Redistribution of membrane-bound and cytosolic actin in rabbit polymorphonuclear leucocytes during phagocytosis

Donald I. H. STEWART* and Neville CRAWFORD†

Department of Biochemistry, Institute of Basic Medical Sciences, Royal College of Surgeons of England,
35/43 Lincoln's Inn Fields, London WC2A 3PN, U.K.

(Received 24 September 1984/Accepted 8 October 1984)

In the analysis of highly purified surface membrane from both resting and phagocytosing neutrophils an increase in the surface membrane associated actin has been demonstrated. This change at the cell periphery is associated with a coincident increase in the F-actin content of the cells following stimulation of the cells by exposure to opsonized Oil Red O droplets. The actin which is newly associated with the surface membrane of the phagocytosing cells was more susceptible to removal by detergent than the membrane-associated actin in resting cells and it was also noted that the F-actin associated with phagosomes was readily disrupted by detergent. A redistribution of the surface membrane glycoprotein 5'-nucleotidase was observed during phagocytosis, but no change in distribution of a ^{125}I -labelled *Lens culinaris* lectin was observed during the entire phagocytic process.

The internalization of particles by phagocytosis is an important property of specialized cells which participate in the generalized host-defence mechanisms of the body, removing dead cells, bacteria and other harmful particulate substances from the circulation. The kinetic aspects, divalent-cation dependence and energy requirements of the phagocytic process have been well documented (Sbarra & Karnovsky, 1959; Michell *et al.*, 1969; Silverstein *et al.*, 1977; Hallgren *et al.*, 1978; Besterman & Low, 1983), but much less is known about the more detailed molecular changes that take place at the surface membrane which are involved in the particle engulfment. One feature that has received some attention, however, has been the relationship between certain surface membrane constituents, which are intrinsic to the bilayer, and the adjacent cytoskeletal elements extending into the cytoplasm of the phagocytic cell (Boxer *et al.*, 1974; Stossel *et al.*, 1976; Berlin & Oliver, 1978; Berlin *et al.*, 1979).

Abbreviations used: F-actin, filamentous actin; G-actin, unpolymerized (subunit) actin; DNAase I, deoxyribonuclease type I; 5'-nucleotidase, 5'-ribonucleotide phosphohydrolase.

* Present address: Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

† To whom reprint requests should be addressed.

Electron microscopic observations and fluorescent antibody studies have confirmed that the submembranous network contains actin filaments which extend throughout the pseudopodia as they surround the particle to be phagocytosed. It was also seen that in the area immediately beneath the particle the organization and amount of actin appeared to be much reduced or even lost when the pseudopods fuse and particle engulfment has been completed.

In the present study we have focused our attention upon this redistribution of actin during phagocytosis and have used the paraffin oil droplet procedure of Stossel *et al.* (1972) to follow phagocytosis quantitatively in rabbit polymorphonuclear leucocytes. We have explored not only the gross changes in actin distribution but also the level of organization of the actin associated with surface membrane domains and in the cytosol.

Materials and methods

Isolation of membrane fractions

Polymorphonuclear leucocytes were collected from the peritoneal cavity of rabbits by the method of Stewart & Crawford (1981). Washed cells were stimulated to phagocytose by the paraffin oil droplet method of Stossel *et al.* (1972).

Surface membrane fractions were isolated from both resting and phagocytosing cells by the

procedure of Stewart *et al.* (1983) which involves a disruption of the cells by homogenization and a sorbitol density gradient fractionation of the nuclei-free homogenate to give a mixed membrane fraction substantially enriched in surface membranes. This mixed membrane fraction was purified further by free flow electrophoresis.

Measurement of G- and F-actin

Actin was measured in the subcellular fractions by both area measurements of scanned polyacrylamide gel separations and also by a DNAase I inhibition assay. Polyacrylamide slab gels were prepared according to the method of Laemmli (1970) and consisted of 14cm 10% or 13% acrylamide resolving gels overlaid by 1.5cm 5.6% acrylamide stacking gel. The gels were scanned on a Joyce-Loebl 200/210 densitometric scanner at a wavelength of 575nm. The actin peak was identified by comigration with samples of purified leucocyte actin or platelet actin and the amount of actin present was estimated by cutting and weighing.

The DNAase-I inhibition assay for G-actin was performed as described by Blikstad *et al.* (1978).

Initially measurements of the soluble and particulate phase distribution of total actin in both resting and phagocytosing cells were carried out using high speed supernatants and pellets prepared from the homogenates and treated with 3M guanidinium chloride.

Surface membrane markers

5'-Nucleotidase (Avruch & Wallach, 1971), acid and alkaline phosphatase (Fernley & Walker, 1965) and glucose-6-phosphatase (Hubscher & West, 1965) were measured as markers for the various subcellular fractions. These were chosen from a range of seven enzymes that had been more comprehensively investigated in leucocyte fractions earlier (Stewart *et al.*, 1983). In some experiments the cells were prelabelled with ^{125}I -labelled *Lens culinaris* lectin at a non-aggregating concentration as described by Menashi *et al.* (1981).

Nucleic acid and protein analysis

DNA and RNA were determined by the ethidium bromide fluorescence procedure of Blackburn *et al.* (1973) and protein was routinely determined by either the microtannin turbidity procedure of Mejbaum-Katzenellenbogen & Drobyszczka (1959) or the method of Lowry *et al.* (1951). The choice of the protein assay was determined by the sensitivity requirement and/or the presence of interfering substances. Standard curves were prepared from purified bovine serum albumin (Armour).

Results

Standardization of the phagocytic model *in vitro*

Both the rate of particle uptake and its extent were assessed by measuring spectrophotometrically the amount of opsonized Oil Red O dye taken up by the cells. The uptake of the oil droplets during incubation of the cells with the Oil Red O emulsion reaches a maximum at around 10min and thereafter reaches a plateau (Fig. 1). This phagocytic model was highly reproducible in replicate experiments using the same cell harvest. The particle size of the droplets generally lay in the range 0.5–2.0 μm in diameter with the small sizes predominating. In routine experiments, in which resting phagocytosing cells were compared, the phagocytosis was stopped after at least 15min to ensure that the plateau level has been reached. Neither the rate nor the extent of particle uptake were affected by the ^{125}I -labelled *Lens culinaris* lectin label which was applied at the whole cell level in some experiments, as a surface membrane marker.

Isolation and purification of surface membrane from resting and phagocytosing cells

Density gradient centrifugation. In the isolation of surface membrane from homogenates of resting cells, the homogenate was applied to the 13–55% sorbitol gradient and, after centrifugation, the mixed membrane fraction, which was rich in

Fig. 1. Time curve for the uptake of opsonized Oil Red O droplets by phagocytosing neutrophils

Cells were incubated at 37°C with a sonicated suspension of paraffin oil containing Oil Red O dye. At each time point a sample of cells was diluted with ice-cold buffer, washed five times and lyophilized. The dye was extracted with redistilled dioxan for 16h and measured spectrophotometrically.

surface membrane markers and located in the density range 1.08–1.11, was isolated. The membrane fraction from resting cells was shown by transmission electron microscopy to consist entirely of sealed membrane vesicles with no identifiable granule contamination (Stewart *et al.*, 1983). This fraction was also free of contamination by granule constituents.

Homogenates from phagocytosing cells gave a surface membrane-rich fraction in exactly the same density region as that from the resting cells. This fraction, which was similar morphologically to that prepared from control cells, was uncontaminated by Oil Red O and we believe represented the regions of the surface membrane not involved in the phagocytic process. There was, however, a prominent narrow Oil Red O-containing zone located at the top of the gradient close to the meniscus. This was shown by transmission electron microscopy to consist of oil-loaded membrane vesicles with a clear boundary membrane. Since this fraction contained almost all of the Oil Red O (>96%) present in the gradient we believe it represents the phagocytic vesicles. In our studies with phagocytosing cells this fraction at the top of the gradient was always harvested separately and referred to as the phagocytic vesicle fraction.

Free flow electrophoresis. The mixed membrane fractions taken from the density gradients were concentrated by centrifugation, resuspended in electrophoresis buffer and applied to the chamber of the high voltage free flow electrophoresis apparatus.

With membranes from the resting cells the mixed membrane fraction from the gradient resolved into two components, F_1 and F_2 , the one with higher anodal mobility, F_1 generally predominating (Fig. 2a). In all experiments, this most electronegative fraction accounted for all the surface membrane marker enzyme 5'-nucleotidase and ^{125}I -labelled *Lens culinaris* lectin label. These surface membrane markers were undetectable in the less electronegative fraction. This latter fraction has been characterized previously (Stewart *et al.*, 1983) and shown to be enriched in triacylglycerols.

The mixed membrane fraction derived from phagocytosing cells and taken from the same gradient density range showed an almost identical two-component separation (F_1' and F_2') in the electrophoresis chamber (Fig. 2b). As with the non-phagocytosing cells, there was no detectable enzymic activity associated with this least electronegative peak F_2' .

In studies with resting cells the F_1 electrophoretic peak was pooled and centrifuged to give a pellet PF_1 and the F_1' peak from the phagocytic cells was similarly pooled, PF_1' . Both these pooled fractions

Fig. 2. Profiles for the separation of mixed membrane fractions by free flow electrophoresis

Protein (○) and (●) is shown for both resting (a) and phagocytosing (b) cells. ● represents phagosomes which were electrophoresed separately from the 'uninvolved' surface membrane. Typical profiles for surface membrane markers are shown for ^{125}I -lectin (▲) and 5'-nucleotidase (△).

were located at exactly their original peak positions in the electrophoretic profile when they were re-electrophoresed. The PF_1' fraction from the phagocytosing cells, representing surface membrane domains not involved in phagocytic vesicle formation, showed an enrichment of 5'-nucleotidase with respect to homogenate activity of over twice the enrichment values for the purified surface membrane from the resting cells (Table 1). However, the enrichment values for ^{125}I -lectin were approximately the same in both the phagocytic and resting cell membranes (Table 1).

Changes in soluble and particulate phase distribution of actin during phagocytosis

Whole cells (resting and phagocytosing) were homogenized and separated into a soluble (cytosol) and a particulate phase by high-speed centrifugation (100000g, 90 min). Measurements were then made of the total (G+F)-actin content after treatment with guanidinium chloride by both gel electrophoresis and DNAase I inhibition assay methods. After 20 min of phagocytosis there was a significant redistribution of the actin pools within the cells (Table 2). In the resting cells, 69% of the total cell actin was sedimentable, whereas after phagocytosis, 84% of the total cell actin sedimented. The total actin content of the resting

Table 1. *Distribution of intracellular markers in the homogenate and uninvolved surface membrane of resting and phagocytosing neutrophils*

Values indicate relative enrichment of the marker with respect to homogenate, 1.0. 5'-Nucleotidase and ^{125}I -labelled *Lens culinaris* lectin are surface markers, while acid phosphatase, glucose-6-phosphatase and DNA are indicators of intracellular organelles and membrane.

Constituent	Resting cells		Phagocytosing cells	
	Homogenate	Membrane	Homogenate	Membrane
5'-Nucleotidase	1.0	18.0 (± 0.6)	1.0	38 (± 3.2)
^{125}I -labelled <i>Lens culinaris</i> lectin	1.0	11.0 (± 0.7)	1.0	10 (± 1.1)
Acid phosphatase	1.0	1.2	1.0	n.d.
Glucose-6-phosphatase	1.0	1.3	1.0	n.d.
DNA	1.0	<0.1	1.0	n.d.

Table 2. *Total actin content of cytosolic and particulate fractions prepared from resting and phagocytosing neutrophils*
Actin concentration was determined by both DNAase I inhibition and gel scanning methods. The actin content in the fractions was measured, in the case of the DNAase inhibition experiments, after depolymerization of the F-actin with 3M-guanidinium chloride. The total amount of actin in resting and phagocytosing cells was determined independently by gel scanning and was found to be the same in both cases.

	Total actin		Cytosol actin		Particulate actin	
	(μg)	(%)	(μg)	(%)	(μg)	(%)
Resting cells	56 $\pm$ 3.6	100	17 $\pm$ 1.8	31	39 $\pm$ 2.0	69
Phagocytosing cells	57 $\pm$ 5.0	100	9 $\pm$ 0.7	16	48 $\pm$ 5.0	84

and phagocytosing cells was also measured and found to be unchanged after phagocytosis.

Changes in the quantity of actin associated with the purified surface membrane after phagocytic stimulation

Quantitative determination of the amount of actin associated with the electrophoretically purified surface membrane vesicles were made on the PF_1 and PF_1' fractions from the pooled free flow electrophoresis separations.

Fig. 3 shows densitometric scans of the polypeptide profiles from polyacrylamide-gel electrophoretic separations of purified resting cell surface membranes and the uninvolved surface membranes from phagocytosing cells. In qualitative terms there is no significant difference in these scans with respect to the range of polypeptides seen. There are, however, significant quantitative changes and in particular a substantial increase, in the phagocytic cell membranes, of a component of M_r 42000 which comigrates with a similar peak in an authentic sample of purified actin (lower gel scan in Fig. 3). Two-dimensional isoelectric focusing/polyacrylamide slab gel electrophoresis confirmed that the M_r 42000 band was almost entirely accounted for by actin.

Table 3 shows the quantitative measurements of actin with the DNAase inhibition assay. There is an increase of 43% in the amount of actin associated with the uninvolved surface membrane

Fig. 3. Densitometric scans of Coomassie Blue-stained gels of uninvolved surface membrane from (a) resting cells and (b) phagocytosing cells, demonstrating the increase in actin associated with the membrane of stimulated cells; standard actin is also shown (c).

Table 3. Surface-membrane-associated actin in resting and phagocytosing neutrophils determined by the DNAase inhibition assay on purified membrane fractions

Expt. no. . . .	Actin ($\mu\text{g}/100\ \mu\text{g}$ of protein)						Mean $\pm$ S.D.
	1	2	3	4	5	6	
Resting cells	10.9	5.4	8.2	5.7	4.1	8.5	7.1 $\pm$ 2.5
Phagocytosing cells	15.7	8.0	12.3	7.9	5.4	12.3	10.2 $\pm$ 3.8
Increase (%)	+44	+48	+50	+38	+28	+45	+43 $\pm$ 6.5%

Table 4. Distribution of G- and F-actin in purified surface membranes and phagosomal membranes of resting and phagocytosing cells as determined by the DNAase I inhibition method

The F-actin content was determined by subtraction of the G-actin content from a total G- and F-actin content measured after depolymerizing the F-actin with 3M-guanidinium chloride.

Membrane fraction	Actin ($\mu\text{g}/100\ \mu\text{g}$ of protein)	
	G-actin	F-actin
Purified surface membrane from resting cells	<0.5	8.6
Purified surface membrane from phagocytosing cells	<0.5	12.3
Phagosomal membranes prior to washing	5.0	6.3
Phagosomal membranes after washing	<0.5	6.1

when expressed as μg of actin/ $100\ \mu\text{g}$ of membrane protein as compared with the amount present in the resting cell surface membranes.

In order to determine the level of organization of the actin associated with the surface membrane fractions, measurements of G-actin were made by using the DNAase-I inhibition assay before and after treatment with guanidinium chloride to dissociate F-actin. Table 4 shows the distribution of these two actins in the membrane fractions from a typical experiment. From the Table it is clear that all the actin associated with these membranes is filamentous, the content of G-actin being below the limits of detection. Similar measurements were made on the phagocytic vesicle membrane. Although about 50–60% of the total actin associated with these vesicles was lost during the washing, only a small proportion of the F-actin was removed during washing with iso-osmotic saline, implying a loose absorption of soluble phase G-actin to the phagocytic vesicle membrane. The uninvolved surface membrane isolated from the phago-

cytosing cells has twice the content of F-actin of the phagocytic vesicle membranes when the data are expressed as μg of actin/ $100\ \mu\text{g}$ of membrane protein.

Detergent treatment of surface membrane vesicles: effect upon actin

In some preparations, the purified surface membranes were treated with Triton X-100. This detergent dissection removes only 10–15% of the membrane-associated actin in resting cells (Table 5). In the phagocytosing cells, significantly more actin is removed (20–25%) by the detergent treatment but this in quantitative terms accounts for slightly less than the newly assembled F-actin attached to the membrane during the phagocytic event.

Treatment of the phagocytic vesicle membrane with Triton X-100 removes all the actin, suggesting that, although associated sufficiently tightly with the lipid bilayer to withstand gradient separation and extensive washing, the F-actin is not stabilized in the same way as the Triton-resistant filamentous actin intrinsic to the uninvolved or resting cell surface membranes.

Distribution of 5'-nucleotidase in the resting and phagocytosing cell surface membranes

Table 6 shows that the activity of the surface ectoenzyme 5'-nucleotidase in the leucocyte surface membrane domain not involved in the phagocytic process was increased substantially during phagocytosis. Expressed as specific activities, there is a 3.4-fold enrichment in this enzyme with respect to its activity in the resting cells. Concomitant with this enrichment, there is a very significant depletion of 5'-nucleotidase activity in the phagocytic vesicle membranes, from a resting cell membrane level of around 13.0 down to 1.6 nmol/h per mg of membrane protein. This latter activity level is not significantly different from the activities found in the whole cell homogenates, and taken in the context of the activities seen in the resting cell surface membrane represent a substantial depletion in activity.

Table 5. *Effect of Triton X-100 on surface membrane and phagosomal membrane actin content in resting and phagocytosing neutrophils*

The membranes were treated with a final concentration of 1% Triton X-100 for 30 min. The membranes were then isolated by centrifugation at 100 000g for 90 min. The total actin content of the membrane fractions was measured by gel scanning.

Membrane fraction	Actin content ($\mu\text{g}/100 \mu\text{g}$ of protein)		Actin removed by Triton X-100 (%)
	Before treatment	After treatment	
Surface membrane from resting cells	8.6	7.5	12.8
Surface membrane from phagocytosing cells	12.3	9.3	24.4
Washed phagosomal membranes	6.1	<0.5	~100.0

Table 6. *Surface membrane 5'-nucleotidase activity in resting and phagocytosing neutrophils*
Values in parentheses represent enrichments of the specific activities with respect to activities in the corresponding homogenate.

Cellular fraction	Expt. no.	5'-Nucleotidase activity (nmol of AMP/h per mg of protein)	
		Resting cells	Phagocytosing cells
Homogenate		1.2	1.0
Surface membrane preparations	1	7.7	36.0
	2	14.6	42.0
	3	16.7	49.0
	Mean	13.0 (10.8)	45.0 (45.0)
Phagosomal membrane preparations	1	—	1.8
	2	—	1.4
	3	—	1.7
	Mean	—	1.6 (1.6)

Discussion

Our present approach to the problem of investigating the dynamic aspects of actin structure and function has been by focusing interest on the presence and level of organization of actin in the cytosol of the rabbit leucocyte together with a study of the concomitant changes in membrane-associated actin. Our investigations have revealed that during phagocytosis there is an overall assembly of G-actin into filamentous forms and this equilibrium shift appears to coincide with an increased assembly of F-actin on the surface membrane and particularly on the surface domain uninvolved in the formation of the phagocytic vesicles.

Triton X-100 dissection of the surface membranes from resting and phagocytosing cells revealed that a substantial amount of the newly assembled F-actin in the uninvolved surface domain of the phagocytosing cells was removable by Triton

X-100, and virtually all of the significantly lower amount of actin associated with the phagocytic vesicle membrane was also non-sedimentable. These findings suggest that, although the actin assembled on the membrane during phagocytosis results in a fairly tight association and is accountable for as newly assembled polymer, it is not apparently stabilized in the same way as the endogenous network of actin present in the membrane of both the resting and phagocytosing leucocytes.

One current view of all cells that display motile activities is that the steady state equilibrium that normally exists in the cytosol between filamentous actin and a critical concentration of G-actin monomer is in some way disturbed during the motile event. Although the ionic conditions in the normal cytosolic environment are generally considered to favour actin filament formation, the two forms of actin do exist but the equilibrium conditions will also be affected or controlled by the

action of other proteins such as profilin, which binds to G-actin preventing filament assembly, or actin filament shortening proteins such as gelsolin (Lind *et al.*, 1982) and the actin destabilizing factors of Chaponnier *et al.* (1979) and Harris & Weeds (1978). The whole field of actin accessory proteins has recently been well reviewed by Weeds (1982).

In a recent study of a detergent-insoluble matrix prepared from murine tumour cells and lymphocytes, Mescher *et al.* (1981) showed that the major component of the matrix was actin but also associated with it was at least one major cell surface glycoprotein, 5'-nucleotidase. Although no functional role for these protein interactions was revealed in these studies of Mescher *et al.* (1981), it is interesting to note that in the present investigations of the change in distribution of 5'-nucleotidase activity during the phagocytic process there is a greater than 3-fold enrichment of the activity of this enzyme in the uninvolved plasma membrane compared with the membrane of the resting leucocyte. In addition, there is a substantial depletion of 5'-nucleotidase in the boundary membranes of the phagocytic vesicles. The nature of any relationship between 5'-nucleotidase and the cytoskeleton lying adjacent to the plasma membrane is not clear. However, it has been shown that the inhibitory characteristics of concanavalin A towards 5'-nucleotidase activity are completely different if cells are pretreated with the microfilament disrupting agents cytochalasin B or D (Carraway *et al.*, 1979). In their studies the inhibition appears to be non-co-operative in control cells and co-operative in the cytochalasin-treated cells. Similarly, an association between actin and 5'-nucleotidase has been demonstrated in earlier studies by Mannherz & Magener (1979). These authors clearly showed that the nucleotidase accelerated the rate of actin polymerization and that concanavalin A inhibition of the enzyme activity suppressed its ability to interact with actin.

It is of interest that the changes in distribution of the 5'-nucleotidase are not reflected during phagocytosis by the ^{125}I -lectin. This would suggest that there is a distinction made between membrane components where some, such as 5'-nucleotidase, may be redistributed away from phagolysosomes while under the same conditions others, such as the ^{125}I -lectin-associated protein, show no change in position in the membrane and so this protein which is in the region of engulfed membrane becomes introduced with it.

Our results show that membrane-associated F-actin in the phagocytosing leucocyte appears to exist in two forms, one soluble in detergent and the other tightly incorporated and resistant to detergent. Gruenstein *et al.* (1975) have also reported

two membrane pools of actin in 3T3 cells and HeLa cells; the treatment of these cells under conditions which favour filament disassembly resulted in the removal of 20–40% of the membrane-associated actin leaving the rest tightly attached to the membrane. How this detergent-insoluble membrane actin is stabilized remains a matter of conjecture; whether newly assembled F-actin locates onto existing filaments or onto new nucleating sites which are revealed during the motile events has yet to be established.

In conclusion, the present studies have shown that the distribution of actin, the major cytoskeletal protein present in the polymorphonuclear leucocyte, alters significantly during phagocytosis. There is a net assembly of F-actin onto the plasma membrane with a concomitant decrease in actin in the cytosol compartment. Associated with this cytoskeletal event is a significant redistribution of the membrane ectoenzyme 5'-nucleotidase a major glycoprotein of the rabbit polymorphonuclear leucocyte surface membrane. Whether these two phenomena are linked in any structural way either directly or through some accessory protein is at present uncertain.

We are grateful for the support of the Cancer Research Campaign during the early part of these studies. D. I. H. S. was a recipient of a M. R. C. Studentship during the course of the work.

References

- Avruch, J. & Wallach, D. F. M. (1971) *Biochim. Biophys. Acta* **223**, 334–339
- Berlin, R. D. & Oliver, J. M. (1978) *J. Cell Biol.* **77**, 789–804
- Berlin, R. D., Caron, J. M. & Oliver, J. M. (1979) in *Microtubules* (Roberts, K. & Hyams, J. S., eds.), pp. 443–485. Academic Press, New York
- Besterman, J. M. & Low, R. B. (1983) *Biochem. J.* **210**, 1–13
- Blackburn, M. J., Andrews, T. M. & Watts, R. W. E. (1973) *Anal. Biochem.* **51**, 1–10
- Blikstad, I., Markey, F., Carlsson, L., Persson, T. & Lindberg, U. (1978) *Cell* **15**, 935–941
- Boxer, L. A., Hedley-Whyte, E. T. & Stossel, T. P. (1974) *N. Engl. J. Med.* **293**, 1093–1098
- Carraway, K. L., Doss, R. C., Huggins, J. W., Chesnut, R. W. & Carraway, C. A. C. (1979) *J. Cell Biol.* **83**, 529–543
- Chaponnier, C., Borgia, R., Rungger-Brandle, F., Weil, R. & Gabbiani, G. (1979) *Experientia* **35**, 1039–1049
- Fernley, H. N. & Walker, P. G. (1965) *Biochem. J.* **97**, 95–103
- Gruenstein, E., Rich, A. & Weibring, R. R. (1975) *J. Cell Biol.* **64**, 223–234
- Hallgren, R., Sjöström, P. & Bill, A. (1978) *Immunology* **34**, 347–351

- Harris, H. E. & Weeds, A. G. (1978) *FEBS Lett.* **90**, 84–88
- Hubscher, G. & West, G. R. (1965) *Nature (London)* **205**, 799–801
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Lind, S. E., Yin, H. L. & Stossel, T. P. (1982) *J. Clin. Invest.* **69**, 1384–1387
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mannherz, H. G. & Magener, M. (1979) *FEBS Lett.* **103**, 77–80
- Mejbaum-Katzenellenbogen, W. & Dobryszcka, W. M. (1959) *Clin. Chim. Acta* **4**, 515–522
- Menashi, S., Weintraub, H. & Crawford, N. (1981) *J. Biol. Chem.* **256**, 4095–4101
- Mescher, M. F., Jose, M. J. & Back, S. P. (1981) *Nature (London)* **289**, 139–144
- Michell, R. M., Pancake, S. J., Noseworthy, J. & Karnovsky, M. L. (1969) *J. Cell Biol.* **40**, 216–224
- Sbarra, A. J. & Karnovsky, M. L. (1959) *J. Biol. Chem.* **234**, 1355–1362
- Silverstein, S. C. Steinman, R. M. & Cohn, Z. A. (1977) *Annu. Rev. Biochem.* **46**, 669–772
- Stewart, D. I. H. & Crawford, N. (1981) *FEBS Lett.* **126**, 175–179
- Stewart, D. I. H., Emes, C. H. & Crawford, N. (1983) *Biochim. Biophys. Acta* **733**, 154–162
- Stossel, T. P., Mason, R. J., Hartwig, J. & Vaughan, M. (1972) *J. Clin. Invest.* **51**, 615–624
- Stossel, T. P., Hartwig, J. H. & Boxer, L. A. (1976) in *Contractile Systems in Non-Muscle Tissues* (Perry, S. V. et al., eds.), pp. 323–329, Elsevier/North-Holland Biomedical Press, Amsterdam
- Weeds, A. (1982) *Nature (London)* **296**, 811–816