and tubulin systems.

Exposure of lymphocytes to microtubule depolymerizing drugs such as colcemid, colchicine and nocodazole resulted in the segregated accumulation of vimentin filaments into a ring-like arrangement. As previously reported by De Brabander *et al.* (1977), nocodazole did not appear to be an efficient microtubule-disrupting agent in lymphocytes, particularly in resting cells. Differential sensitivity of microtubules to drugs has been extensively documented in the literature (for a review see Raff, 1979) and may be related to different factors that regulate tubulin dynamics *in vivo* such as interactions of

microtubules with other proteins and postranslational modifications of tubulin. Brooks and Richmond (1983) had previously reported in a study on the effects of colcemid on mouse 3T3 cells, that quiescent cells generally exhibit more drug-resistant microtubules than stimulated cells. These authors suggested that such differential sensitivity could be attributed either to the smaller pool of ATP in quiescent cells or to the more rapid reorganization of microtubules in stimulated cells. In any case, the equal potency of nocodazole, compared to colchicine and colcemid, in promoting the segregated reorganization of the vimentin system indicates that complete disassembly of microtubules is not required to uncouple the relationship between the two filamentous systems.

Aside from their microtubule-disrupting action, these drugs have been shown to cause structural alterations of cytoplasmic organelles such as displacement of the centrosome and fragmentation of the Golgi apparatus in different cell types including lymphocytes (Thyberg *et al.*, 1977; Rudd *et al.*, 1979; Thyberg *et al.*, 1980; Sandoval *et al.*, 1984). It appears possible that these drugs may affect also the functional integrity of these organelles (Thyberg, 1984). Therefore, the effects of microtubule-disrupting drugs on the organization of the vimentin system could be related either to reduced direct interactions with microtubules and/or to a functional alteration of the centrosome/Golgi complex.

It has been previously reported that removal of microtubules upon cold treatment of lymphocytes does not alter the structural integrity nor the positioning of the centrosome/Golgi complex even though it causes a dispersion of satellite bodies (Schweitzer and Brown, 1984). I have shown that removal of microtubules by cold does not affect the normal organization of the vimentin system in both resting and stimulated lymphocytes. These observations indicate

that vimentin organization may be dependent more on the structural integrity of the centrosome/Golgi complex than on direct interactions with microtubules. Two laboratories have previously observed that cold exposure of He La cells also results in the complete disappearance of microtubules without causing any change in vimentin distribution (Virtanen *et al.*, 1983; Maro *et al.*, 1983). Based on this observation, Virtanen *et al.* (1983) concluded that normal vimentin organization is independent of microtubules. In contrast, Maro *et al.* (1983) have argued that vimentin organization is dependent on direct interactions with microtubules and that absence of vimentin reorganization upon cold disassembly of microtubules in He La cells reflects the temperature-dependency of such a reorganization. The argument was based on the following experimental situation: He La cells were exposed for 1 hour to nocodazole (10^{-6} M) at 37°C, a treatment which was sufficient to disrupt microtubules without aggregating vimentin filaments; the cells were then further exposed to the drug for 6 hours either at 37°C or 4°C. As perinuclear accumulation of vimentin was observed after the second exposure to the drug at 37°C but not at 4°C, the authors concluded that such a reorganization is temperature-dependent. In my view, disassembly of microtubules without aggregation of the vimentin system in He La cells exposed to nocodazole for 1 hour at 37°C demonstrates that vimentin organization does not depend on direct interactions with microtubules. Furthermore, microtubule-depolymerizing drugs such as nocodazole have been shown to alter the structural integrity of cytoplasmic organelles at 37°C, but their side effects at 4°C is completely unknown. The experimental situation designed by Maro *et al.* (1983) does not therefore appear appropriate to test the importance of direct interactions between vimentin filaments and microtubules for the maintenance of normal vimentin organization.

Observations on the effects of the microtubule assembly-promoting drug taxol also suggest that the ability of the centrosome to act as a functional organizing center for microtubules may contribute to the maintenance of a spatial relationship between intermediate filaments and microtubules. In cultured cells, taxol has been shown to induce massive assembly of microtubules at sites other than MTOCs (e.g. centrosome, poles and kinetochores) (De Brabander *et al.*, 1981a; Horwitz *et al.*, 1982; Manfredi *et al.*, 1982; Geuens *et al.*, 1983; Maro *et al.*, 1984) and, in some cases, to lead to the disappearance of centrosome-associated microtubules (De Brabander *et al.*, 1981a; De Brabander *et al.*, 1981b). In those cells, taxol has been reported to result in partial retraction of vimentin filaments into a perinuclear focus with the rest of the vimentin filaments being observed along the non-centrosomal bundles of microtubules (Maro *et al.*, 1984). It has been demonstrated in previous studies (Paatero and Brown, 1982; Brown *et al.*, 1985), and confirmed here, that the effects of taxol on microtubule organization of lymphocytes are quite different. In resting and stimulated lymphocytes, taxol induces the rearrangement of microtubule patterns into large bundles exclusively assembled from the centrosome. The extent of bundling is dependent on times of exposure to the drug. After a 4-hour exposure, microtubules show maximal lateral aggregation and appear as one or few large bundles terminating on the centrosome. Under these conditions, vimentin filaments are reorganized in parallel with microtubule bundles and perinuclear accumulation of vimentin filaments is not observed. Observations performed on cells treated for 1 hour with taxol further demonstrate that reorganization of the vimentin system lags behind that of microtubules. These data therefore indicate that taxol-induced co-distribution of the two filamentous systems is probably due neither to direct interactions between microtubules and vimentin filaments nor to a passive trapping of the vimentin system during reorganization of microtubules.

ORGANIZATIONAL FATE OF VIMENTIN DURING REDISTRIBUTION OF SURFACE IMMUNOGLOBULIN

Over the past years, studies of the cellular mechanisms implicated in the redistribution of membrane receptors have been focused on the possible role of microfilaments and microtubules in cap formation (Edelman *et al.*, 1973; Yahara and Edelman, 1973; Yahara and Kakimoto-Sameshima, 1978; Rogers *et al.*, 1981; Paatero and Brown, 1982; Braun, 1983; Braun and Unanue, 1983). In the case of sIg redistribution on lymphocytes, the current view is that the microfilament system generates the motive force for the translocation while the microtubule system provides the spatial information to direct the microfilament-dependent migration of the receptors. The possible involvement of intermediate filaments in the capping process has been considered previously only by a few investigators (Zucker-Franklin *et al.*, 1979; Bourguignon and Bourguignon, 1981; Dellagi and Brouet, 1982). Using electron microscopy, Zucker-Franklin *et al.* (1979) first observed the presence of intermediate filaments in human lymphocytes and showed that ligand-induced sIg redistribution is accompanied by extensive accumulation of the filaments in the cytoplasm of the uropod in these cells. Using double immunofluorescence, Dellagi and Brouet (1982) have also reported that capping of sIg induces a redistribution of vimentin intermediate filaments in human and murine lymphocytes. From a filamentous network, the vimentin system reorganizes underneath the cap into a diffuse aggregate often devoid of detectable filamentous structure. Similar observations were performed during redistribution of β_2 -microglobulin on lymphoblastoid T cells (Dellagi and Brouet, 1982) and of con A receptors on murine lymphocytes (Bourguignon and Bourguignon, 1981).

The accumulation of vimentin at the site of the cap during redistribution of sIg in murine lymphocytes has been confirmed and further documented in the present study. Using double immunofluorescence, the organizational fate of vimentin was observed at different stages of the redistribution process, from the diffuse localization of ligand-receptor complexes through their clustering into patches and their aggregation into a cap. An intact filamentous network of vimentin is retained in cells that show a diffuse or patchy sIg distribution and still display a rounded morphology. As translocation of patches proceeds, vimentin filaments progressively retract from other cytoplasmic regions and reorganize as a diffuse aggregate in the newly formed uropod at a site close to the centrosome/Golgi complex underneath the sIg cap. These results indicate that vimentin reorganization is related to the active translocation of sIg patches. This conclusion is compatible with the report of Zucker-Franklin *et al.* (1979) that anti-Ig-induced accumulation of intermediate filaments is not observed in chronic leukemia lymphocytes, which show no capping but only patching of ligand-receptor complexes.

By electron microscopy, the vimentin aggregate detected in capped cells by immunofluorescence did not appear to correspond to an accumulation of intermediate filaments but rather to a fibrogranular mass directly apposed to the plasma membrane at the site of the sIg cap. This fibrogranular mass is most likely not composed exclusively of vimentin, but may represent the site of inclusion of different proteins previously detected at the site of the cap by immunofluorescence. Arrays of intermediate filaments were rarely visualized in these cells. These observations contrast with those of Zucker-Franklin *et al.* (1979) who demonstrated the presence of thick bundles of intermediate filaments in the uropod of capped human lymphocytes. Such a discrepancy between the two

studies cannot be explained. Differences in cell types, experimental conditions (temperature, time of exposure to the ligand) or ligands can lead to very different results in studies of cap formation and might be involved here.

Under normal conditions, as reported previously (Rogers *et al.*, 1981) and confirmed here, the microtubule system retains its radial pattern through the entire process of sIg redistribution. Accumulation of the vimentin system at the site of the cap occurs therefore independently of microtubule rearrangements. On the other hand, as the sIg cap is invariably formed over the pole containing the centrosome/Golgi complex in murine lymphocytes, it appears possible that the vimentin system may be passively trapped underneath the cap due to its spatial relationship to the position of centrosome. Observations on cells that were exposed to colcemid prior to addition of anti-Ig excluded this first possibility. It has been demonstrated that, in resting lymphocytes exposed to colcemid, the vimentin system is organized as a ring-like structure showing no spatial relationship to the position of the centrosome. Following redistribution of sIg on such treated cells, the vimentin system is nevertheless consistently accumulated at the site of the cap. As the normal spatial relationship between the site of the cap and the position of the centrosome/Golgi complex has been disrupted by the drug, these observations strongly support a dynamic reorganization of the vimentin system during cap formation. Furthermore, they demonstrate that colcemid-induced vimentin reorganization is not "fixed" but can be disrupted rapidly (7 minutes) in response to certain extracellular signals.

The behavior of vimentin during sIg cap formation in colcemid-treated cells raised the possibility that the vimentin system may be directly involved in the capping process. This second possibility was nevertheless not supported by

the organizational fate of vimentin during sIg cap formation on cells pretreated with taxol. It has been shown that, in resting lymphocytes exposed to taxol, vimentin filaments are rearranged into one or few thick arrays that parallel microtubule organization. Like colcemid, taxol does not prevent sIg cap formation but uncouples the normal relationship between the site of the cap and the position of the centrosome. In taxol capped cells, vimentin does not accumulate underneath the cap but is detected in few thick arrays, extending from a focus close to the plasma membrane towards the site of the cap. Such a pattern is reminiscent of the microtubule organization in taxol-capped cells, indicating that taxol-induced co-localization of the two filamentous systems is maintained during the capping process. Without excluding a possible involvement of the vimentin system in the capping process, these observations on the other hand clearly demonstrate that cap formation is not directly dependent on the accumulation of vimentin.

In light of this series of parallel observations in normal and drug-treated cells, it is proposed here that the vimentin system may be involved in the transmission of spatial information from the microtubule system (microtubules and centrosome) to the contractile machinery of microfilaments during normal translocation of ligand-sIg receptor complexes. Under normal conditions, the vimentin network of resting lymphocytes extends from the centrosomal region to the cell periphery in a pattern partially coincident with the organization of microtubules. Retraction/disintegration of this network towards the centrosome during migration of sIg patches could orient the microfilament-dependent movement of receptors, either directly by physical interactions between vimentin and actin filaments or indirectly by creating tracks of reduced resistance in the cortical layer along which the microfilament system would more easily

translocate the receptors. In colcemid-treated cells, the vimentin organization is independent of the microtubule system and its disintegration would not provide the same spatial information to the microfilaments resulting in cap formation anywhere on the cell surface. In taxol-treated cells, in contrast, the vimentin organization is more strictly dependent on the microtubule system than normally. Binding of anti-Ig does not affect this organization and the microfilament-dependent translocation of receptors would not receive spatial directives, still resulting in cap formation anywhere on the cell surface.

Mangeat and Burridge (1984) have recently presented data suggesting that the accessory protein spectrin can play the role of a linker between intermediate filaments and actin filaments in cultured cells. Although there is still no direct evidence demonstrating interactions between intermediate filaments and microfilaments in lymphocytes, the co-relocalization of spectrin (Nelson *et al.*, 1983), actin (Gabbiani *et al.*, 1977) and vimentin underneath the site of the cap during sIg redistribution strongly suggests a functional association between the two filamentous systems in that cellular process. The recent observation by Repasky and coworkers (Lee *et al.*, 1984; Repasky *et al.*, 1984) of an in situ subset of lymphocytes that show a natural, non-induced, cytoplasmic cap containing vimentin and spectrin also supports the possibility of functional interactions between intermediate filaments and microfilaments in lymphocytes.

**ORGANIZATIONAL FATE OF VIMENTIN AND ITS SPATIAL
RELATIONSHIP TO THE CENTROSOME DURING DIVISION IN CELLS
GROWN ATTACHED AND IN SUSPENSION**

Double immunofluorescence has shown that the distribution of vimentin is spatially related to the position of the centrosome throughout the cell cycle in lymphocytes. Found as an aggregate close to the centrosome in stimulated cells, vimentin is detected during mitosis as a transient filamentous cage which encloses the mitotic apparatus. An unexpected but interesting observation was the concomittant aggregation of vimentin and relocalization of the centrosomes at a position close to the midbody in each daughter cell during cytokinesis. Comparative observations on the behavior of the vimentin system and the centrosomes during cytokinesis in different attached and suspension cell lines as well as in mouse L cells that grow attached and in suspension have further indicated that : 1) relocalization of the centrosomes near the midbody during cytokinesis is not peculiar to lymphocytes but may represent a more general feature of cells that grow in suspension; and 2) aggregation of the vimentin system, which occurs concomittant with the relocalization of the centrosomes in some lymphoid cells, is not required for centrosome relocalization in other suspension cells.

Relocalization of the centrosomes during cytokinesis was not observed in mouse 3T3, PtK2 and attached mouse L cells. In these cells, the centrosomes consistently remain at the poles distant from the midbody. In contrast, this process appears of general occurrence in all suspension cells examined here including mouse L cells, indicating that it may represent an adaptative response of the cells to growth conditions. A similar process of relocalization of the

centrosomes during cytokinesis had been also described by Wilson (1925) in the spermatogonia of the beetle *Blabps* and defined as a type of telokinetic movement: "In another type of telokinetic movement, both centrioles, still closely associated, migrate around the periphery of the nucleus until they may come to lie at a position on the spindle side of the nucleus (instead of opposite to it) and nearly 180° away from their original position. The meaning of this remarkable process is quite unknown."

What are the mechanisms involved in the relocalization of the centrosomes during cytokinesis? What is the functional significance of such a process for cells that grow in suspension? These questions are beyond the scope of the present study. Nevertheless, some observations appear worthy of comment here.

Relocalization of the centrosomes does not necessarily proceed synchronously between the two daughter cells but rather appears to be related to nuclear events in each daughter cell. In all suspension cells examined, chromosomes are massed at the poles in a cup-like arrangement during early telophase. In each daughter cell, the convex part of the chromosome cup is facing the midbody and the polar centrosome is located inside its concave part at nearly 180° from the midbody. In late telophase, the nuclear membrane is reformed around the decondensing chromosomes. The nucleus retains a cup shape but its concave part which still encloses the centrosome has been displaced from its original position to any angle between the midbody and the opposite pole of the cell. Later during cytokinesis, chromosome decondensation is completed and distribution of centromeres resembles that observed in interphase nucleus. The concave part of the nucleus is then facing the midbody and the centrosome is

located close to it. These data are consistent with considerable evidence demonstrating the existence of a close centriole-nucleus association in mammalian cells, including lymphoid cells and mouse L cells (Policard and Bessis, 1953; Bornens, 1977; Fais *et al.*, 1984). Furthermore, they suggest a possible mechanical contribution of the nucleus to the relocalization of the centrosome during cytokinesis. Centrosome relocalization could be effected by a rotation of the nucleus as such movements have been observed in a variety of cells (Albrecht-Buehler, 1984; Bard *et al.*, 1985). Movement of the centrosome could be rather facilitated by the displacement of the nuclear cleft over the nuclear surface. These possibilities should be considered in further examinations of mechanisms and functional significance of centrosome relocalization during division of suspension cells.

Recently, centrosome relocalization has been also shown to occur in some cell types in response to certain stimuli. Mouse 3T3 cells, polymorphonuclear leukocytes and newt eosinophils reposition the centrosome between the nucleus and the leading lamella during migration (Albrecht-Buehler and Bushnell, 1979; Malech *et al.*, 1977; Koonce *et al.*, 1984). Cultured endothelial cells migrating into an experimental wound move the centrosome to the cell side facing the wound (Gotlieb *et al.*, 1983). These observations have led to the suggestion that direction of migration is determined by the position of the centrosome. Few studies however have examined the position of the centrosome during motility of suspension cells. In motile lymphocytes, the centrosome is not located in the leading part of the cell but rather is found in the uropod behind the nucleus. The relocalization of centrosomes during cytokinesis could theoretically be related to the ability of this organelle in directing migration. At either side of midbody, centrosomes would operate migration of daughter cells in opposite

directions and would facilitate their separation. The relevance of this suggestion will await additional data on the position of the centrosome during motility of other suspension cells.

BEHAVIOR OF VIMENTIN DURING MITOSIS

While the specific functions of vimentin filaments during mitosis have not yet been defined, it is intriguing to note that, from distinct interphase organizations in attached and suspension cells, this filamentous system is consistently rearranged with the onset of mitosis into a filamentous cage-like structure in both types of cells. Among the cell lines examined in this study, the only exception was SP2/0 which retained a diffuse vimentin distribution through its entire cell cycle. The vimentin cage structure persists in all other cell lines from prophase to late anaphase. The organizational fate of vimentin at later stages of mitosis apparently followed two patterns : 1) the vimentin cage is retained in mouse 3T3, PtK2 and both attached and suspension mouse L cells; and 2) in contrast, this structure is dismantled and vimentin is aggregated in lymphocytes and EL4. My observations on SP2/0 cells together with the results of antibody injection experiments (Gawlitta *et al.*, 1981; Klymkowsky, 1981; Lin and Feramisco, 1981; Klymkowsky *et al.*, 1983) clearly demonstrate that some cells can undergo mitosis in the absence of extended arrays of intermediate filaments. In contrast, the formation of a transient vimentin cage-like structure with the onset of prophase in lymphocytes and EL4, which do not display cytoplasmic vimentin networks prior to or after mitosis, strongly supports a functional involvement of vimentin filaments in some mitotic events. In these cells, a vimentin cage is present from prometaphase till late anaphase. It appears

possible that vimentin filaments may be involved in the spatial ordering of the chromosomes at the metaphase plate and/or in the first steps of chromosome movement to the poles. Although there is considerable evidence for the spatial ordering of chromosomes at the metaphase plate (Comings, 1968; Heslop-Harrison and Bennett, 1983), the mechanisms involved in the establishment of the metaphase order remains to be determined. It has been shown in this study and few others (Jones *et al.*, 1985) that intermediate filaments can be observed in close proximity to chromosomes. These filaments in association with lamins or other nuclear matrix components could act as a physical constraint to direct the ordering of the chromosomes from interphase to metaphase. Although not obligatory, their contribution to such events would offer important advantages. Organized as a filamentous cage around the mitotic apparatus, intermediate filaments could indeed coordinate the intracellular arrangement of the chromosomes and other cellular organelles relatively to the position of the microtubule spindle. Similarly, Jones *et al.* (1985) have recently proposed that intermediate filaments could act as a brake to impede the movement of chromosomes towards the pole during anaphase.

CONCLUSIONS

In the introduction, I addressed different questions concerning the cellular dynamics and cytoplasmic roles of intermediate filaments. Therefore I will draw hereafter the conclusions that resulted from the experimental work presented in this thesis in the form of answers to these questions.

- 1° Based on structural and pharmacological evidence, vimentin filaments have been shown to be spatially related to the microtubule system in well flattened cultured cells. Is there such a relationship between the two filamentous systems in lymphocytes which are primary culture cells in suspension?

Using double immunofluorescence with antibodies to vimentin and tubulin, it has been demonstrated that, through the entire cell cycle of lymphocytes, the distribution of vimentin is spatially related to the microtubule system (microtubule and centrosome). Exposure of resting and stimulated lymphocytes to treatments that alter microtubule organization has provided further evidence of a close spatial relationship between the two filamentous systems and also indicated that the structural integrity of the centrosome/Golgi complex may be crucial for the organization of the vimentin system.

What is the spatial fate of the vimentin system in relation to the important changes in microtubule organization that occur during mitogenic stimulation?

In resting lymphocytes, vimentin forms a filamentous network partially coincident with the radial pattern of microtubules. During stimulation, there are progressive changes in centrosome organization and up to a 5-fold increase in numbers of microtubules assembled from the centrosome (Schweitzer and Brown, 1984). In fully stimulated cells, a vimentin network is not observed but rather vimentin is detected as a diffuse aggregate. Using double immunofluorescence and electron microscopy, it has been shown that the vimentin aggregate is located in the vicinity of the centrosome. Time course of the organizational fate of vimentin during mitogenic stimulation has indicated that the aggregation of the vimentin system does not parallel temporally the progressive changes in tubulin expression but rather occurs as a late event during blastogenesis.

- 2° Because they form an extensive network between the nucleus and the plasma membrane during interphase in flattened cultured cells, intermediate filaments have been suggested to play a role in the cytoplasmic integration of organelles or in the transmission of signals. Is there a correlation between the organization of the vimentin system and the increase in numbers of organelles during mitogenic stimulation of lymphocytes?

By immunofluorescence or electron microscopy, extended cytoplasmic arrays of intermediate filaments are not observed in fully stimulated lymphocytes which display a higher degree of cytoplasmic complexity than resting cells. Vimentin rather appears as a diffuse aggregate usually devoid of any filamentous structure and located near the centrosome. These observations together with those performed on

lymphocytes treated with microtubule-specific drugs argue against a structural role of the vimentin system during mitogenic stimulation. On the other hand, they provide evidence that microtubules constitute the major architectural elements of the cytoplasm in lymphocytes.

Is the vimentin system involved in the short-term response of B lymphocytes to binding of anti-Ig? Does accumulation of vimentin at the site of the cap, as reported by Dellagi and Brouet (1982), occur during sIg redistribution in cells treated with anti-microtubule drugs which uncouple the normal relationship between the site of the cap and the position of the centrosome/Golgi complex?

Using double immunofluorescence, it has been shown that the filamentous network of vimentin present in resting B lymphocytes is affected neither by the binding of anti-Ig to surface receptors nor by the clustering of ligand-receptor complexes into patches. In contrast, the vimentin system is accumulated into a diffuse aggregate underneath the cap close to the position of the centrosome during sIg cap formation. These data indicate that vimentin reorganization is related to the active translocation of patches.

In resting lymphocytes exposed for 4 hours to colcemid, the vimentin system is organized as a ring-like structure showing no spatial relationship to the position of the centrosome. When these cells are exposed to anti-Ig, the vimentin system is nevertheless consistently accumulated underneath the site of the cap which is formed anywhere on the cell surface without relation to the position of the centrosome. These observations further indicate that anti-Ig-induced accumulation of vimentin corresponds

to a dynamic rearrangement of this filamentous system that can occur independently of the microtubules or the centrosome.

In resting lymphocytes treated for 4 hours with taxol, the vimentin system is arranged in a few thick arrays that parallel the drug-induced microtubule bundles. During sIg cap formation in such treated cells, vimentin does not accumulate underneath the cap which shows no spatial relationship with the position of the centrosome. Vimentin is rather detected in few thick arrays extending from a focus close to the plasma membrane towards the site of the cap. Without excluding a possible involvement of the vimentin system in the sIg capping process, these observations clearly demonstrate that cap formation is not directly dependent on the accumulation of vimentin.

In the light of this series of parallel observations in normal and drug-treated cells, it is proposed that the vimentin system may be involved in the transmission of spatial information from the microtubule system to the contractile machinery of microfilaments during sIg cap formation.

Is the vimentin system involved in long-term response of lymphocytes to binding of the mitogen con A? What is the organizational fate of the vimentin system in cells stimulated in the continued presence of colcemid or taxol?

It has been shown that aggregation of the vimentin system late during blastogenesis constitutes a specific response of lymphocytes to con A. During mitogenic stimulation in the continued presence of colcemid, the

entry of lymphocytes into S phase is markedly inhibited. In these cells, the centrosome/Golgi complex is disrupted upon removal of microtubules and vimentin is present in filamentous arrays through the stimulation process. In contrast, in the continued presence of taxol, stimulation of lymphocytes proceeds normally up to mitosis. Structural integrity of the centrosome/Golgi complex is not altered by taxol-induced reorganization of microtubules and vimentin shows a sequence of rearrangements during progression in the cell cycle that resembles that observed during normal stimulation. These results strongly suggest that vimentin rearrangements are more important than changes in microtubule organization for the progression of lymphocytes through the cell cycle in response to mitogen.

- 3° Extensive rearrangements of intermediate filaments, particularly of vimentin-type, have been observed during mitosis in flattened cultured cells. What is the organizational fate of the vimentin system in relation to the microtubule patterns during mitosis of lymphocytes which are primary culture cells in suspension?

When stimulated lymphocytes enter mitosis, vimentin reappears as filamentous arrays extending from the polar centrosomes during prophase and then forms a cage-like structure that transiently encloses the mitotic apparatus. During cytokinesis, the polar centrosomes relocate to a position adjacent to the midbody and vimentin is detected as an aggregate, similar to that seen prior to mitosis, close to the centrosome in each daughter cell.

As the concomittant aggregation of the vimentin system and relocation of the polar centrosomes close to the midbody observed during

cytokinesis in lymphocytes had not been previously reported in other cells, a comparative study has been performed on several attached and suspension cell lines. It has been demonstrated that : 1) relocation of the centrosomes near the midbody during cytokinesis is not peculiar to lymphocytes but may represent a more general feature of cells that grow in suspension; 2) aggregation of the vimentin system, which occurs concomittant with the relocation of the centrosomes in lymphocytes and lymphoid cells, is not required for centrosome relocation in other suspension cells. This comparative study also provides support for a functional involvement of this filamentous system in mitotic events.

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