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The specificity of anti-actin serum

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Summary. The immunogenicity of smooth muscle actin is increased by 'ageing' at 4° for at least a week. Rabbits lacking natural smooth muscle antibodies were injected with 1 mg of aged purified actin in adjuvant. Fourteen out of thirty-six rabbits produced serum antibodies which precipitated with actin solution, but not with smooth muscle tropomyosin, myosin, light or heavy meromyosin or with other unidentified non-actin proteins in crude extracts. Analysis of crude actin extract before and after precipitation by antiserum (i) by Sephadex G-200 chromatography and (ii) for its stimulating effect on myosin ATPase activity showed that actin was selectively removed. The precipitate itself, analysed on SDS–polyacrylamide gel, showed one band in the actin position, and otherwise only bands representing immunoglobulins. The antiserum also inhibited the ability of actin to stimulate myosin ATPase activity, and prevented polymerization of G-actin to F-actin, as shown by viscosity and EM studies.

On immunofluorescence with cryostat tissue sections or cell cultures, anti-actin serum stained smooth muscle fibres and many non-muscle cells, in the latter staining the microfilaments. The staining was prevented by absorbing the antiserum with actin (16 μg per 5 μl serum), and was abolished by pretreatment of the cells with cytochalasin B. No species specificity was demonstrated for these anti-actin antibodies.

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INTRODUCTION

The recent increase of interest in actin, one of the contractile proteins, stems from findings that practically all mammalian cells from any tissue contain it (Pollard & Weihing, 1974; Trenchev, Sneyd & Holborow, 1974; Holborow, Trenchev, Dorling & Webb, 1975). The content varies in different types of cell and the morphological appearance of intracellular actin structures depends upon its physiological state and upon the presence of pathological changes (Pollack, Osborn & Weber, 1975; Gabbiani, Trenchev, & Holborow, 1975). The widespread presence of actin in cells which normally display only limited mobility suggests that it performs functions additional to those called on for cell movement.

A striking feature of actin in non-muscle cells is that it aggregates into filaments which may change in pattern and appearance in a matter of hours (Lampert, Trenchev & Holborow, 1974). Some of these changes have been noted in pathological tissues (Gabbiani *et al.*, 1975) but they have been poorly studied and are ill-understood.

Anti-actin serum is a potentially useful tool for the study of intracellular actin but doubts have been raised about the specificity of presumed anti-actin antibody, and it has indeed been thought that native actin is non-immunogenic (Nachmias & Kessler, 1976; Bray, 1974). However, we have reported success in raising anti-actin antibodies of characterized reactivity in rabbits (Trenchev *et al.*, 1974; Holborow

et al., 1975), and Lazarides & Weber (1974) have subsequently made a similar claim.

The purification of actin from extracts containing other non-actin proteins is laborious. Even with the most successful techniques of purification traces of other proteins e.g. tropomyosin, actinin, myosin or other less well identified proteins are difficult to eliminate, and one or more of them may be a more powerful antigenic stimulus than actin.

Our preliminary experiments showed that actin prepared from human uterus is a poor antigen, rabbits failing to produce detectable anti-actin antibody as judged by immunofluorescence and by precipitation, when injected with native smooth muscle actin. The use of immunofluorescence as a means of detection of anti-actin is complicated by the fact that many normal rabbits (in some colonies more than 50%) have spontaneous anti-smooth muscle autoantibodies, which appear to be a mixture of autoantibodies against various contractile proteins including actin.

We found (Trenchev *et al.*, 1974; Holborow *et al.*, 1975) that anti-actin antibody is produced by rabbits injected with smooth muscle actin which had been kept at 4° for at least a week, while rabbits receiving fresh native actin produced no detectable antibody. Lazarides *et al.*, (1974) also obtained anti-actin antibody by immunizing with denatured actin. We show below that purified actin, partially denatured by 'ageing' at 4°, stimulates production of specific anti-actin antibody when injected into rabbits negative for smooth muscle auto-antibodies. This paper reports on the specificity of the antibody produced.

MATERIALS AND METHODS

All proteins except skeletal muscle actin were isolated from human uterus, either fresh or kept frozen at −20°. The smooth muscle tissue was separated from connective tissue and from the endometrium, washed with cold saline and homogenized prior to extraction. An acetone-dried powder was prepared from the homogenate according to Iyengar & Weber (1964). Skeletal muscle actin was prepared from white and red rabbit muscle.

Preparation of actin

The acetone-dried powder was extracted at 0° with a solution of 0·08 M ATP, 0·1 M CaCl, 0·01 M dithiothreitol in deionized water, the pH being adjusted to 8·2 with solid sodium bicarbonate. Actin was prepared from this extract by each of the following procedures:

(a) passage through a Sephadex G-200 column (Adelstein, Godfrey & Kielley, 1963). The second peak monitored by absorbance at 200 nm was collected.

(b) The aggregation-disaggregation method of Spudich & Watt (1971) modified by extending the time allowed for aggregation to 40 h and by using 0·01 M dithiothreitol instead of ascorbic acid.

(c) Precipitation at the isoelectric point of actin (pH 5·0). The precipitate was redissolved in distilled water (pH 8·2) and solid ammonium sulphate added to 20 per cent concentration (108 g/l). The precipitate was discarded and the concentration of ammonium sulphate in the supernatant raised to 30% (36 g/l). The resulting precipitate contained the actin.

(d) Method (c) followed by method (a).

Actin was identified by SDS gel electrophoresis, by EM examination of filaments (Huxley, 1963) and by its enhancing effect on the ATPase activity of myosin (Pollard & Weihing, 1974).

Preparation of other proteins

(1) *Tropomyosin* was prepared from ethanol–ether dried smooth muscle tissue homogenate according to Bailey (1948). The procedure was repeated, and the tropomyosin preparation obtained was analysed by SDS polyacrylamide gel electrophoresis (Fig. 1a).

(2) *Myosin* was extracted from the initial homogenate in Guba–Staub solution (Offer, Moos & Starr, 1973) and precipitated by 14-fold dilution with water (pH 7·2) at 4°. The precipitate was redissolved in Guba–Staub buffer, and myosin was precipitated between 33 and 40% ammonium sulphate saturation. After dialysis, myosin was further purified by gradient chromatography on DEAE–Sephadex A-50 (Offer *et al.*, 1973). Myosin was identified in SDS gels (Fig. 1c), and also on electronmicroscopy by negative staining of the filaments.

(3) *Heavy and light meromyosin* were obtained from myosin after trypsin digestion. Digestion was prolonged to 40 minutes because of the greater resistance of smooth muscle myosin to trypsin (Hamoire, 1973). Heavy and light meromyosin were separated from the digest by precipitation with ammonium sulphate. Heavy meromyosin was re-

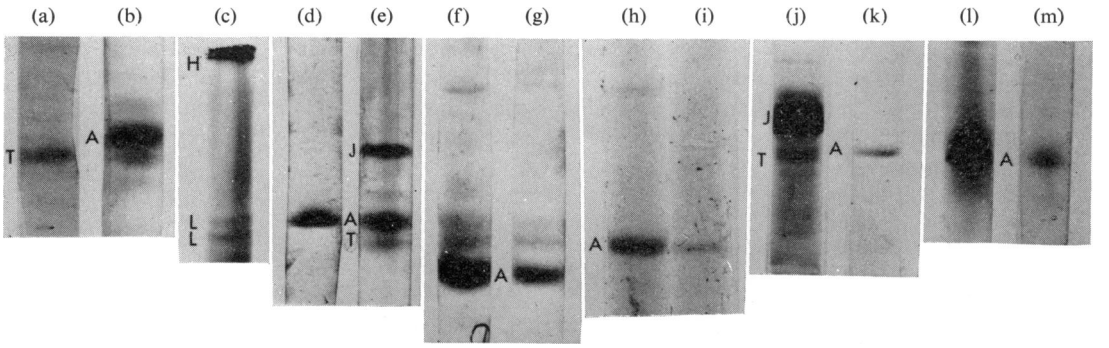

Fig. 1. SDS–polyacrylamide gel electrophoresis patterns of proteins from smooth muscle. (A = actin, T = tropomyosin, J = J band, H = heavy chain of myosin, L = light chain myosin). (a) Purified tropomyosin (10 μg); (b) crude actin extract, partially purified; (c) purified myosin (10 μg); (d) actin prepared by procedure (d) (10 μg); (e) crude actin extract, partially purified (10 μg); (f) purified actin (Spudich and Watt method) (30 μg); (g) purified actin (Spudich and Watt method) (10 μg); (h) purified actin from Sephadex G-200 (20 μg); (i) purified actin from Sephadex G-200 (10 μg); (j) proteins precipitated at pH 5·2 from Guba–Staub extract after myosin precipitation; (k) actin purified from (j); (l) actin prepared by procedure (c) (30 μg); (m) actin prepared by procedure (c) (10 μg).

covered between 47 and 53% and light meromyosin between 53 and 70% saturation.

Tissue cultures of lens epithelial cells and BDH fibroblasts were as described by Hughes, Laurent, Lonchampt & Courtois, (1975).

SDS–polyacrylamide gel electrophoresis was carried out according to Weber & Osborn (1969) in 7·5% gels.

Double diffusion was performed in 1% agar, which contained 0·5 M KCl when myosin was included in tests.

The immunofluorescent technique was as previously described (Trenchev *et al.*, 1974).

Measurement of effect of antiserum on viscosity

Equal volumes of actin solution and antiserum dilutions (1:1, 1:10, 1:20) were mixed for ten minutes at room temperature before adding $MgCl_2$ (20 mM) and KCl (0·1 M) final concentration to the protein mixture.

Immunizing procedure

Rabbits were screened by immunofluorescence for serum smooth muscle or other auto-antibodies and only negative animals were injected. The rabbits received actin solution which had been stored for at least a week at 4°. Each animal received 1 mg of actin purified by procedure (d) above, in either Freund's complete or incomplete adjuvant. Blood samples were collected 21 days later.

Anti-tropomyosin antiserum was prepared as previously described (Trenchev *et al.*, 1974).

Estimation of ATPase activity

Activation of smooth muscle myosin ATPase by actin was measured at 25° by mixing equal volumes of solutions of actin (0·8 mg/ml) and myosin (0·42 mg/ml). To test the effect of antisera equal volumes of actin solution and neat serum were mixed and after 15 min incubation at room temperature the mixture was added to the myosin. The final mixture was adjusted to 0·3 M KCl, 1 mM ATP, 1 mM $MgCl_2$, pH 7·4, and additionally contained either 0·1 mM $CaCl_2$ or 0·1 mM EDTA. The estimation of inorganic phosphorus was carried out as described by Rockstein & Herron (1951). The ATPase activity of the antiserum alone was determined and subtracted from the total ATPase activity of the mixture.

The purity of actin preparations was checked by SDS gel electrophoresis and by chromatography on Sephadex G-200.

Repeated aggregation and disaggregation of actin (Spudich & Watt, 1971) gave only partial purification. After two aggregation-disaggregation cycles preparations of smooth muscle actin still showed bands of other proteins in SDS gel, especially in the region where actinin is seen in skeletal muscle preparations (Fig. 1f,g).

Chromatography on Sephadex G-200 gave better separation (Fig. 1h,i). At high loading, however, an additional band of molecular weight about 90,000 (Fig. 1h) was seen. Attempts to remove this second polypeptide by repeated elution on Sephadex G-200 were unsuccessful. A similar band was seen in the aggregation-disaggregation preparations of actin

(Fig. 1f) so that a combination of both techniques was not attempted. From crude actin extract or from the supernatant solution after myosin extraction the method of pI precipitation and ammonium sulphate precipitation (method (c)), although simple, sometimes produced surprisingly pure actin (Fig. 1j,k,l,m). This technique is still under investigation.

Isoelectric point precipitation followed by gel filtration on Sephadex G-200 usually gave reasonably purified actin. Isoelectric point precipitation at pH 5·0 does not separate actin from tropomyosin or from some other proteins (Fig. 1e) but it removes contaminating proteins of molecular weight above 60,000. The actin obtained by combination of these two methods appeared homogeneous in SDS gels (Fig. 1d) and was used for immunizing most of the rabbits. It was left at 4° to age for 1 or 2 weeks before being used injected.

RESULTS

Precipitation of actin by antisera

The sera of fourteen of the thirty-six rabbits injected with actin gave precipitation in tubes with dilutions of smooth muscle actin solution (initial concentration 1·0 mg/ml) up to 1:128, and with skeletal muscle actin (1·0 mg/ml) up to a dilution of 1:64. There was no precipitation with tropomyosin or buffer solution. No precipitation was obtained with the pre-immune sera when tested against actin.

In double diffusion in agar the anti-actin serum gave a precipitation line with purified actin, but not with myosin, heavy meromyosin, light meromyosin, tropomyosin, or buffer solution (Fig. 2a). The precipitin line moved away from the antiserum well with diminishing concentration of actin (Fig. 2b). Fig. 2c shows the precipitation reactions of the antiserum with partially purified actin preparations and with tropomyosin. The presence of actin was necessary to produce a precipitation line in agar and this was invariably a single line (Fig. 2c). The presence or absence of tropomyosin or other impurities did not change the reaction.

Fig. 2c shows the precipitation reactions obtained with various preparations obtained from crude actin by precipitation with magnesium acetate. The antiserum is in the central well. Well 1 contains 100 mM magnesium acetate solution. In well 2 is the supernatant after precipitation with 50 mM Mg acetate, and containing actin, tropomyosin and at least three other unidentified components as shown in Fig. 3d. Wells 4 and 5 contain supernatants after precipitation with 20 and 100 mM Mg acetate respectively (Fig. 3c,b). Both contain a component not hitherto described, which we have provisionally designated 'J' band. This is absent in wells 2 and 6 (Fig. 3c). Tropomyosin is present in well 4 but not in well 5 (Fig. 3 c,b). The solution in well 6 is apparently free from the 'J' band component (Fig. 3a) but contains tropomyosin and smaller amounts of other components, differing from those in well 2 (Fig. 3d). The precipitin line in Fig. 2c shows a reaction of identity for wells 4, 5 and 6, and is unaffected by the presence or absence of tropomyosin, 'J' band or other components. Well 3, containing purified tropomyosin (Fig. 1a) again gave no precipitation.

The anti-actin sera gave no precipitation with human serum proteins in tubes or in double

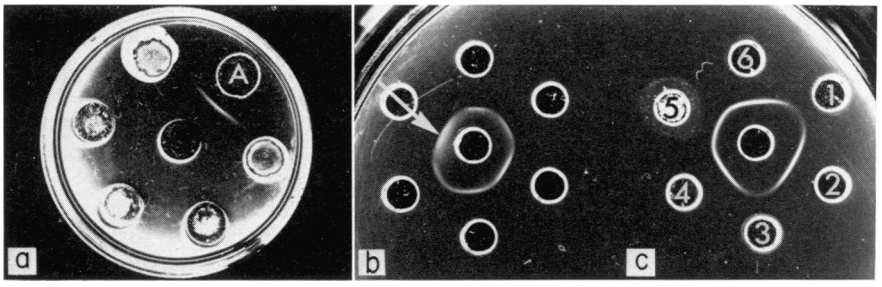

Fig. 2. Double diffusion in agar. Anti-actin serum in centre wells. (a) Purified actin (as in Fig. 1d) in well A. The remaining wells clockwise were filled with myosin, heavy meromyosin, light meromyosin, tropomyosin and buffer used for actin extraction; (b) in the outer wells falling dilutions of purified actin, clockwise 1:1 (arrow) to 1:6; (c) the outer wells contain 100 mM Mg acetate solution (1), actin solution (as in Fig. 3d) (2), tropomyosin solution (3), actin solutions (as in Fig. 3c, 3b and 3a).

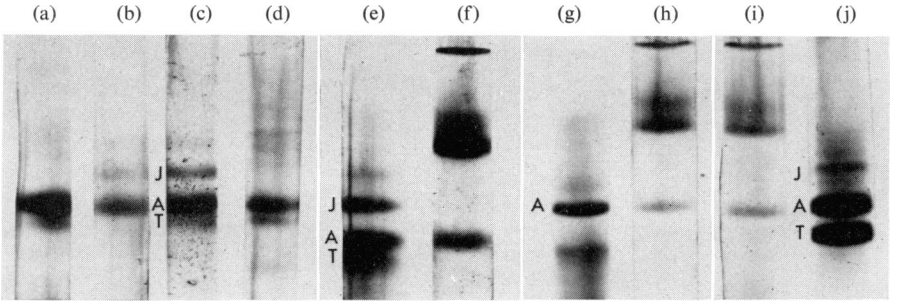

Fig. 3. SDS gel patterns of partially purified actin. (A = actin, T = tropomyosin, J = J band). (a) Precipitated by 100 mM Mg acetate; (b) supernatant from (a); (c) supernatant after precipitation with 20 mM Mg acetate; (d) supernatant after precipitation with 50 mM Mg acetate; (e) partially purified crude actin (10 μg); (f) immune precipitate with anti-actin serum from (e) (15 μg); (g) partially purified actin (10 μg); (h) immune precipitate with anti-actin serum from (g); (i) partially purified actin with added tropomyosin (20 μg); (j) immune precipitate with anti-actin serum from (i).

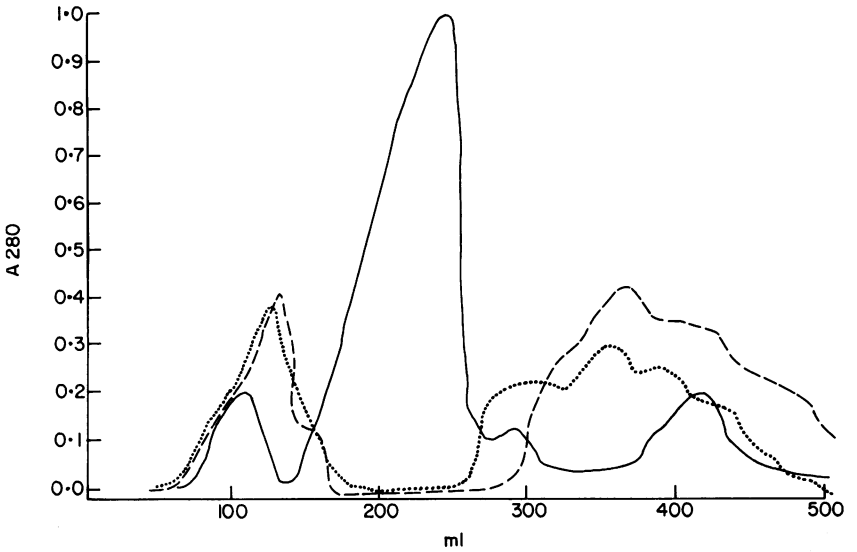

Fig. 4. Elution pattern on Sephadex G-200 of crude actin extract (——), the same extract after treatment with anti-actin gamma-globulin fractions (. . . .), and the gamma-globulin fraction itself (– – –).

diffusion in agar at various dilutions of human serum from 1:1 to 1:16.

A gammaglobulin fraction was separated from one of the anti-actin sera by 33% ammonium sulphate precipitation and mixed at optimal ratio with crude actin extract. The mixture was incubated overnight at 4° and the precipitate removed by centrifugation at 20,000 *g* for 10 min. The supernatant was then applied to a Sephadex G-200 column. For comparison, crude actin extract alone or rabbit serum gammaglobulin fraction alone were run on similar columns with the same buffer. Crude actin extract gave three peaks, the second identified as actin (Fig. 4). After precipitation of crude actin with antibody, the second peak was absent, while the first and third were unchanged (Fig. 4). The gamma-globulin fraction from pre-immune serum did not produce this effect.

The precipitate obtained on mixing the gamma-globulin fraction of anti-actin serum with crude actin extract was washed thoroughly in saline and run in SDS polyacrylamide gel electrophoresis. Apart from the gammaglobulin bands, only one other band, corresponding in position to actin, was seen. This result was obtained with three different preparations of crude actin (Fig. 3e,f,g,h,i,j).

Inhibition of polymerization of G-actin

Anti-actin antibodies also react with G-actin, preventing polymerization of rabbit skeletal muscle actin into filaments in 0·1 M KCl, 2 mM $MgCl_2$. This was demonstrated by electron microscopy and by measurement of viscosity of actin solutions. It was specific for anti-actin sera and correlated with the amount of anti-actin antibody added (Table 1).

Table 1. Effect of rabbit antiserum on skeletal rabbit actin polymerization as measured by the V_{rel} (water) at 25° in an Oswald viscometer

Proteins	Anti-serum dil.	V_{rel}
Actin solution (0·825 mg/ml)		1·37
Anti-actin serum		1·75
Anti-tropomyosin serum		1·80
Actin + Mg^{2+} + KCl		12·5
Actin + Mg^{2+} + KCl	Anti-actin 1:1	1·45
Actin + Mg^{2+} + KCl	Anti-actin 1:10	2·69
Actin + Mg^{2+} + KCl	Anti-actin 1:20	6·50
Actin + Mg^{2+} + KCl	Anti-tropomyosin 1:1	8·06
Actin + Mg^{2+} + KCl	Anti-tropomyosin 1:10	6·68
Actin + Mg^{2+} + KCl	Anti-tropomyosin 1:20	6·55
Actin + Mg^{2+} + KCl	Preimmune serum 1:1	9·02

Inhibition of enhancement of myosin ATPase activity by actin

Addition of smooth muscle actin increased the Ca^{2+}-dependent ATPase activity of smooth muscle myosin three- to four-fold. When actin was pre-treated with anti-actin serum, this enhancing effect was prevented in proportion to the amount of antiserum added (Table 2). The pre-immune serum produced no inhibition.

When crude actin extract was precipitated with anti-actin gammaglobulin and the precipitate removed, the supernatant had no enhancing effect on myosin ATPase activity.

Immunofluorescence

By immunofluorescence, the anti-actin antisera stained smooth muscle and skeletal muscle and also heart muscle. Skeletal and heart muscle showed striational staining (Fig. 5a) but staining of smooth muscle fibres appeared homogeneous (Fig. 5b). Similar patterns of staining were seen with swine anti-whole rabbit immunoglobulin, with goat anti-rabbit IgG and with sheep anti-rabbit IgM conjugates (Nordic). Staining was specifically abolished by absorption with purified actin (16 μg per 5 μl of antiserum) but not with tropomyosin, myosin, heavy

Table 2. Effect of anti-actin on activation of smooth muscle myosin ATPase by purified actin, or crude actin solution. The conditions are described in the text

Protein(s)	Antiserum(dil)	μmol Pi/mg protein/min	
		With 0·1 mM Ca	With 0·1 mM EDTA
Myosin	—	0·030	0·30
Myosin + actin	—	0·108	0·102
Myosin + actin	Anti-actin 1:1	0·030	0·030
Myosin + actin	Anti-actin 1:10	0·051	0·50
Myosin + actin	Anti-actin 1:100	0·098	0·92
Myosin + actin	Preimmune serum 1:1	0·105	0·100
Myosin + actin	Preimmune serum 1:10	0·106	0·101
Myosin + actin	Preimmune serum 1:100	0·110	0·102
Myosin + actin	Anti-tropomyosin 1:1	0·110	0·100
Myosin + actin	Anti-tropomyosin 1:10	0·109	0·100
Myosin + actin	Anti-tropomyosin 1:100	0·110	0·099
Myosin + crude actin	—	0·123	0·040
Myosin + crude actin	Anti-actin 1:1	0·030	0·032
Myosin + crude actin	Anti-actin 1:10	0·060	0·039
Myosin + crude actin	Anti-actin 1:100	0·110	0·041
Myosin + crude actin	Preimmune serum 1:1	0·120	0·040
Myosin + crude actin	Preimmune serum 1:10	0·120	0·042
Myosin + crude actin	Preimmune serum 1:100	0·120	0·040

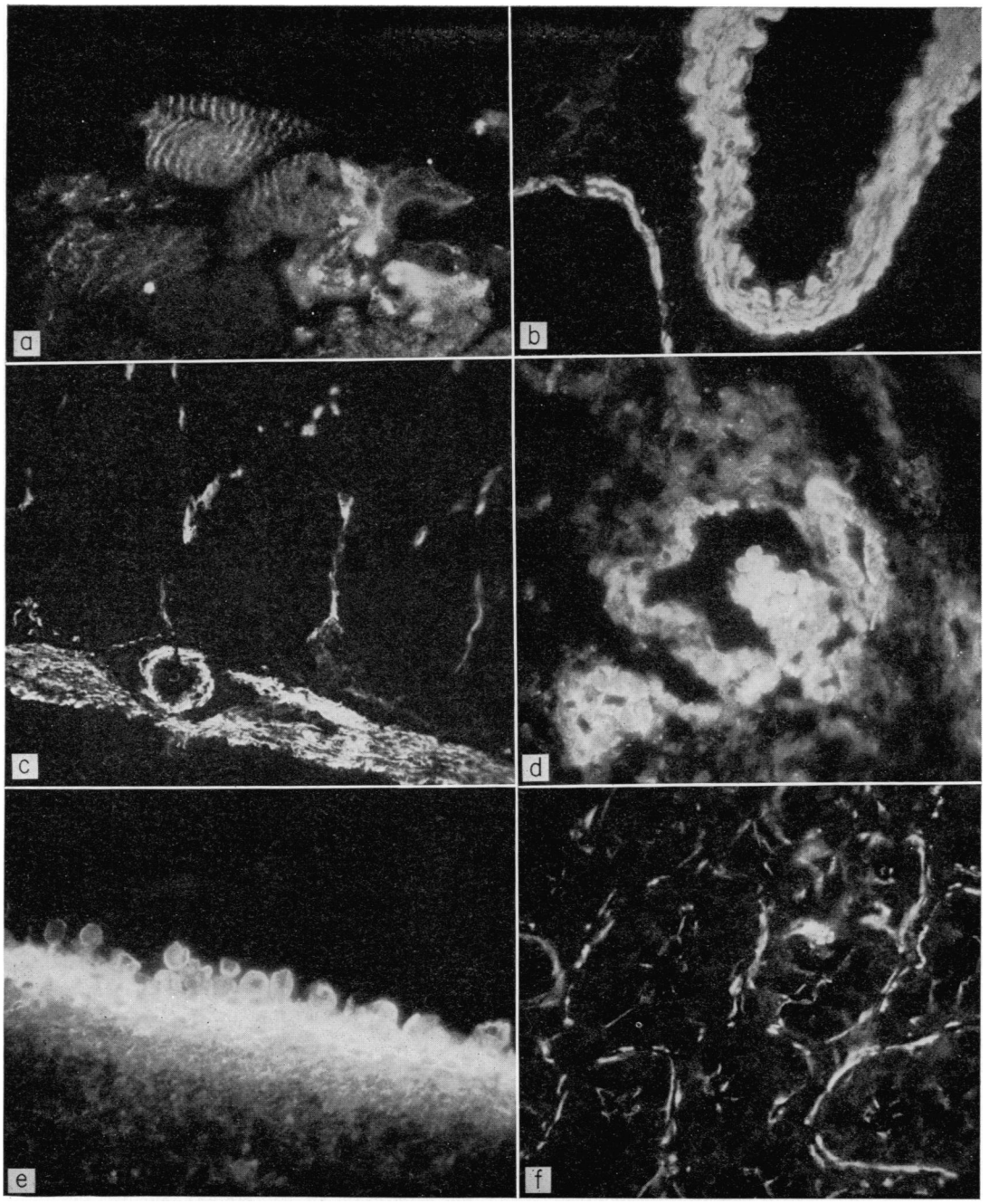

Fig. 5. (a) Immunofluorescent micrograph of Drosophila skeletal muscle stained with anti-smooth muscle actin antiserum (magnification ×230); (b) Immunofluorescent micrograph of rat liver section stained with anti-actin serum. Specific fluorescence in the smooth muscle coat of an artery and a vein (magnification ×115); (c) Immunofluorescent micrograph of a rat stomach section stained with anti-actin serum. Specific fluorescence in muscle fibres, muscularis mucosa and artery muscle coat (magnification ×115); (d) Tissue section of kidney from a 10 cm human embryo, stained with anti-actin serum. Specific staining in the cells of a glomerulus, tubular cells and the cells lining the future Bowman's capsule (magnification ×115); (e) Cryostat tissue section of skin from a 10 cm human embryo. Note the specific fluorescence of the skin epithelial cells (magnification ×115). The same cells stain very poorly in adult skin; (f) Tissue section of rat salivary gland, stained with anti-actin. Bright fluorescence is seen in the myo-epithelial cells (magnification ×230).

meromyosin, or light meromyosin. The absorbing power of purified actin was about three times greater than that of crude actin.

When 4 parts of purified actin and 1 part of myosin were mixed at 0° in 0·05 M KCl, incubated for 30 min, centrifuged and the precipitated actomyosin discarded, the remaining supernatant failed to absorb out the anti-actin antibody.

Tissues showing specific staining

The anti-actin sera diluted up to 1:100 gave specific fluorescence of various types of cells and tissues. Actin was demonstrated in cells of lung, thyroid, oesophagus, stomach (Fig. 4c), duodenum, bladder, ureter, testis, ovary, peritoneum, spleen, salivary gland (Fig. 4f), liver (Holborow *et al.*, 1975), kidney Trenchev, Dorling, Webb & Holborow, 1976), skin and placenta. No species specificity was found. The species tested were mouse, rat, rabbit, guinea-pig, bovine and human. Brighter fluorescence was obtained with foetal tissues (Fig. 4d and 4e) than with adult. More fluorescence was seen in active cells; for example, fibroblasts were more highly stained than fibrocytes. Also cells in carcinoma tissue (Gabbiani *et al.*, 1975) contained more actin.

In tissue culture cells, the actin staining was mainly in the form of filamentous structures (Lonchampt *et al.*, (1976), increased in concentration around the nucleus and under the cell membrane, but not usually on the membrane surface itself (Meager, Nairn, Ungkitchanukit, Hughes & Trenchev, 1976). When cytochalasin B was added to the culture, staining for actin became weak or negative (Lonchampt *et al.*, 1976).

In contrast, cells grown in medium containing colchicine gave the same staining for actin as non-treated cells.

All anti-actin staining activity was found in gamma-globulin fractions of anti-actin sera.

DISCUSSION

These results show that antibodies raised in rabbits by injection of partially denatured smooth muscle actin have specific anti-actin activity. Partially denatured actin presumably differs sufficiently from the rabbits' own actin to break tolerance if such exists. Denaturing must not be carried too far if immunization is to be successful. For example, in our hands, a single injection of 0·5 mg of actin treated with 0·2% SDS, did not produce detectable antibody. The optimal extent of denaturation is not known, but since antibodies against partly denatured actin cross react with native actin it appears that the procedure needs to leave parts of the antigenic mosaic intact. Whatever the mechanism of immunogenicity, it appears from the results of precipitation tests, from analysis by SDS–polyacrylamide gel electrophoresis of the immune precipitates obtained with the antiserum, and from the ability of the anti-sera to selectively remove the actin peak from chromatography of crude extract, that the immunization procedure described produces antibody directed against actin, which does not react with other non-actin proteins present in crude extracts, including myosin, heavy meromyosin, light meromyosin and tropomyosin.

Further support is provided by the immunofluorescence results. The antibody gives the same patterns and stains the same structures as those identified by heavy meromyosin binding—i.e. the apical region of intestinal epithelial cells (Tilney & Mooseker, 1971; Ishikawa, Bischoff & Holtzer, 1969), the basal part of kidney tubular cells (Rostgaard, Kristensen & Nielsen, 1972) and fibroblasts, smooth muscle cells and epidermal embryonic cells (Ishikawa *et al.*, 1969).

Anti-actin antibody prevents the polymerisation of G actin into filaments, and also prevents both actin and crude extract of the acetone-dried powder from enhancing the ATPase activity of myosin. This effect on the biological properties of actin is unlikely to be brought about in any way other than by direct action on actin molecules.

Cytochalasin B is known to disrupt actin microfilaments and its effect on fluorescence produced by the antiserum is further evidence that the specificity of the antibody is anti-actin. The lack of a colchicine effect indicates antigenic differences between actin and tubulin.

The failure of tropomyosin to absorb the anti-actin antibody or precipitate with it shows that anti-actin antibody does not cross react with tropomyosin, and a tropomyosin band was not seen on SDS gel separation of the components in the immune precipitate formed by the anti-actin gammaglobulin fraction and a crude actin extract which contained tropomyosin. In addition, on Sephadex G-200 analysis of the supernatant after precipitation, the first peak, containing tropomyosin, remained while the actin peak disappeared. Neither myosin nor its

sub-fragments heavy and light meromyosin showed any cross-reaction with the anti-actin sera. Anti-actin sera did not react with a skeletal muscle troponin complex. It is not yet established whether or not troponin is present in the smooth muscle cell.

It thus appears unlikely that these anti-actin sera contain antibodies against other non-actin proteins present in crude actin extract. In double-diffusion tests the various unidentified proteins in the latter which were seen on SDS electrophoresis, did not precipitate with anti-actin, and they were not revealed by SDS electrophoresis of the immune precipitate. The anti-actin antibody did not react with the non-actin proteins, which emerged as a first peak on Sephadex G-200 chromatography. Finally, the immunofluorescent staining was completely absorbed by isolated actin which on SDS electrophoresis gave a single band and not by the unidentified proteins from crude extract.

Since no species specificity was found, it appears that specific anti-actin antibody could be raised against actin obtained from other sources. It provides a new and useful tool in studying the distribution of actin, particularly in structures such as microfilaments. The implications of such a study cannot be foreseen with any degree of certainty, but it will not be surprising to find that actin may be involved in the pathology of various types of non-muscle cell.

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