J. Cell Sci. **48**, 241–257 (1981)
Printed in Great Britain © *Company of Biologists Limited 1981*

A PROBE FOR FLAGELLAR DYNEIN IN THE MAMMALIAN MITOTIC APPARATUS

GARY W. ZIEVE* AND J. RICHARD McINTOSH†

* *Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts* 02139, *and* † *Department of Molecular, Cellular and Developmental Biology, University of Colarado, Boulder, Colorado* 80309, *U.S.A.*

SUMMARY

An anti-serum has been prepared in rabbits that precipitates high-molecular-weight bovine sperm proteins, including the dyneins. The activity of the serum against the dyneins is demonstrated by the recognition of dynein polypeptides in stained electrophoretic profiles of sperm proteins and in immunoprecipitates of radiolabelled sperm proteins. In addition, the serum stains the sperm flagella when used in indirect immunofluorescence and quantitatively inhibits the motility of demembranated sperm reactivated with ATP. However, the serum has additional anti-sperm activities besides those directed against the dyneins as demonstrated by the staining of the acrosome in indirect immunofluorescence. When used to immunoprecipitate proteins from extracts of cultured cells, the serum precipitates 2 polypeptides; one has a molecular weight higher than the flagellar dyneins, one lower. No specific staining of cultured cells is observed when an affinity-purified anti-dynein fraction IgG is used to stain a variety of cultured cells including bovine fibroblasts. We interpret these data to suggest that flagellar dynein is not a component of the mammalian mitotic spindle and discuss how this conclusion is consistent with recent genetic and structural studies on the mitotic spindle.

INTRODUCTION

Wave propagation by eukaryotic flagella is a well-characterized form of microtubule-mediated motility. Work in many laboratories has suggested that mechanochemically active cross-bridges between adjacent microtubules in the flagellum generate a sliding force between the tubules which is transformed into a bending motion by the flagellar structure (Satir, 1968; Summers & Gibbons, 1971; Kincaid, Gibbons & Gibbons, 1973; Warner & Satir, 1974; Warner & Mitchell, 1978). Gibbons & Rowe (1965) first demonstrated that the mechanochemically active arms in flagella possess an ATPase activity which they named dynein. Since the first identification of dynein, it has been characterized as a cluster of at least 5 polypeptides with molecular weights ranging from 270000 to 300000 daltons and which possess ATPase activity (Linck, 1973; Kincaid *et al.* 1973; Ogawa & Mohri, 1975; Gibbons, Franks, Gibbons & Ogawa, 1976; Piperno & Luck, 1979). Recent analysis has revealed that the mechanochemically active cross-bridges of flagella include several polypeptides of low molecular weight in addition to the dyneins (Piperno & Luck, 1979; Huang, Piperno & Luck, 1979). The ATPase activity of the dyneins requires Ca^{2+} and Mg^{2+} and is inhibited by vanadate ions in the V+ oxidation state (Kobayashi, Mortensen, Nath & Flavin, 1978; Sale & Gibbons, 1979).

The mitotic spindle is a microtubule-containing machine, present in all eukaryotic cells, which functions to separate chromosomes during cell division. In higher eukaryotes, including mammals, the actions of the spindle include both the movement of the chromosomes to the spindle poles and also an increase in the separation of the poles (Schrader, 1953; Oppenheim, Hauschka & McIntosh, 1973; Inoué & Ritter, 1975). The molecular mechanisms responsible for producing these movements are not known. It has been suggested that force production may be the result of the controlled disassembly of microtubules, or active sliding between adjacent microtubules, or some combination of both mechanisms (McIntosh, Hepler & Van Wie, 1969; Inoué, 1976; Margolis, Wilson & Kiefer, 1978; Oakley & Heath, 1978; Borisy, 1978). Recently, Cande & Wolniak (1978) have demonstrated that spindle motility is inhibited by vanadate ions, suggesting that dynein-like ATPase activity is involved in the production of motile forces.

To test the hypothesis that dynein is a mechanically active component of the mammalian spindle we have prepared an anti-serum that includes an activity directed against mammalian flagellar dynein. The serum does not stain the mammalian mitotic spindle nor does it immunoprecipitate polypeptides with molecular weight similar to dynein from cultured cells. These data are discussed in the light of what is currently known about the structure, chemistry and genetics of mitotic spindles.

MATERIALS AND METHODS

Cell culture

Bovine cells, a primary line of fibroblasts isolated by Deborah Turnbull, 3T3 and PTK_1 cells, were cultured as monolayers in Ham's F12 supplemented with 10% foetal calf serum. HeLa cells were cultured as monolayers in Dulbecco's modified Eagle's medium supplemented with 7% calf serum. For the metabolic labelling of cellular proteins, cells were incubated with 20 μ/ml of [^{35}S]methionine (Amersham) in a medium with 10% of the normal concentration of methionine supplemented with dialysed serum. All cell culture reagents were purchased from GIBCO (Santa Clara, CA).

Sperm lysis and extraction of dynein

Frozen ampoules of bovine sperm, obtained from the Artificial Insemination Facility at Colorado State University in Fort Collins, were slowly thawed and the sperm were rinsed free of storage medium by sedimentation at 8000 **g** for 12 min through a cushion of 30% sucrose, 30 mM Tris pH 8. 3×10^9 sperm were resuspended in 10 ml of lysis buffer (1% sodium dodecyl sulphate (SDS), 2 mM ethylenediamine tetracetic acid (EDTA), 40 mM sodium acetate, 30 mM Tris pH 8, 2 mM phyenyl methyl sulphonate (PMSF), 1% Aprotinin (Sigma) and sedimented by centrifugation at 4000 **g** for 5 min. Aprotinin, a basic polypeptide derived from bovine lung, was an effective inhibitor of sperm proteases and an essential component of the lysis buffer. The supernatant fluid was collected and the sperm were reextracted with 5 ml of the same buffer and again sedimented. The pooled supernatants contained greater than 90% of the sperm flagellar protein. The accessory fibres of the sperm tail were solubilized by an additional extraction of lysed sperm in lysis buffer supplemented with 10 mM dithiothreitol (DTT) (Bedford & Calvin, 1974). After 15 min of extraction, the tails were totally solubilized as observed by light microscopy and sperm heads were sedimented by centrifugation at 10000 **g** for 10 min. Solubilized sperm proteins were precipitated with ten volumes of acetone and collected by centrifugation at 10000 **g** for 15 min.

Preparation of antisera

Precipitated sperm flagellar protein was resuspended in protein electrophoresis sample buffer and electrophoresed on 5 mm thick 8 % polyacrylamide gels as described by Laemmli (1970). The gels were stained briefly with Coomassie blue and a thin strip from the high-molecular-weight region of the gels that contained the cluster of dynein polypeptides was cut out with a razor blade. The high-molecular-weight proteins in this strip of gel were electro-eluted and prepared as an immunogen according to the procedure of Lazarides & Hubbard (1976). Hereafter these proteins will be called the dynein fraction. Serum was collected from several New Zealand White Rabbits and used to stain cultured cells using indirect immuno-fluorescence. Two rabbits whose preimmune sera gave a low level of staining were used for antibody production. The dynein fraction was emulsified in complete Freund's adjuvant and injected into the popliteal lymph nodes of the rabbits. After 3 weeks, and at intervals of 10 days thereafter, injections were repeated with the dynein fraction emulsified in incomplete Freund's adjuvant. Faint staining of the sperm flagellum was observed after the second injection and strong staining after the fourth. 200–500 μg of antigen was used per injection. Serum from a single rabbit was used for the experiments reported here; however, serum from a second rabbit gave similar results.

Serum was fractionated by precipitation with 50 % $(NH_4)_2SO_4$. After the second precipitation the precipitate was dissolved in one half the original vol. of 0·15 M NaCl, 20 mM Tris pH 7·7, and stored in aliquots at −20 °C.

An affinity-purified anti-dynein fraction IgG was prepared according to the procedure of Fujiwara & Pollard (1976). Two milligrammes of electroeluted dynein fraction protein was coupled to 0·2 g of cyanogen bromide-activated Sepharose (Pharmacia) and used as an immunoabsorbent. Serum was processed in batch.

Characterization of antisera

Antigens were localized in SDS-polyacrylamide gels according to the procedure of Burridge (1978). Briefly, sperm extracts were run on polyacrylamide gels according to the procedure of Laemmli (1970). The gels were fixed but not stained, neutralized and overlaid with a 1–15 dilution of immune or preimmune serum for 16–24 h. The gels were then rinsed for 6 days and overlaid with ^{125}I-labelled Staph A protein (Pharmacia) for 16–24 h and rinsed for another 6 days. Gels were then dried and analysed by autoradiography.

Ouchterlony double diffusion analysis was performed according to the procedure of Yen, Dahl, Schachner & Shelanski (1976). The dynein fraction and whole sperm flagellar protein were dissolved in 0·5 % SDS at concentrations of 1 mg/ml and 20 mg/ml respectively and used as antigens. Gels were rinsed in 0·3 M NaCl and stained with Coomassie blue.

Preparation of modelled sperm

Motile demembranated bovine sperm were prepared according to the procedures of Lindemann (1978). Frozen sperm were thawed and maintained at 25 °C throughout the experiment. Sperm were diluted with rinse buffer (0·097 M sodium citrate, 5 mM $MgSO_4$, 2 mM fructose and 1 mM $CaCl_2$ pH 7·4) and pelletted by centrifugation at 300 **g** for 5 min. Rinsed sperm were resuspended in reactivation buffer (0·024 M potassium glutamate, 0·132 M sucrose, 0·02 M Tris, 1 mM DTT, 0·1 % Triton X-100 (w/w), 1 mM ATP, 20 μM cATP pH 7·9) at a concentration of 3×10^7 sperm/ml. Thawed sperm were approximately 35 % active and after demembranation in reactivation buffer motility varied from 10 to 30 %. To assay the effect of antibodies on the modelled sperm the immunoglobin fraction prepared as described above was added to a final concentration of 10 % or approximately 2 mg/ml. Reactivated sperm were placed under a coverslip and observed with phase optics. For cinematographic analysis, sperm were pinned between the coverslip and the slide and reactivation buffer containing 10 % immunoglobin fraction was flushed under the coverslip using capillary action.

Immunoprecipitations

Immunoprecipitations were performed by a modification of the method of Kessler (1975). 100 μg of ^{35}S-labelled cell protein in immunoprecipitation lysis buffer (10 mM Tris pH 7·2, 150 mM NaCl, 1 % sodium deoxycholate, 1 % Triton X-100, 0·1 % SDS, 1 mM PMSP, 1 % Aprotinin) were incubated with 100 μg of the immunoglobin fraction for 60 min at 4 °C. The immunoglobins were adsorbed onto Staph A Sepharose (Pharmacia) and purified by extensive rinsing with lysis buffer. Bound antibody and antigen were released from the beads by boiling in sample buffer and the released material was analysed on 5–11 % gradient polyacrylamide slab gels. Similar polypeptides were immunoprecipitated if cells were lysed in sperm lysis buffer and diluted with a 10-fold excess of immunoprecipitation lysis buffer before adding antibody.

Twenty microgrammes of sperm flagellar protein in sperm lysis buffer were iodinated with 400 μCi ^{125}I using chloramine T (Aldrich) as described by Hunter & Greenwood (1962). The iodinated protein was diluted with 10 vol. of the immunoprecipitation lysis buffer and 1 μg of labelled extract in 0·2 ml was incubated with 10 μg immunoglobins for 60 min at 40 °C. Immunoglobin complexes were adsorbed with formaldehyde-fixed *Staphylococcus aureus* and analysed as described above.

Immunofluorescence

Rinsed sperm were fixed in 0·1 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethane-sulphonic acid (HEPES), pH 7·4, 3 mM $MgSO_4$, 2 % formaldehyde for 10 min. Fixed sperm were collected by centrifugation at 800 **g** for 5 min and rinsed in phosphate-buffered saline lacking divalent cations (PBSa, 0·171 M NaCl, 3 mM KCl, 10 mM Na_2HPO_4 pH 7·2) and permeabilized with 95 % ethanol for 5 min at room temperature. The sperm were then rinsed and incubated in serum for 40 min at 37 °C, rinsed extensively and then incubated with fluorescein-conjugated goat anti-rabbit IMgG (iles) for 35 min at 37 °C. The sperm were rinsed and allowed to settle on to polylysine-coated coverslips and mounted in Elvanol. Fixation with either acetone or methanol at −20 °C gave similar results.

For immunofluorescence, bovine cells were grown on glass coverslips in small Petri dishes. Coverslips with cells attached were rinsed in PBSa and then fixed in 0·1 M HEPES buffer, 3 mM $MgSO_4$, 2 % formaldehyde for 30 min. Coverslips were then rinsed in PBSa, permeabilized with 95 % ethanol for 5 min and rinsed extensively in PBSa. Cells were then incubated with serum for 40 min at 37 °C, rinsed extensively in PBSa and then incubated with fluorescein-conjugated goat anti-rabbit IgG (Miles). Coverslips were then rinsed in PBSa followed by distilled H_2O and mounted in Elvanol. Alternative fixation protocols utilizing acetone or methanol at −20 °C gave similar results. Fluorescent-labelled preparations were viewed in a Zeiss Universal Microscope equipped with epi-fluorescence optics. All photographs were taken with Kodak Plus-X film developed with Diafine.

RESULTS

Sperm fractionation and preparation of antigen

Bovine sperm flagellar proteins were solubilized by extracting sperm in lysis buffer containing 1 % SDS and several reagents selected to inhibit proteolytic activity (Kassell & Kay, 1973). As first described by Bedford & Calvin (1974), the major morphological alteration in mammalian sperm (Fig. 1 A, B) exposed to SDS is a selective extraction of the flagellar structural proteins and also the membranous components of the sperm head and tail (Fig. 1 C). The remaining sperm tail is maintained by the coarse fibres which are stabilized by disulphide bonds (Bedford & Calvin, 1974). The tail can be completely solubilized in the presence of reducing agents leaving the compact head structure (Fig. 1 D). The protein composition of the SDS-solubilized sperm flagella (Fig. 2) includes the abundant tubulin polypeptides of approximately

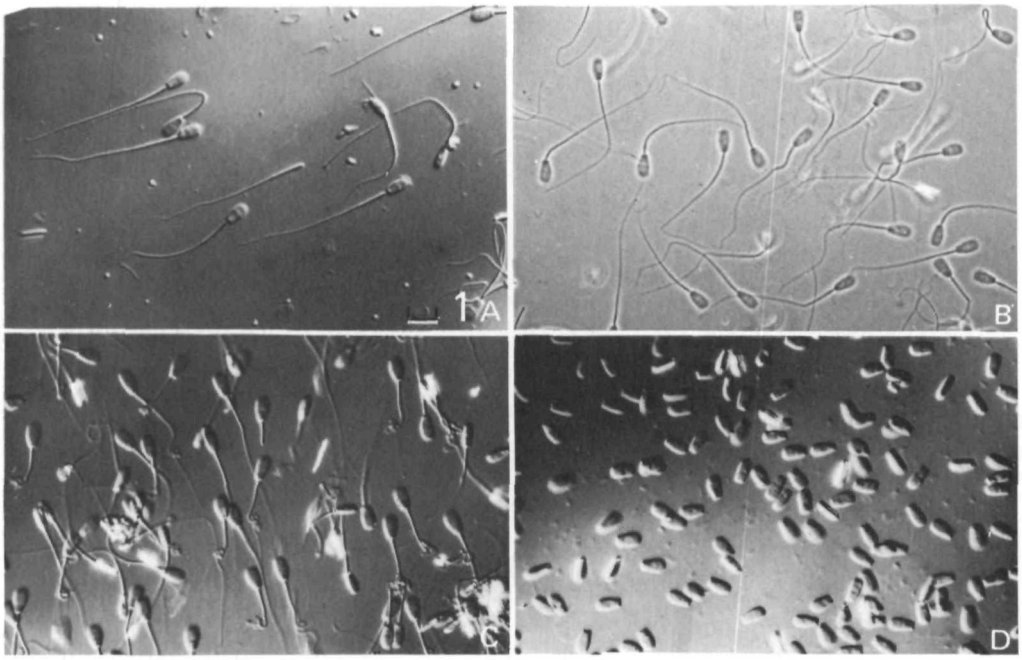


Fig. 1. Nomarski (A) and phase-contrast micrographs (B) of intact bovine sperm and Nomarski micrographs of sperm after extraction in lysis buffer (C) followed by lysis buffer containing 10 mM DTT (D). × 340; bar 10 μm.

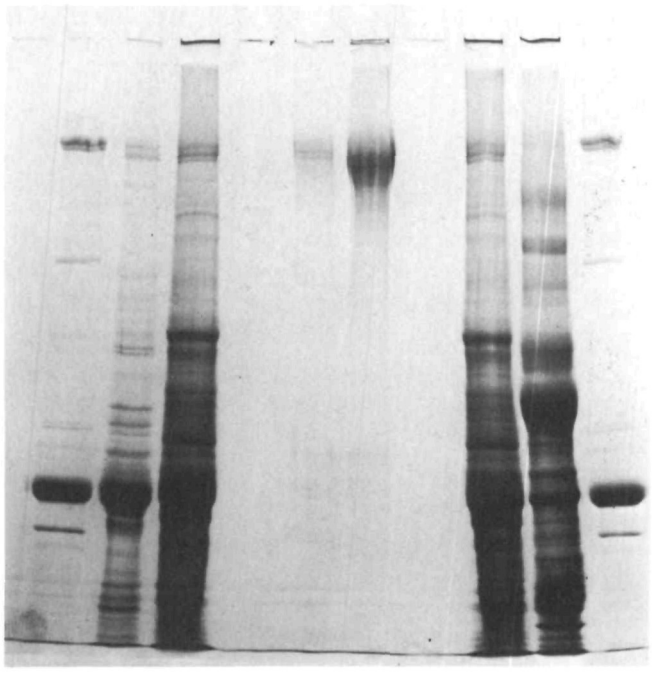


Fig. 2. Polyacrylamide gel electrophoresis of: A, porcine brain microtubule protein; B, sea-urchin flagella; C, bovine sperm proteins solubilized by lysis buffer; D, 2 μg electroeluted dynein fraction; E, 20 μg electroeluted dynein fraction; F, bovine sperm proteins solubilized by lysis buffer; G, additional bovine sperm proteins solubilized in lysis buffer plus 10 mM DTT; and H, porcine brain microtubule protein.

55000 daltons and a cluster of high-molecular-weight components of 260000–300000 daltons which have been identified in other flagellar extracts as the dynein polypeptides (Linck, 1973; Kincaid *et al.* 1973; Ogawa & Mohri, 1975; Piperno & Luck, 1979). The bovine flagellar proteins are similar to the well characterized sea-urchin sperm flagellar proteins (Fig. 2B). The tail fraction solubilized by reducing agents includes a major polypeptide of about 90000 daltons and a small amount of tubulin (Fig. 2G).

The dynein polypeptides were prepared for use as an antigen by extracting flagellar protein from approximately 4 l of bovine semen (12×10^{10} sperm) and fractionating

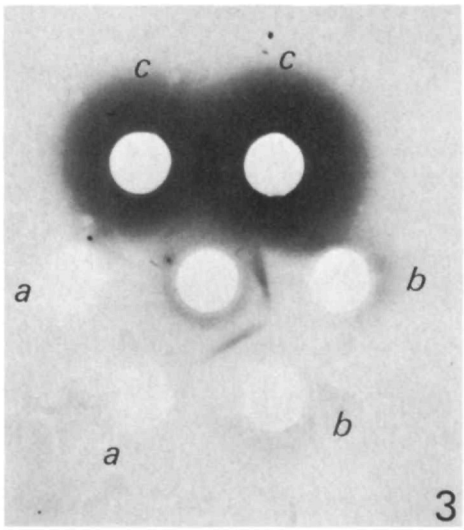


Fig. 3. Ouchterlony double diffusion analysis of immune serum. Immune serum was placed in the centre well and lysis buffer (*a*), electroeluted dynein fraction (*b*) and total lysis buffer solubilized sperm proteins (*c*) were placed in outer wells.

the solubilized material on preparative polyacrylamide gels. The dynein-containing region of the gels was then excised and the polypeptides were electroeluted from the gels and prepared for injection into rabbits as described above. This 'dynein fraction' consisted of the dynein polypeptides and possibly additional proteins of similar molecular weight (Fig. 2D, E). Minor polypeptides of approximately 60000 daltons are also evident in the electrophoretic profile of the eluted dynein fraction. These polypeptides increased in abundance after repeated freezing and thawing of the electroeluted dynein fraction, suggesting that they may represent degradation products of higher-molecular-weight proteins.

Characterization of the anti-serum

The anti-serum raised against the electroeluted dynein fraction precipitated when reacted with the antigen preparation in Ouchterlony double diffusion tests (Fig. 3B). No precipitation line was obtained when preimmune serum was substituted for immune serum or in the absence of antigen (Fig. 3A). A single faint precipitin line

was observed when anti-serum was reacted against whole flagellar extracts. However, our inability to rinse out all the unreacted protein made photographing of this precipitin line difficult (Fig. 3C).

The specificity of the anti-serum was better tested when whole serum was used to stain electrophoretic profiles of bull sperm flagellar extracts, following the method of Burridge (1978). The immune anti-serum recognized the high-molecular-weight

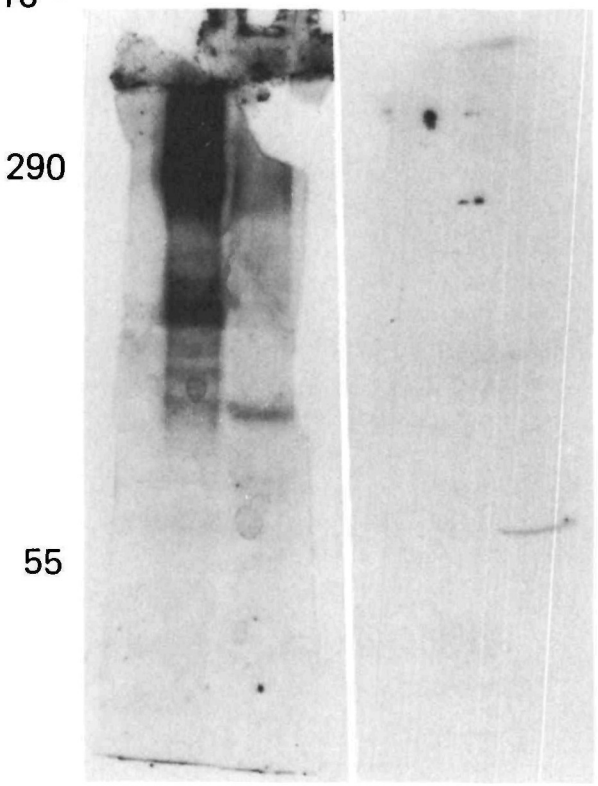


Fig. 4. Staining of electrophoretic profiles of solubilized sperm fraction with immune and pre-immune sera according to the procedure of Burridge (1978). A, sperm proteins in lysis buffer; B, a fraction extracted from the residue by means of lysis buffer containing 10 mM DTT; A and B were stained with immune serum, C and D, respectively, are similar fractions stained with pre-immune serum.

sperm polypeptides that included dynein and a few other sperm proteins (Fig. 4A). No major activity was detected against low-molecular-weight sperm proteins or against coarse fibre proteins (Fig. 4B). Approximately 70% of the immune reaction was directed against the cluster of dynein polypeptides but about 30% of the activity was directed against a region of the gels at approximately 200000 daltons. By Coomassie blue staining this lower-molecular-weight region of the gel contained less than 2% of the mass of protein found in the dynein region, so this activity probably represents some highly antigenic proteins that copurify with the dynein in our procedure. The

preimmune serum does not recognize any of the high-molecular-weight proteins of the flagellum (Fig. 4C); however, one lower-molecular-weight polypeptide in the coarse fibre extract is stained (Fig. 4D).

An additional indication of the efficacy of the anti-serum was provided by its ability to immunoprecipitate high-molecular-weight polypeptides, including the

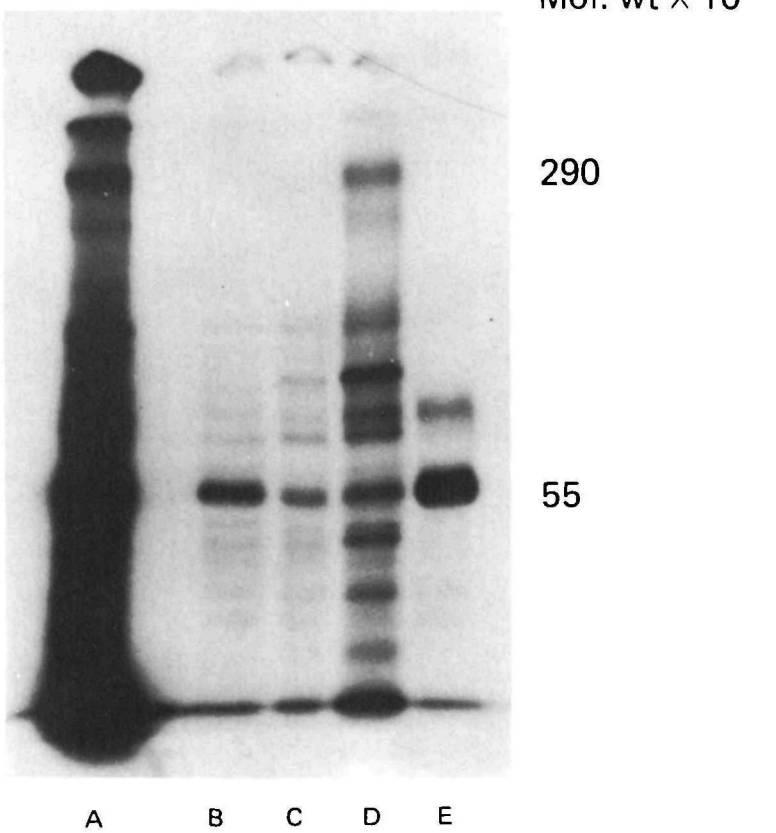
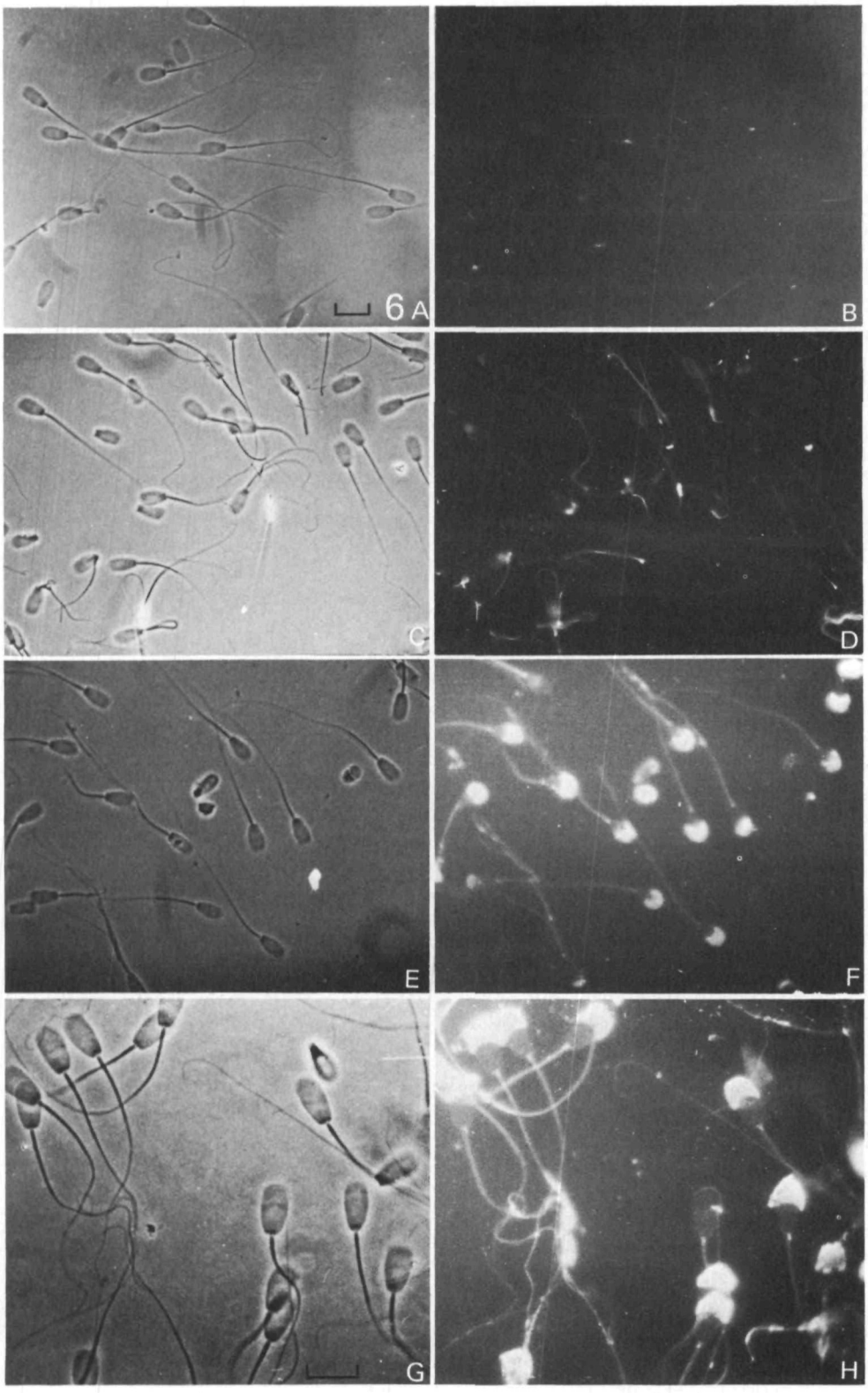


Fig. 5. Immunoprecipitation of lysis buffer solubilized sperm proteins labelled with ^{125}I. Labelled sperm proteins (A) were incubated with no serum (B), pre-immune (C), immune serum (D) and antibody against tubulin (E), and precipitated and analysed as described in Materials and methods.

dyneins from extracts of total sperm flagellar protein (Fig. 5D). The preimmune serum does not precipitate these polypeptides, suggesting that they represent a specific immune response. Several polypeptides of lower molecular weight are also precipitated by the immune serum. The basis for this activity is not understood; the low-molecular-weight components may represent degradation products of higher-

---

Fig. 6. Indirect immunofluorescent staining of bovine sperm. Phase and fluorescent micrographs of sperm stained with pre-immune serum (A, B), anti-tubulin antibody (C, D), whole serum (E, F) and an affinity-purified anti-dynein IgG (G, H). A–F, × 500, bar 10 μm: G–H, × 750, bar 10 μm.


6
A
B
C
D
E
F
G
H

molecular-weight polypeptides that are produced during the multiple rinses that are required for the immunoprecipitation reaction.

Immunofluorescent staining of bovine sperm

Bovine sperm were stained with the immune serum to localize the antigens recognized by the serum. Whole serum and an affinity-purified anti-dynein fraction IgG were used to stain fixed and permeabilized sperm. Both antibody preparations gave similar indirect innumofluorescent staining (Fig. 6). Both the sperm flagella and the sperm acrosome are stained by the immune serum. Preimmune serum gives no detectable staining of any components of the sperm and antibody directed against tubulin stains only the sperm flagellum, as would be expected. The intense staining of the acrosome occurs both in whole serum and when the affinity-purified IgG fraction is used, suggesting that the acrosomal antigen is a high-molecular-weight polypeptide which

Table 1. *Effects of serum on motility of intact and demembranated sperm*

Material	1		2	
	Motile, %	*n*	Motile, %	*n*
Rinsed sperm	35	121	37	141
Rinse sperm + 10 % immune serum	32	149	36	145
Modelled sperm	16	123	15	191
Modelled sperm + 10 % preimmune serum	15	123	11	170
Modelled sperm + 10 % immune serum	0	210	0	180
Modelled sperm + 10 % anti-tubulin serum	18	92	10	220

Sperm were rinsed and reactivated in lysis buffer containing ATP as described in Materials and methods. Sperm were combined with indicated sera and observed using phase optics. *n* is the number of sperm scored in each assay.

copurifies with the dynein polypeptides in the electroeluted dynein fraction. The staining of both the acrosome and the flagella is abolished if sperm are extracted in 1 % SDS prior to staining, confirming that the antigens responsible for the staining are present in the SDS-solubilized fraction.

Inhibitory activity of anti-serum against motile sperm

Several systems have been developed to reactivate flagellar motility following the removal of the cell membrane and these models have been useful in determining the physiological conditions necessary for flagellar motility. Recently, Lindemann (1978) has developed such a system for reactivating demembranated bovine sperm in the presence of cAMP and ATP. The data in Table 1 demonstrate that the immune serum quantitatively inhibits the motility of the lysed 'model' sperm. Cinematographic analysis demonstrates that the inhibition of sperm motility occurs within 1 min after addition of the immune serum to the reactivated sperm. Pre-immune serum and antibody directed against tubulin have almost no effect on the reactivated sperm. On the other hand, the immune serum has no effect on the motility of sperm

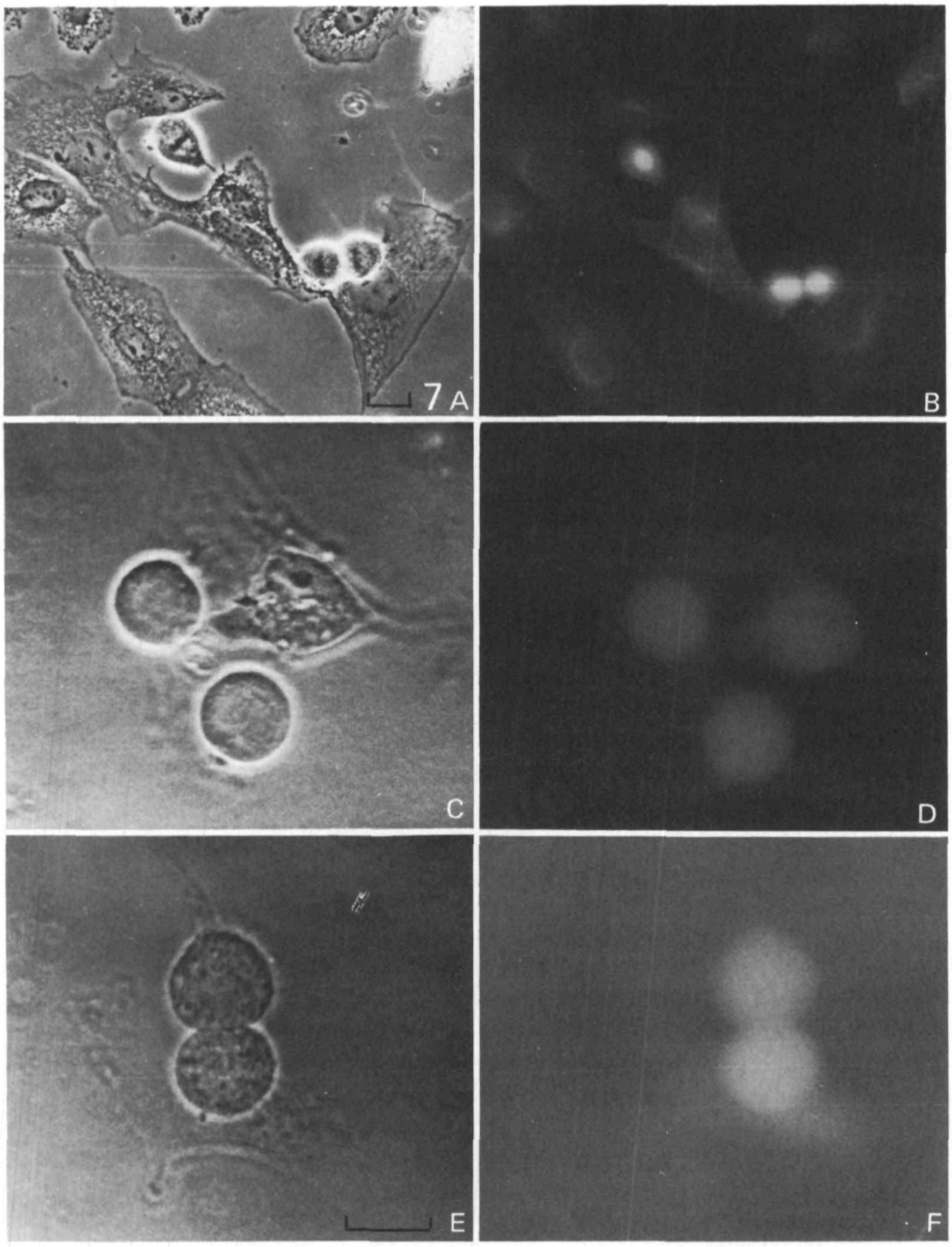


Fig. 7. Indirect immunofluorescent staining of mitotic bovine fibroblasts. Fibroblasts were stained with an affinity-purified anti-dynein fraction IgG and antibody against tubulin. Phase (A) and fluorescent (B) micrograph of cells stained with anti-tubulin antibody. × 600, bar 10 μm. Phase (C, E) and fluorescent (D, F) micrographs of cells stained with anti-dynein fraction IgG. × 1200, bar 10 μm.

with intact membranes. These data suggest that the immune serum is capable of interacting directly with active motile elements in the flagella and inhibiting their motility.

Localization of dynein fraction antigens in cultured cells

To determine whether components of the dynein fraction are present in non-flagellated mammalian cells bovine fibroblasts were stained using the affinity purified

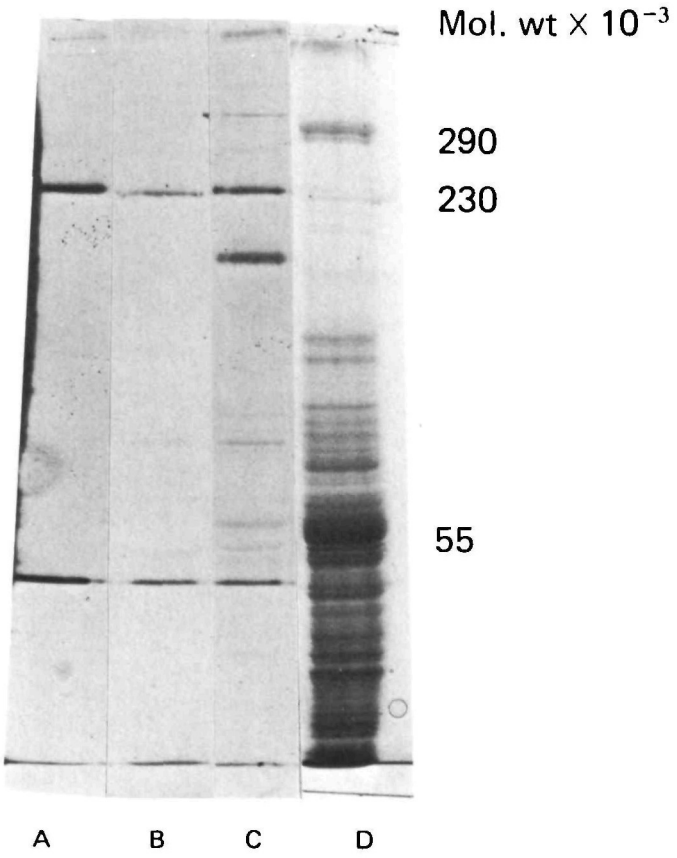


Fig. 8. Immunoprecipitation of whole bovine cell proteins. [^{35}S]methionine-labelled cell proteins were incubated with no serum (A), pre-immune serum (B), and immune serum (C) and precipitated and analysed as described in Materials and methods Coomassie blue staining of sperm flagellar proteins from same gel (D). The polypeptide of approximately 230000 daltons which binds non-specifically to Staph A protein is fibronectin.

anti-dynein fraction IgG (Fig. 7). No specific staining was detected in any region of mitotic or interphase bovine fibroblasts following several different methods of fixation. No specific staining was observed in any of the cell types investigated, including the extremely flat PTK_1 cells and $3T3$ mouse fibroblasts. Control preparations stained simultaneously with antibodies directed against tubulin gave a strong positive

staining of microtubule components of the cells, suggesting the dynein fraction polypeptides are not abundant components of cultured cells.

In an alternative procedure to search for dynein fraction polypeptides in cultured cells, *in vivo* labelled bovine cells were immunoprecipitated with the immune serum. The serum precipitates 2 specific high-molecular-weight polypeptides of approximately 200000 and 300000 daltons from the cell extracts (Fig. 8). Proteins of similar molecular weight are immunoprecipitated from the labelled HeLa cells (Zieve, unpublished). No cluster of dynein-like polypeptides was observed, even after extremely long exposures when other regions of the gel had saturated the film.

DISCUSSION

The anti-serum described here was raised against a group of high-molecular-weight sperm proteins that included the flagellar dyneins. Several criteria suggest that the anti-serum is directed against dynein in addition to other high-molecular-weight sperm antigens. The quantitative inhibition by our immune serum of demembranated reactivated sperm, combined with the lack of inhibitory activity when used against intact motile sperm, suggests that the immune antibodies are penetrating into the flagella of the demembranated sperm and interfering with the action of a component significant for motility, most likely the dyneins. The fact that anti-tubulin antibody stains the sperm tail, but does not affect flagellar motility in our system argues for the motile significance of the components recognized by our anti-serum. In addition, the staining of the sperm tail by our immune serum in indirect immunofluorescence and the recognition of dynein polypeptides in SDS-polyacrylamide gels and in immunoprecipitates of radiolabelled sperm proteins suggests that components of the anti-serum bind to flagellar dynein. However, the recognition of other high-molecular-weight sperm proteins in sperm extracts in addition to dynein and the intense staining of the sperm acrosome by the immune-serum demonstrate that the serum contains other anti-sperm activities.

The strong staining of the acrosome is most likely due to the inclusion of acrosomal proteins in the electroeluted dynein fraction. Several of the high-molecular-weight polypeptides in the electroeluted dynein fraction, with differing electrophoretic mobilities from the cluster of dynein polypeptides, are recognized by the anti-serum and may be responsible for the staining of the acrosome. This is consistent with the observed staining of the acrosome by an IgG fraction purified by its affinity for the dynein fraction proteins.

The inability of the immune serum to immunoprecipitate polypeptides from cultured cells with the same molecular weight as the flagellar dyneins suggests that flagellar dynein is not a component of the cells. Two high-molecular-weight polypeptides, one of higher and one of lower molecular weight than the flagellar dyneins, are immunoprecipitated from the cultured cells and probably represent antigens common to the sperm and cultured cells. However, our inability to detect specific staining of the mitotic spindle or any other component in the cultured cells by immunofluorescence supports the conclusion that flagellar dynein is not a component

of the mitotic spindle. The lack of specific staining was observed after several different methods of fixation, suggesting that the 2 high-molecular-weight polypeptides in cell extracts that do react with the immune serum are either not abundant enough or organized in a manner that makes them undetectable by indirect immunofluorescence. The conclusion that the dyneins are not present in the cultured cells based upon the lack of immunofluorescent staining is of course subject to the caveat that dynein might be present but in a form which is masked. This possibility would seem unlikely, since if dynein is present in the spindle in such a way that it can function mechanically, such a function would require access to the surface of the microtubules and hence exposure to antibodies.

Our results are in disagreement with several recent reports of anti-dynein staining of the mitotic spindle in sea urchins (Mohri *et al.* 1976; Kobayashi, Ogawa & Mohri, 1978). It is possible that there is a dynein in the sea-urchin spindle, but that in mammals, the somatic spindle dynein is antigenically distinct from flagellar dynein. It seems to us more likely though, that the flagellar dynein found in sea-urchin spindles is there because these egg cells and blastomeres will be forming flagella after several rounds of cell division and the dynein is present simply as a soluble component of the ooplasm which collects in the region of the spindle.

Structural studies on mitotic spindles suggest that sliding between microtubules occurs during mitotic movements (McDonald, Pickett-Heaps, McIntosh & Tippit, 1977; Tippit, Schulz & Pickett-Heaps, 1978; McDonald, Edwards & McIntosh 1979), and it has been hypothesized that mechanochemically active links between microtubules are active in generating movements (McIntosh, Cande & Snyder, 1975). The recent demonstration that spindle motility in a lysed mammalian cell is sensitive to vanadate ions supports the suggestion that a mechanochemically active ATPase is involved (Cande & Wolniak, 1978). Serial section analysis of the mitotic spindle interzonal microtubules in plant and animal cells has demonstrated that spindle microtubules which lie next to one another often originate from opposite poles (McDonald *et al.* 1977; Tippit *et al.* 1978; McIntosh, McDonald, Edwards & Ross, 1979; McDonald *et al.* 1979). These data are consistent with a sliding mechanism operating during mitosis. The tubules sliding past one another in such a mechanism would necessarily originate from opposite poles and thus probbaly be of opposite polarities. This is distinctly different from the organization of the microtubules in a flagellum, where all the doublet microtubules originate from the basal body, so the dynein arms make bridges between microtubules of similar polarity (Allen & Borisy, 1974; Binder, Dentler & Rosenbaum, 1975). These considerations suggest that a mechanochemically active cross-link between microtubules which appear to slide in the mitotic spindle would have different properties from the dynein arms in flagella.

Structural studies on human patients with congenitally paralysed cilia and flagella have demonstrated the lack of dynein arms on axonemal doublets. These patients suffered primarily from respiratory and reproductive problems (Afzelius, 1976*a*, *b*) while all other metabolic functions were apparently normal. Also several mutants with paralysed flagella have been isolated in *Chlamydomonas reinhardtii* which lack dynein arms yet go through normal cell division (Huang *et al.* 1979). The genetic

defect responsible for the lack of dynein arms in either system could be in the high-molecular-weight dynein polypeptides or any of several accessory proteins responsible for the assembly of the dynein arms. However, the failure of this lesion to inhibit cell division suggests that the mechanochemically active system in the mitotic spindle is not based on flagellar dynein.

Our immunological data, the structural considerations of anti-parallel interactions and available genetic data suggest that flagellar dynein is not responsible for generating sliding between adjacent microtubules in the mitotic spindle. The structure of the mitotic spindle and the alterations that occur in this fibrillar array during cell division strongly suggest that sliding does occur between adjacent microtubules during anaphase and it seems likely that mechanochemically active crosslinks between adjacent microtubules generate this motion. Crosslinks have been observed between adjacent microtubules in several organisms (McIntosh, 1974) and they are easily seen in the interzone of mammalian spindles (Hepler, McIntosh & Cleland, 1970; Brinkley & Cartwright, 1971). We are presently attempting to isolate biochemically useful quantities of mitotic spindles in an effort to identify proteins that might mediate tubule–tubule sliding.

We would like to thank F. Solomon in whose laboratory this work was completed, K. Fujiwara for the generous gift of antibody directed against tubulin and G. Herring for valuable technical assistance. This investigation was supported by grant number PCM-77-14716 from the National Science Foundation and UCM 154C from the American Cancer Society to J.R.M. G.W.Z. was the recipient of a Helen Hay Whitney Postdoctoral Fellowship.

REFERENCES

AFZELIUS, B. A. (1976*a*). The Role of Cilia in Man. In *Contractile Systems in Non-muscle Cells* (ed. S. V. Perry, A. Margreth & R. S. Adelstein), pp. 275–282. New York: North-Holland.

AFZELIUS, B. A. (1976*b*). A human syndrome caused by immotile cilia. *Science, N.Y.* **193**, 317–319.

ALLEN, C. & BORISY, G. G. (1974). Structural polarity and directional growth of microtubules of *Chlamydomonas* flagella. *J. molec. Biol.* **90**, 381–402.

BEDFORD, J. M. & CALVIN, H. I. (1974). Changes in S–S linked structures of the sperm tail during epididymal maturation with comparative observations in sub-mammalian species. *J. exp. Zool.* **187**, 181–204.

BINDER, L. I., DENTLER, W. L. & ROSENBAUM, J. L. (1975). Assembly of chick brain tubulin onto flagellar microtubules from *Chlamydomonas* and sea urchin sperm. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1122–1226.

BORISY, G. G. (1978). Polarity of microtubules of the mitotic spindle. *J. molec. Biol.* **124**, 565–570.

BRINKLEY, B. R. & CARTWRIGHT, J. (1971). Ultrastructural analysis of mitotic spindle elongation in mammalian cells *in vitro*. *J. Cell Biol.* **50**, 416–431.

BURRIDGE, K. (1978). Direct identification of specific glycoproteins and antigens in sodium dodecyl sulfate gels. *Meth. Enzym.* **50**, 54–64.

CANDE, W. Z. & WOLNIAK, S. M. (1978). Chromosome movement in lysed mitotic cells is inhibited by vanadate. *J. Cell Biol.* **79**, 573–580.

FUJIWARA, K. & POLLARD, T. D. (1976). Fluorescent antibody localization of myosin in the cytoplasm, cleavage furrow and mitotic spindle of human cells. *J. Cell Biol.* **71**, 848–875.

GIBBONS, I. R., FRANKS, E., GIBBONS, B. H. & OGAWA, K. (1976). Multiple forms of dynein in sea urchin sperm flagella. In *Cell Motility* (ed. R. Goldman, T. Pollard & J. Rosenbaum), pp. 915–932. New York: Cold Spring Harbor Laboratory.

GIBBONS, I. R. & ROWE, A. J. (1965). Dynein: A protein with adenosine triphosphate activity from cilia. *Science, N.Y.* **149**, 424–426.

HEPLER, P. K., MCINTOSH, J. R. & CLELAND, S. (1970). Intermicrotubule bridges in mitotic spindle apparatus. *J. Cell Biol.* **45**, 438–444.

HUANG, B., PIPERNO, G. & LUCK, D. J. L. (1979). Paralyzed flagella mutants of *Chlamydomonas reinhardtti*. *J. biol. Chem.* **254**, 3091–3099.

HUNTER, W. M. & GREENWOOD, F. C. (1962). Preparation of iodine-131 labeled human growth hormone of high specific gravity. *Nature, Lond.* **194**, 495–496.

INOUÉ, S. (1976). Chromosome movement by reversible assembly of microtubules. In *Cell Motility* (ed. R. Goldman, T. Pollard & J. Rosenbaum), pp. 1317–1328. New York: Cold Spring Harbor Laboratory.

INOUÉ, S. & RITTER, H. (1975). Dynamics of mitotic spindle organization and function. In *Molecules and Cell Movement* (ed. S. Inoué & R. E. Stephens), pp. 3–30. New York: Raven Press.

KASSELL, B. & KAY, J. (1973). Zymogens of proteolytic enzymes. *Science, N.Y.* **180**, 1022–1027.

KESSLER, S. W. (1975). Rapid isolation of antigens from cells with a staphylococcal protein A-antibody adsorbent. *J. Immun.* **115**, 1617–1624.

KINCAID, H. L., GIBBONS, B. H. & GIBBONS, I. R. (1973). The salt-extractable fraction of dynein from sea urchin flagella. An analysis by gel electrophoresis and by adenosine triphosphatase activity. *J. supramolec. Struct.* **1** 461–470.

KOBAYASHI, T., MARTENSEN, T., NATH, J. & FLAVIN, M. (1978). Inhibition of dynein ATPase by vanadate and its possible use as a probe for the role of dynein in cytoplasmic motility. *Biochem. biophys. Res. Commun.* **81**, 1313–1318.

KOBAYASHI, Y., OGAWA, K. & MOHRI, H. (1978). Evidence that the Mg-ATPase in the cortical layer of sea urchin egg is dynein. *Expl Cell Res.* **114**, 285–292.

LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, Lond.* **227**, 680–685.

LAZARIDES, E. & HUBBARD, B. D. (1976). Immunological characterization of the sub unit of the 100 A° filaments from muscle cells. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4344–4348.

LINCK, R. W. (1973). Chemical and structural differences between cilia and flagella from the Lammellibranch mollusc, *Aequipecten irradians*. *J. Cell Sci.* **12**, 951–981.

LINDEMANN, C. B. (1978). A cAMP-induced increase in the motility of demembranated bull sperm models. *Cell* **13**, 9–18.

MARGOLIS, R. L., WILSON, L. & KIEFER, B. I. (1978). Mitotic mechanism based on intrinsic microtubule behavior. *Nature, Lond.* **272**, 450–452.

MCDONALD, K. L., EDWARDS, M. K. & MCINTOSH, J. R. (1979). Cross-sectional structure of the central mitotic spindle of *Diatoma vulgare*. *J. Cell Biol.* **83**, 443–461.

MCDONALD, K. L., PICKETT-HEAPS, J. D., MCINTOSH, J. R. & TIPPIT, D. H. (1977). On the mechanism of anaphase spindle elongation in *Diatoma vulgare*. *J. Cell Biol.* **74**, 377–388.

MCINTOSH, J. R. (1974). Bridges between microtubules. *J. Cell Biol.* **61**, 166–187.

MCINTOSH, J. R., CANDE, W. Z. & SNYDER, J. A. (1975). Structure and physiology of the mammalian mitotic spindle. In *Molecules and Cell Movement* (ed. S. Inoué & R. E. Stephens), pp. 31–77. New York: Raven Press.

MCINTOSH, J. R., HEPLER, P. K. & VAN WIE, D. G. (1964). Model for mitosis. *Nature, Lond.* **224**, 659–663.

MCINTOSH, J. R., MCDONALD, K. L., EDWARDS, M. K. & ROSS, B. M. (1979). Three-dimensional structure of the central spindle of *Diatoma vulgare*. *J. Cell Biol.* **83**, 428–442.

MOHRI, H., MABUCHI, I., OGAWA, K., KURIYAMA, R. & SAKAI, H. (1976). Evidence for participation of dynein in chromsome movement in mitosis. In *Contractile Systems in Non-Muscle Cells* (ed. S. V. Perry, A. Margreth, R. S. Adelstein). New York: North-Holland.

OAKLEY, B. R. & HEATH, I. B. (1978). The arrangement of microtubules in serially sectioned spindles of the alga *Cryptomonas*. *J. Cell Sci.* **31**, 53–70.

OGAWA, K. & MOHRI, H. (1975). Preparation of antiserum against a trypsin fragment (Fragment A) of dynein and an immunological approach to the subunit composition of dynein. *J. biol. Chem.* **250**, 6476–6483.

OPPENHEIM, D. S., HAUSCHKA, B. T. & MCINTOSH, J. R. (1973). Anaphase motions in dilute colchicine: Evidence of two phases in chromosome segregation. *Expl Cell Res.* **79**, 95–105.

PIPERNO, G. & LUCK, D. J. L. (1979). Axonemal adenosine triphosphatases from flagella of *Chlamydomonas reinhardtii*. *J. biol. Chem.* **254**, 3084–3090.

SALE, W. S. & GIBBONS, I. R. (1979). Study of the mechanism of vanadate inhibition of the dynein cross-bridge cycle in sea urchin sperm flagella. *J. Cell Biol.* **82**, 291–298.

SATIR, P. (1968). Studies on cilia: III. *J. Cell Biol.* **39**, 77–94.

SCHRADER, F. (1953). *Mitosis: The Movements of Chromosomes in Cell Division*, 2nd ed. New York: Columbia University Press.

SUMMERS, K. E. & GIBBONS, I. R. (1971). ATP-induced sliding of tubules in trypsin-treated flagella of sea urchin sperm. *Proc. natn. Acad. Sci. U.S.A.* **68**, 3092–3096.

TIPPIT, D. H., SCHULZ, D. & PICKETT-HEAPS, J. D. (1978). Analysis of the distribution of spindle microtubules in diatom *Fragilaria*. *J. Cell Biol.* **79**, 737–763.

WARNER, F. D. & SATIR, P. (1974). The structural basis of ciliary bend formation: Radial spoke positional changes accompanying microtubule sliding. *J. Cell Biol.* **63**, 35–63.

WARNER, F. D. & MITCHELL, D. R. (1978). Structural conformation of ciliary dynein arms and the generation of sliding forces in *Tetrahymena* cilia. *J. Cell Biol.* **76**, 261–277.

YEN, S., DAHL, D., SCHACHNER, M. & SHELANSKI, M. L. (1976). Biochemistry of the filaments of brain. *Proc. natn. Acad. Sci. U.S.A.* **73**, 529–533.

(*Received* 17 *March* 1980 – *Revised* 23 *July* 1980)