

Actin in Human Spermatozoa

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ABSTRACT

An actin-like material was extracted from human spermatozoa. The material comigrated with actin on sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis, and showed specific affinity for myosin. Purified anti-actin antibodies were used to localize actin to the posterior region of the sperm head and to the connecting piece, midpiece and mainpiece of the tail. Determination of the function of sperm actin requires further investigation.

INTRODUCTION

Actin has been detected in a wide variety of nonmuscle cells and has been associated with many aspects of cell motility (Clarke and Spudich, 1977). The possible involvement of actin in sperm function has been suggested by a number of reports of its presence in both invertebrate (Tilney, 1975) and vertebrate sperm (Clarke and Yanagimachi, 1978; Talbot and Kleve, 1978). Tilney and co-workers (Tilney, 1975) have isolated and characterized the actin from the sperm of two marine invertebrate species, and have demonstrated its involvement in the acrosome reaction and sperm-egg fusion. There has been no comparable work with mammalian spermatozoa.

Recent evidence for the presence and localization of actin in mammalian sperm has rested largely on a number of immunofluorescent studies using anti-actin antisera (Clarke and Yanagimachi, 1978; Talbot and Kleve, 1978; Campanella et al., 1979). Although these studies have suggested the presence of an actin-like protein in mammalian sperm, comparison of the reported localization of the protein in the sperm reveals a number of discrepancies. In order to clarify this situation we have used biochemical techniques to identify the actin of human sperm and specific antibodies to reveal its localization within the sperm.

MATERIALS AND METHODS

Sperm Preparation

Sperm suspensions containing 90-100% motile sperm with less than 0.01% contamination by other cell types, were prepared essentially as described by Hellema and Rumke (1978). Briefly, 1.0 ml of semen ($> 80 \times 10^6$ /ml, $> 50\%$ motile) was overlaid with 2.0 ml of phosphate buffered saline (PBS), pH=7.4, containing 5% AB positive human serum. The tube was then incubated in a tilted position (approx. 45°) for 1 h at 37°C . The top 1.0 ml containing motile sperm was carefully pipetted off and washed twice with PBS. Sperm prepared in this way were used for extraction of sperm actin.

Anti-Actin Antibodies

Autoantibodies specific for human actin were derived from two sources.

1) *Chronic active hepatitis (CAH) serum.* Purified anti-actin antibodies (AAA) were obtained by affinity chromatography of a chronic active hepatitis (CAH) serum containing anti-actin antibodies (Toh et al., 1976). Purified rabbit skeletal muscle actin was coupled to Sepharose 4B as described in a following section. The Sepharose-actin column (0.5×1.0 cm) was equilibrated with a buffer containing 2 mM Tris HCl, 1 mM ATP, 0.5 mM CaCl_2 , and 0.5 mM DTT, pH 8.0. CAH serum diluted 1:4 with the same buffer was then run through the column at 4°C (approx. 2 ml over 60 min). Unbound material was eliminated from the column by repeated washing with the buffer, then bound antibody was eluted with 0.2 M glycine-HCl, pH 2.8. The neutralized eluate was dialysed against PBS and stored at -70°C .

2) *Monoclonal myeloma serum.* Immunoglobulin A (IgA) anti-actin antibody was obtained from the serum of a patient with monoclonal IgA myeloma, the anti-actin specificity of which has previously been described (Toh et al., 1977). Specific antibodies were prepared by mixing 100 μl of serum with 1.5 mg rabbit skeletal muscle G-actin in a total volume of 0.6 ml. The mixture was incubated at 37°C for 30 min, and 4°C overnight, then centrifuged at 4,000 g for 1 h at 4°C . The supernatant was removed and stored at

Accepted October 1, 1981.

Received May 20, 1981.

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–70°C (serum absorbed with actin). Specific anti-actin antibodies were eluted from the precipitate with 0.2 M glycine HCl pH 2.8 (37°C, 30 min). Any precipitate was removed by a further centrifugation step. The neutralized supernatant was stored at –70°C (designated IgA-AAA).

Mouse stomach smooth muscle. Blocks (2 × 2 mm) of mouse stomach were snap-frozen in a liquid nitrogen isopentane slurry, and stored at –70°C until sectioned. Four micron sections were cut at –20°C on a Tissue-Tek II Cryostat (Miles). The sections were air-dried at room temperature prior to staining.

Immunofluorescence. Sperm smears were prepared, fixed in PAF (Stefanini et al., 1967) and stained by standard indirect immunofluorescence procedures as described previously (Clarke and Yanagimachi, 1978). Air-dried sections of mouse stomach were stained by the same procedure. Membrane immunofluorescence and general procedures were as described by Nairn (1976). Fluorescein conjugated sheep antibodies directed against human immunoglobulin classes were obtained from Wellcome Research Laboratories (Beckenham, England) and had fluorescein:protein molar ratios of 3.0–4.0 and protein concentrations of 6.0–8.0 mg/ml. Conjugates were used at a concentration of 0.5 mg/ml in PBS.

Immunobead reaction. Immunobead reagents were obtained from Bio-Rad Laboratories and were used for detection of membrane bound immunoglobulins. They consisted of polyacrylamide beads with covalently bound rabbit antibodies directed against human immunoglobulin classes: IgG (Cat. No. 170–5100), IgA (Cat. No. 170–5114) or IgM (Cat. No. 170–5120).

Motile sperm (50 µl containing 5 × 10⁶ sperm) were added to 200 µl of the sperm or antibody solution to be tested. After 60 min incubation at 37°C to allow antibody binding to the sperm membrane, the sperm were pelleted and washed three times (500 g, 10 min, R. T.) in PBS or Tyrode's solution containing 0.3% bovine serum albumin (CSL, Melbourne, Australia). After final resuspension of the sperm in 100 µl of buffer, one drop was mixed with a drop of immunobead reagent on a glass slide and covered with a coverslip. After 10 min in a moist chamber at R. T. to allow interaction between the beads and sensitized sperm, the reaction was observed under phase-contrast optics at magnification of 500 ×. Motile sperm with two or more beads attached were rated as positive. Control experiments included parallel incubation of sperm in PBS, Tyrode's solution or normal serum, and inhibition of positive reaction by prior incubation of immunobeads with immunoglobulins precipitated from normal serum by 50% ammonium sulphate (Nairn, 1976).

Protein preparations. Rabbit skeletal muscle was used for the preparation of actin (Spudich and Watt, 1971) and myosin. Myosin was prepared by the procedure of Briskey and Fukazawa (1971) and freed of residual actin contamination by chromatography on an Ultrogel AcA 34 column equilibrated and eluted with 0.6 M KI, 0.5 mM ATP, 1 mM DTT, 10 mM sodium pyrophosphate, pH 8.0. Under these conditions (Pollard et al., 1974), actin is depolymerized and completely dissociated. Pure myosin elutes in the void volume completely free of actin which is retarded on the column. Analysis of this column-purified myosin

on overloaded SDS gels revealed the complete absence of actin (Fig. 3). The concentrations of G-actin and myosin were estimated using E values at 280 nm of 1.11 mg.ml^{–1} (Houk and Ve, 1974) and 0.543 mg.ml^{–1} (Briskey and Fukazawa, 1971) respectively. Myosin subfragment S-1 was prepared according to Weeds and Taylor (1975) and its concentration estimated using an E value at 280 nm of 0.77 mg.ml^{–1}.

Coupling of proteins to Sepharose. G-actin was coupled to CN Br-activated-Sepharose 4B using standard procedures (March et al., 1974) except that the coupling buffer used was 2 mM Tris, 1 mM ATP, 0.2 mM CaCl₂, pH 8.0. The protein-coupled Sepharose gels were stored at 4°C in PBS containing azide. Before use the azide was removed by washing with PBS.

Preparation of sperm extracts. Motile human sperm were prepared as described above and stored frozen at –70°C at a concentration of 10⁸/ml in PBS containing 1 mM PMSF, 0.1 mg/ml trypsin inhibitor and 1–5 mM DTT. To prepare the sperm extract (see Results) the frozen sperm suspensions were thawed and then sonicated at full power on a Beckman sonicator in 6 × 15 sec bursts with 1 min on ice in between each burst. The suspensions were then centrifuged at 40,000 rpm × 30 mins in a 50 Ti rotor in a Beckman ultracentrifuge. The resulting supernatants were collected and designated the sperm extract. Generally, these extracts were concentrated three- to fivefold on an Amicon PM-10 membrane before further analysis. If not used immediately, the extracts were stored frozen at –50°C.

Myosin affinity technique. To identify actin in the sperm extracts, the myosin affinity technique of Koch and Smith (1978) was used. The strategy of this technique is to make use of the strong and specific affinity of myosin filaments for actin. If a sample contains actin it should bind to and be precipitated with myosin filaments. Using column purified myosin, myosin filaments were prepared in 10 mM Tris Cl, pH 7.5 as described by Koch and Smith (1978). Sperm extract was prepared as above, dialysed against 10 mM Tris Cl, pH 7.5 and then concentrated fivefold using a Minicon concentration cell. 100 µl of this concentrated extract was then mixed with 200–300 µg of myosin filaments and incubated for 1 h at 4°C. The mixtures were then centrifuged at 100 g for 5 min and the resulting pellets of myosin filaments were washed twice in buffer before dissolving in SDS sample buffer and analysis on SDS polyacrylamide gel electrophoresis.

Polyacrylamide gel electrophoresis. Electrophoresis in the presence of SDS was performed in 1 mm thick 10 or 12.5 cm acrylamide slab gels using the discontinuous buffer system of Laemmli (1970). Prior to electrophoresis, samples of extracts and standards were mixed with an equal volume of sample buffer containing 0.125 M Tris HCl pH 6.8, 5% B-mercaptoethanol, 5% SDS and 10% glycerol. Before application to the gel, these mixtures were heated at 100°C for 1 min to ensure complete dissociation. The gels were calibrated for molecular weight estimation using the following standard mixture (Pharmacia Fine Chemicals, Upsala, Sweden). Thyroglobulin (450,000), ferritin (220,000), myosin heavy chain (200,000), phosphorylase (94,000), bovine serum albumin (68,000), catalase (60,000), ovalbumin (45,000), lactate de-

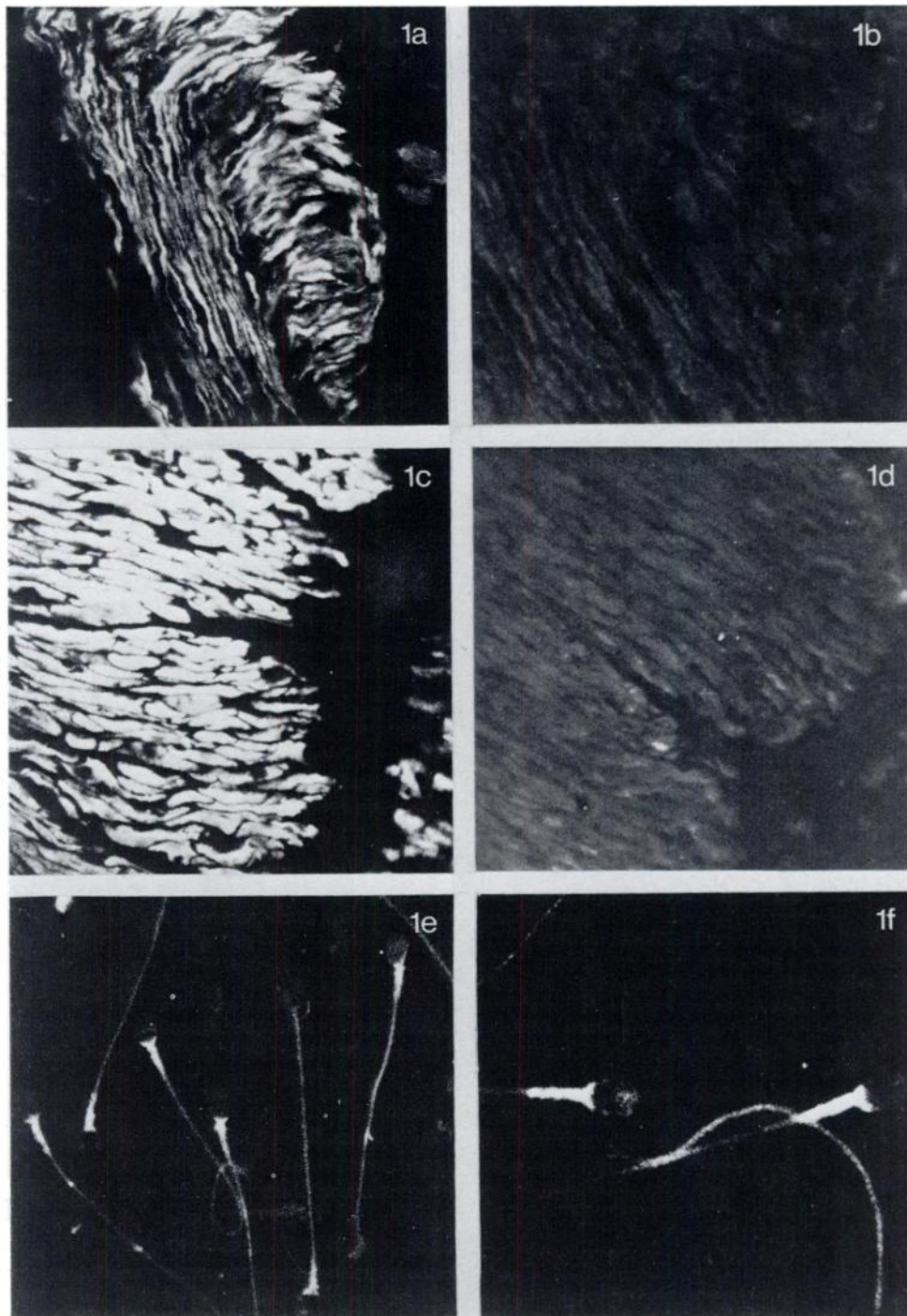


FIG. 1. Indirect immunofluorescence using anti-actin antibodies. (a-d) Mouse stomach smooth muscle sections reacted with: a) CAH anti-actin antibody (AAA) fraction X836; b) CAH serum absorbed by passing through Sepharose-actin column X836; c) Monoclonal myeloma anti-actin antibody (IgA-AAA) fraction X836; and d) monoclonal myeloma serum absorbed with actin X836; e) human spermatozoa reacted with CAH anti-actin antibody (AAA) fraction X912. f) human spermatozoa reacted with monoclonal myeloma anti-actin antibody (IgA-AAA) fraction X1,520.

hydrogenase (36,000), carbonic anhydrase (30,000), trypsin inhibitor (21,500) and α -lactalbumin (14,000).

Following electrophoresis, the gels were stained in 0.25% coomassie blue in 50% methanol, 10% acetic acid for 4–16 h and then completely destained by extensive washing in 5% methanol, 10% acetic acid.

Protein estimation. Protein concentration was determined by the method of Lowry et al. (1951), using bovine serum albumin as standard.

RESULTS

Localization of Actin in Human Sperm

1) *Affinity purified anti-actin antibody (AAA).* In order to eliminate any nonspecific staining due to other serum components, AAA was purified from CAH serum by affinity chromatography.

The fraction eluted from the Sepharose-actin with 0.2 M glycine-HCl (pH 2.8) produced very strong staining of mouse stomach smooth muscle when used in indirect immunofluorescence with an anti-IgG conjugate (Fig. 1a). No staining was observed using anti-IgM or anti-IgA conjugates (not shown). The fraction which did not bind to the column (absorbed serum) produced negligible staining of mouse stomach smooth muscle (Fig. 1b).

In the presence of anti-IgG conjugate, the purified AAA produced strong staining of the sperm midpiece, connecting piece and a saucer-shaped area in the post-nuclear region, weak staining of the tail mainpiece, and weak speckled staining over the acrosomal regions (Fig. 1e). The exact area of staining in the post-nuclear region is difficult to define using immunofluorescence, but appears to extend from the connecting piece to approximately the level of the posterior ring. The absorbed serum did not stain sperm in the presence of anti-IgG conjugate.

The interaction of whole CAH serum, AAA or absorbed serum with viable sperm was investigated using the immunobead reaction. There was no significant reaction between any of these preparations and viable sperm as assessed by immunobead tests.

2) *Monoclonal myeloma IgA anti-actin (IgA-AAA).* IgA-AAA produced very strong staining of mouse stomach smooth muscle in indirect immunofluorescence using a specific anti-IgA conjugate (Fig. 1c). No significant staining was produced using anti-IgG or anti-IgM conjugates (not shown). Serum absorbed with actin did not stain mouse stomach smooth muscle (Fig. 1d).

In the presence of anti-IgA conjugate,

IgA-AAA produced strong staining of the sperm midpiece, connecting piece and a saucer-shaped area in the post-nuclear region, weak staining of the tail mainpiece, and weak speckled staining over the acrosomal region (Fig. 1f). The staining pattern was indistinguishable from that observed using anti-actin antibody purified from CAH serum by affinity chromatography. Absorbed serum did not stain sperm in the presence of anti-IgA conjugate. IgA-AAA did not bind to viable sperm by the criteria of immunobead reaction or membrane immunofluorescence.



FIG. 2. SDS polyacrylamide gel of sperm extracts. 1) PBS extract. 2) PBS plus 0.1% Triton X-100 extract. 3) PBS plus 1% NP-40 extract. 4) PBS plus 2% SDS extract. 5) PBS extract. 6) Sperm residue after extraction 1. 7) Sperm residue after extraction 2. 8) Sperm residue after extraction 3. 9) Sperm residue after extraction 4. A) Skeletal muscle actin. Arrow indicates position of 42,000 dalton band.

Biochemical identification of sperm actin. The presence of an actin-like component in human sperm was further indicated by analysis of sperm extracts on SDS polyacrylamide gel electrophoresis. Equal amounts of sperm were extracted as described in *Materials and Methods* with either PBS, PBS + triton X-100 (0.1%), PBS + 1% NP-40, or PBS + 2% SDS. The sperm suspension in each of these media were sonicated, centrifuged and the supernatants taken for analysis on SDS gels. The corresponding pellets were dissolved by boiling in SDS sample buffer and also analyzed on gels. As shown by the results in Fig. 2, each extract contains a band of MW 42,000 daltons which comigrates with skeletal muscle actin. Comparison of the supernatant and pellet patterns obtained by using the different extraction media suggests that sonication in PBS alone is sufficient to achieve nearly complete extraction of the 42,000 MW band with the addition of detergents being to no further advantage.

The identification of the 42,000 band as sperm actin was achieved by utilizing the myosin-affinity technique of Koch and Smith (1978) which relies upon the strong and specific affinity between myosin and actin. Concentrated sperm extracts were treated with highly purified (actin-free) myosin filaments as described in the *Materials and Methods* section and the resulting well-washed myosin filament pellets were analyzed on SDS gel electrophoresis to detect the presence or absence of actin. If the sperm extracts contained actin it should bind to and be precipitated with the myosin filaments (Koch and Smith, 1978). The results of these experiments are shown in Fig. 3. The electrophoretic patterns in tracks 1, 2 and 3 of this figure illustrate the actin-free character of the column-purified myosin used in these experiments. When concentrated sperm extracts were treated with this myosin, the protein band of MW 42,000 daltons coprecipitated with the myosin filaments (tracks 5 and 7), thus posi-

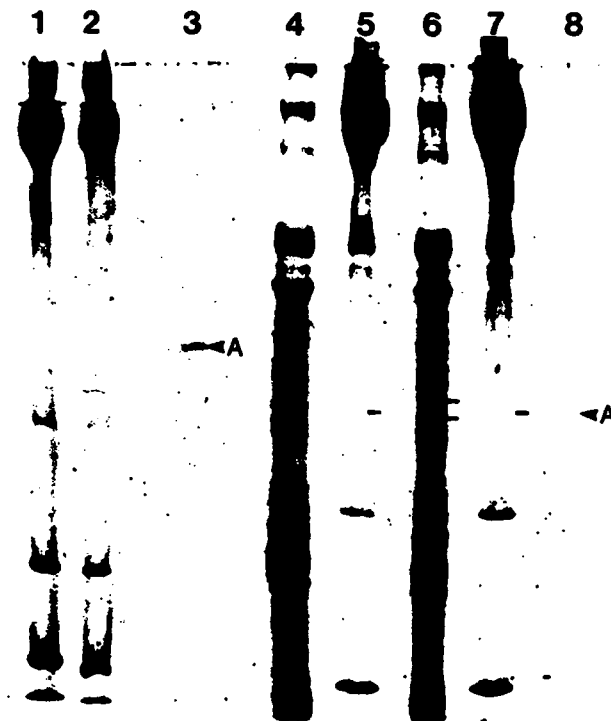


FIG. 3. Myosin affinity precipitation of sperm actin. 1) Column-purified myosin 200µg. 2) Column-purified myosin 300µg. 3) Skeletal muscle actin (A). 4) Sperm extract supernatant after treatment with 200µg myosin filaments. 5) Myosin filament pellet after treatment of sperm extract with 200µg myosin. 6) Sperm extract supernatant after treatment with 300µg myosin filaments. 7) Myosin filament pellet after treatment of sperm extract with 300µg myosin. 8) Skeletal muscle actin (A). Tracks 1–3 were from a different gel run as those from 4–8.

tively identifying this 42,000 band in sperm extracts as sperm actin.

DISCUSSION

The subject of contractile proteins in mammalian sperm has been a controversial one. Initial work indicating the presence of ATPase (Engelhardt and Burnesheva, 1957) and actomyosin-like material (Young and Nelson, 1968) seemed highly promising. Lack of supportive evidence from later work involving chemical analysis of isolated outer dense fibres (ODF) led to a waning of interest in the actomyosin hypothesis (Fawcett, 1975; Baccetti and Afzelius, 1976). The more recent surge of interest in nonmuscle contractile systems, however, and the concomitant availability of a wider variety of biochemical and immunological techniques for identification and localization of contractile proteins, has led to a revival of interest in the question of contractile protein in mammalian sperm.

Recent work by Talbot and Kleve (1978) has produced further support for the presence of actin-like contractile proteins in mammalian sperm. Studying hamster sperm, they were able to demonstrate an electrophoretic band comigrating with actin. In immunodiffusion analysis they observed a line of identity between sperm preparation and purified actins from several sources.

In the present investigation, electrophoretic comigration between a band from solubilized sperm and purified skeletal muscle actin indicated that human sperm contained a macromolecule of similar weight to actin. This evidence was extended by the observation that the band comigrating with actin could be removed by treatment of the sperm extract with myosin filaments. On the basis of its myosin affinity (Koch and Smith, 1978), this band has been identified as sperm actin. The possibility that the actin arose from nonsperm cells in the semen was minimized by the use of purified sperm preparations containing less than 0.01% contamination by round cells or epithelial cells. The present results on human sperm coupled with the observations of Talbot and Kleve (1978) on hamster sperm provide strong support for the belief that mammalian spermatozoa contain a species of nonmuscle actin.

It is important to point out several procedural differences between the present work and earlier work which failed to produce evidence for the existence of sperm actin. In this investi-

gation it was observed that most of the sperm actin was liberated by sonication in PBS alone. The amount released was not significantly increased by other treatments employed. The supernatant from sonicated sperm was then concentrated and analyzed. In the earlier investigations of isolated flagella and ODF, the sperm were usually sonicated, washed and the supernatant fraction discarded (Baccetti et al., 1973).

The localization of sperm actin, apart from the initial work on isolated flagella, rests largely on several investigations employing whole anti-actin serum or crude immunoglobulin precipitates, obtained either from patients with high titer autoantibodies (Clarke and Yanagimachi, 1978) or from immunized experimental animals (Talbot and Kleve, 1978). It is perhaps not surprising, therefore, that there is some confusion as to the exact locality of actin in mammalian sperm. In the present investigations, actin localization has been made using purified antibodies in an attempt to clarify the situation.

The first procedure involved the use of AAA purified from whole CAH serum by affinity chromatography on a Sepharose actin column. The AAA eluted from the Sepharose-actin reacted strongly with the midpiece, connecting piece and posterior region of the sperm head up to approximately the level of the posterior ring. Weaker reaction was observed on the mainpiece of the tail and over the acrosomal region. This staining pattern (Fig. 1e) appeared only with anti-IgG conjugate. The fraction which passed through the column did not produce detectable staining of sperm in the presence of anti-IgG conjugate.

The second localization procedure involved the use of AAA from a patient with IgA monoclonal myeloma (Toh et al., 1977). It has previously been demonstrated that this serum possesses a very high titer against smooth muscle (16,384) and that immunofluorescence induced by the serum could be removed by prior absorption with actin, but not by absorption with myosin, tropomyosin or troponin. When fixed sperm were reacted with IgA anti-actin fraction by indirect immunofluorescence, the localization pattern was identical to the pattern observed with AAA purified from CAH serum by affinity chromatography. Absorbed serum did not stain sperm using anti-IgA conjugate.

The correspondence between the localization results obtained using the two specific anti-

bodies is highly encouraging. It is reasonable to conclude from these observations that actin is located in the human sperm tail, connecting piece, and posterior region of the sperm head. The observation that the specific AAA did not react with the membranes of viable sperm by membrane immunofluorescence or immunobead reaction suggests that the antibodies are binding to intracellular sites. Similar results have been obtained for contractile proteins in other systems (Fujiwara and Pollard, 1976). Determination of the state (i.e. sequestered, monomeric or filamentous) and position of the actin in relation to the sperm membranes and organelles requires much more extensive investigation, particularly using immunoelectronmicroscopy. Tilney's results for invertebrate sperm show that in these cases actin is often associated with the membrane, but not directly connected to intercalated proteins (Tilney, 1976; Tilney and Mooseker, 1976).

The immunofluorescence results obtained in the present investigation agree with those of Campanella et al. (1979) for human ejaculated sperm. However, for several reasons we would be cautious about attributing the acrosomal staining to the presence of actin. Firstly, the use of human autoantibodies in both studies precludes the possibility of using a preimmune control. Secondly, it has been reported that up to 60% of normal human sera produce acrosomal fluorescence (Hjort and Hansen, 1971). The latter may be associated with the observation that the mammalian acrosome can bind immunoglobulins via the Fc regions (Allen and Bourne, 1978). A final reason is the weak nature of the acrosomal staining found in both studies. Thus, we would agree with Talbot and Kleve (1978) that acrosomal localization of actin be regarded as provisional until the observations have been confirmed using highly purified Fab fragments of AAA.

The localization of actin in the post-nuclear region is in accordance with the results of Campanella et al. (1979) who also detected myosin in this area. However, they are at variance with earlier work which indicated that actin was present in the entire posterior region of the mammalian sperm head, including the post-acrosomal region (PAR) proper (Clarke and Yanagimachi, 1978; Tamblyn, 1980). There are several possible reasons for the apparent discrepancy. The earlier work of Clarke and Yanagimachi (1978) involved the use of whole serum from a patient with CAH,

whereas the present work involves the use of antibodies purified from CAH serum by affinity chromatography on rabbit skeletal muscle actin. In the light of a recent report by Lubit and Schwartz (1980), it is feasible that some anti-actin antibodies may be retained on this column, while anti-actin antibodies of a different specificity may pass through. Thus, it was found that the antibody which was retained on the column was of IgG class and reacted strongly with mouse stomach smooth muscle. The material which did not bind to the column included an antibody of IgM class which retained the original post-acrosomal staining, but did not react with mouse stomach smooth muscle. The specificity of this antibody requires further investigation. It is possible that this antibody is directed against an actin determinant which is exposed only when the actin is in a particular state. Thus, it may be that the PAR actin in unreacted sperm is complexed with storage proteins (Tilney, 1976) whereas the actin in the post-nuclear and tail regions is predominantly in a filamentous form. This would also account for the results of Tamblyn (1980) and Peterson et al. (1978) for boar spermatozoa. Actin in mammalian sperm may undergo changes of state during capacitation and acrosome reaction in an analogous manner to the events determined for invertebrate sperm (Tilney, 1975).

The results of Tamblyn (1980) indicate that boar ejaculated sperm contain a material in the post-acrosomal region which can bind DNAase I. The high affinity of DNAase I for monomeric actin makes it seem highly probable that this is the material concerned. In support of this are the results of Peterson et al. (1978) which show that microfilaments appear in the PAR of acrosome-reacted boar sperm. Confirmation of these important observations is necessary before definitive conclusions can be made. They provide tentative support, however, for the hypothesis that (at least in some mammalian sperm) the actin in the PAR is initially in a sequestered or monomeric form such that the DNAase I binding site is exposed. Changes in the internal milieu of the sperm concomitant with the acrosome reaction (e.g. Ca^{++} influx) might be involved in the initiation of polymerization of actin into the filamentous (F-actin) form. The actin would then be able to partake in the sperm-ovum fusion phenomenon which evidently can be initiated in the PAR (Yanagimachi, 1977). Thus a sequence of events not

unlike that observed in several invertebrate systems may pertain (Tilney, 1975).

The present report concurs with that of Campanella et al. (1979) in the observation of actin localized in the human sperm tail and post-nuclear regions. Actin has also been localized to the tail in hamster sperm (Talbot and Kleve, 1978). These reports suggest that actin may be involved in some way in generation of the flagellar beat, as originally proposed by Nelson and co-workers (Young and Nelson, 1968). It has also been suggested (Kleve et al., 1977) that actin situated in the connecting piece/post-nuclear region might be instrumental in determining the angle of orientation between the head and tail, and consequently determine changes in the direction of sperm progression. The results of work with cytochalasins lends support to the possibility that tail actin may play a part in sperm motility (Peterson and Freund, 1977; Chow et al., 1980). This is not definite evidence for the involvement of actin in sperm motility, however, because cytochalasins can, under certain conditions, affect membrane transport of glucose (Lin and Spudich, 1974). It may be rewarding, however, to study the effects of cytochalasins, anti-actin antibodies or other actin-binding substances (e.g. DNAase I, heavy meromyosin or S1 myosin fragments) using a demembrated sperm model (Mohri and Yanagimachi, 1980) in an analogous manner to the investigation of the effect of anti-tubulin antibodies by Asai and Brokaw (1980).

In conclusion, the present work confirms the presence of actin in human sperm and its localization in the post-nuclear and tail regions. Further work is required to clarify the situation regarding the possible existence of actin in the acrosomal and post-acrosomal regions. Extensive investigation is required to determine the state of actin in the different regions of the sperm cell at various stages of development, maturation and the events leading up to fertilization itself, and to begin to understand the functions of sperm actin.

ACKNOWLEDGMENTS

This work was supported by the Royal Women's Hospital Special Purposes Medical Fund. In addition I thank the Teaching Aids Department for preparation of figures, Anne McCartin for patient typing of the manuscript, and Dr. M. N. Cauchi and Dr. R. Hyne for useful criticism of the manuscript.

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