Proc. Natl. Acad. Sci. USA
Vol. 73, No. 11, pp. 4032–4036, November 1976
Cell Biology

Actin-like filaments and membrane rearrangement in oxyntic cells

(heavy meromyosin/membrane translocation/membrane-associated filaments/HCl secretion/electron microscopy)

J. D. VIAL AND J. GARRIDO

Departamento de Biología Celular, Universidad Católica de Chile, Casilla 114-D, Santiago de Chile

Communicated by Keith R. Porter, July 19, 1976

ABSTRACT **The secretory pole of vertebrate oxyntic cells possesses two distinct membrane systems: the apical plasma membrane which presents numerous infoldings, microvilli and processes, and a complex tubulovesicular system located in close proximity to the plasma membrane. These two membrane systems are generally believed to be interconvertible in relation to the functional state of the cell. To determine the role that filaments may play in the interconversion process, the secretory pole of rat and toad oxyntic cells was examined by electron microscopy under conditions designed to demonstrate filamentous structures, i.e., slight cellular swelling and incubation with heavy meromyosin. Filaments 50–80 Å in diameter are present in close association with the plasma membrane to which they are connected by regularly spaced bridges. Heavy meromyosin-treated material reveals "decorated" filaments in topographically corresponding locations. No filaments are seen in association with membranes of the tubulovesicular system. These findings suggest that association with actin-like filaments is a step in the translocation of membranes from the tubulovesicular system to the plasma membrane.**

The extensive membrane rearrangements of the apical pole that accompany the transition from resting state of HCl-secreting state are a common feature of all vertebrate oxyntic cells. In the resting state, the plasma membrane at the secretory pole presents an uncomplicated topography with few projections into the lumen. Beneath the apical membrane, a very well-developed tubulovesicular system is present. The onset of HCl secretion is accompanied by the appearance of complex infoldings and processes which increase the area of the plasma membrane at the apical zone approximately 10-fold (1). At the same time the total area of the membranes that compose the tubulovesicular system is greatly decreased. Extensive work done to clarify the relationship existing between plasma membrane and tubulovesicular system has yielded the following pertinent data: (*i*) morphometric studies show that the sum of the areas of apical and tubulovesicular membranes is approximately constant for all functional states of the cell (1, 2). (*ii*) A definite structural similarity exists between the apical plasma membrane and the membranes of the tubulovesicular system (3). (*iii*) Bicarbonate-activated ATPase activity can be cytochemically demonstrated in relation to the tubulovesicular system in the resting state, while during activity it is found in relation to the plasma membrane of the apical processes (4). (*iv*) Inhibition of protein synthesis does not inhibit the increase of apical membrane area at the onset of secretion (5) which indicates that newly-synthesized membrane components cannot account for this increase.

These data support the widely held opinion that the two membrane systems of the apical pole are interconvertible.

An explanation of the mechanism by which changes at the initial stage of secretion take place should include, among others, at least two events which may probably occur in such an interconvertible system. The first is the transfer of elements of the tubulovesicular system toward the plasma membrane; the second is the incorporation of these elements into the plasma membrane by fusion. Up to the present, attention has been focused almost exclusively upon the membranes themselves. Little consideration has been given to the idea that such extensive changes and rearrangements at the plasma membrane level must, of necessity, entail changes of nonmembrane structures present in the cytoplasm that comes into contact with the membrane systems.

Our work has been directed to the study and characterization of cytoplasmic elements that may participate in the changes that accompany secretion. In this report we describe a system of actin-like filaments that are closely associated with the plasma membrane of the secretory pole of oxyntic cells; these filaments are not associated with membranes of the tubulovesicular system.

MATERIALS AND METHODS

Myosin was prepared from rabbit skeletal muscle as described by Szent-Györgyi (6). After several precipitation and solubilization cycles a 1% solution in 0.6 M KCl was digested with trypsin (Mann Research Laboratories, Inc., specific activity 6600 α-*N*-benzoyl-L-arginine ethyl ester units/mg) by the method of Lowey and Holtzer (7). The digestion was stopped with lima bean trypsin inhibitor (Mann Research Laboratories, Inc.). After dialysis against 1 mM phosphate buffer at pH 7.0, for 3 hr, the digest was centrifuged at 30,000 × *g* for 10 min. The supernatant containing predominantly heavy meromyosin (HMM), without further purification, was employed for the treatment of glycerinated cells.

Gastric gland cell suspensions were prepared from albino rat stomachs by a procedure modified from Romrell *et al.* (8). The modifications introduced were: (*a*) crude papain (BDH Chemicals Ltd., Poole, England) at a concentration of 3 mg/ml was used instead of Pronase; no activator was added to the papain solution and (*b*) no DNase treatment of the initial cell suspension was carried out. This method produces a mixed suspension of gastric gland cells which are 95–98% viable as judged by trypan blue exclusion tests. After washing to eliminate papain, some cells were directly fixed and processed for electron microscopy; others were glycerinated as indicated below.

The method described above was not effective in obtaining similar cell suspensions from the gastric glands of the toad *Bufo spinulosus*. In this animal, the surface epithelium of the mucosa is extremely resistant to dissociation by proteases. This prompted us to adopt a different procedure. Tissue slices (200 μm thick) were obtained by means of a Smith-Farquhar tissue sectioner from freshly excised gastric mucosae. After glycerination, carried out as described below, part of the material was processed for electron microscopy while the rest was treated with HMM.

Abbreviations: HMM, heavy meromyosin; SSB, 100 mM KCl, 5 mM $MgCl_2$, 300 mM sucrose, 600 mM phosphate buffer at pH 7.0.

For glycerination, the material was placed in a salt-sucrose-buffer solution (SSB: KCl, 100 mM; $MgCl_2$, 5 mM; sucrose, 300 mM; 6 mM phosphate buffer at pH 7.0). Glycerol concentration was continuously increased by addition of a 300 mM solution of sucrose in glycerol by means of a gradient maker until the initial volume of SSB had doubled. The passage from 0 to 50% glycerol was completed in 40 min. After 10 min in 50% glycerol, SSB was added stepwise throughout a 30 min period until the concentration of glycerol was 25%. The material was then collected by centrifugation, washed in SSB to remove all glycerol, and incubated at 4° in a 0.4% solution of HMM in 1 mM phosphate buffer at pH 7.0, for 2 hr. The material was subsequently, fixed in 2% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) containing 0.1 M sucrose. After washing, the sample was postfixed in OsO_4, block-stained in uranyl acetate, dehydrated in acetone, and embedded in Epon.

The fine structure of the secretory pole of oxyntic cells was also studied on material which had not undergone glycerol-HMM treatment. This material was obtained and treated by one of the following sequences: (*a*) aliquots of the gastric gland cell suspensions, obtained as described above, were fixed in 1.6% glutaraldehyde in 0.2 M sodium cacodylate buffer at pH 7.2, postfixed in OsO_4, dehydrated in acetone, and embedded in Epon; (*b*) toad gastric mucosae were excised after 45 min of histamine stimulation (10 mg/kg of histamine phosphate injected into the dorsal lymph sac); and (*c*) toad gastric mucosae were quickly removed from the pithed animals and incubated *in vitro* for 1 hr in the solution described by Villegas *et al.* (9) to which 60 μM histamine had been added. Small blocks of tissue from the mucosae obtained in (*b*) and (*c*) were prepared for electron microscopy in the manner described for the cell suspensions.

Silver to gray sections were obtained with diamond knives; they were stained with uranyl acetate in methanol (10) and lead citrate.

RESULTS

The normal structure of the secretory poles of rat parietal cells and toad oxyntic cells is shown in Figs. 1 and 4. While in amphibians the secretory pole corresponds to the apex of the roughly pyramidal cell, in the mammalian cell the secretory pole is represented by the intracellular canaliculus which penetrates deeply into the cytoplasm.

After a variety of procedures, microvilli and processes of the apical poles of both cell types appear slightly swollen. These procedures include prolonged histamine stimulation *in vivo*, incubation with histamine *in vitro*, and slight hypoxia. In these conditions, filaments 50–80 Å in diameter are seen to run parallel to the plasma membrane, separated from it by a rather constant distance of about 300 Å. They are connected to the membrane by bridges having about 100 Å in diameter which are regularly spaced approximately 300 Å apart (Figs. 2 and 5). The membrane-associated filaments are best seen in the microvilli and processes, where favorable sections show that they are arranged parallel to each other. They can also be found, however, in relation to stretches of apical plasma membrane which are devoid of projections. Beneath the plasma membrane and at the bases of the microvilli and processes, similar filaments which are not associated with the membrane form a meshwork of variable thickness. This filamentous layer intervenes between the plasma membrane and the subjacent tubulovesicular system (Fig. 6).

In neither of the cell types studied were filaments seen in association with the elements of the tubulovesicular system. This is especially noticeable in the toad oxyntic cell in which a clear stratification of the structures composing the secretory pole is present. In glycerinated toad mucosae which were not treated with HMM, oxyntic cells which showed an exceptionally good structural preservation were occasionally found. In these cells the presence of 50–80 Å filaments in relation to the plasma membrane and their absence in proximity to the membranes of the tubulovesicular system is patently demonstrated (Fig. 6).

In the mammalian parietal cell, HMM is bound by filaments about 70 Å in diameter which appear "decorated" showing the typical arrowhead pattern. These filaments are present singly or forming loose networks and bundles. The filamentous structures surround the intracellular canaliculus and are especially prominent inside some of the processes that project into its lumen (Fig. 3). In filament bundles a distinct polarity is observed, all arrowhead structures having the same sense in adjacent filaments.

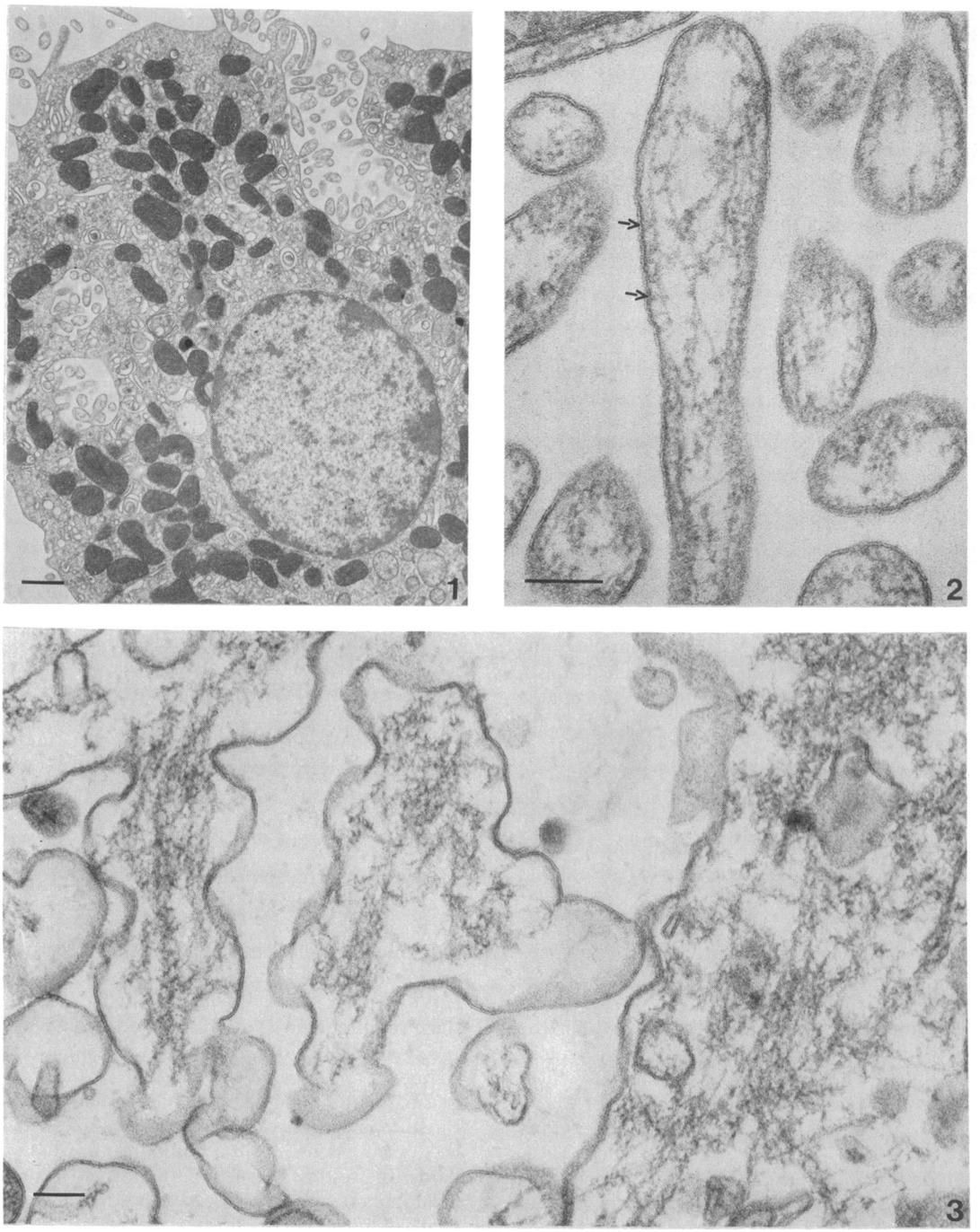
Bundles of filaments approximately 100 Å in diameter which do not bind HMM are also observed in these cells. They are never present in relation to the canaliculus or inside processes projecting into it.

Amphibian oxyntic cells in HMM-treated mucosae characteristically show a poor structural preservation of their secretory poles. Apical processes are almost always extremely swollen and disrupted. In spite of this, thin filaments that bind HMM are seen in relation to the plasma membrane (Fig. 7). The actual presence of these filaments inside apical processes could not be ascertained for the reason given above. Small, well-preserved areas of tubulovesicular system were sometimes found in this material; no "decorated" filaments were present in association to its membranes.

DISCUSSION

Membrane rearrangement at the apical pole of the oxyntic cell would seem to require the presence of a system capable of performing the work necessary for the displacement of the membranous elements that are rearranged, and for the generation of the changes in shape that occur. The system of actin-like filaments we have described here may comply with these requirements from a structural point of view. It is composed of filaments disposed in two ways: membrane-associated filaments, and filaments which form a meshwork beneath the plasma membrane and are not associated with it. Changes in the functional state of the cell entail changes in distribution of these components, from a random meshwork which accompanies the resting state to a parallel array of membrane-associated filaments during secretion. Under conditions which provoke light swelling of the apical microvilli, the filaments remain closely adherent to the plasma membrane, a fact which indicates that the association of these structures is a strong one. This peripheral distribution of the filaments clearly differs from the central core arrangement described for actin-like filaments in the intestinal microvillus (11).

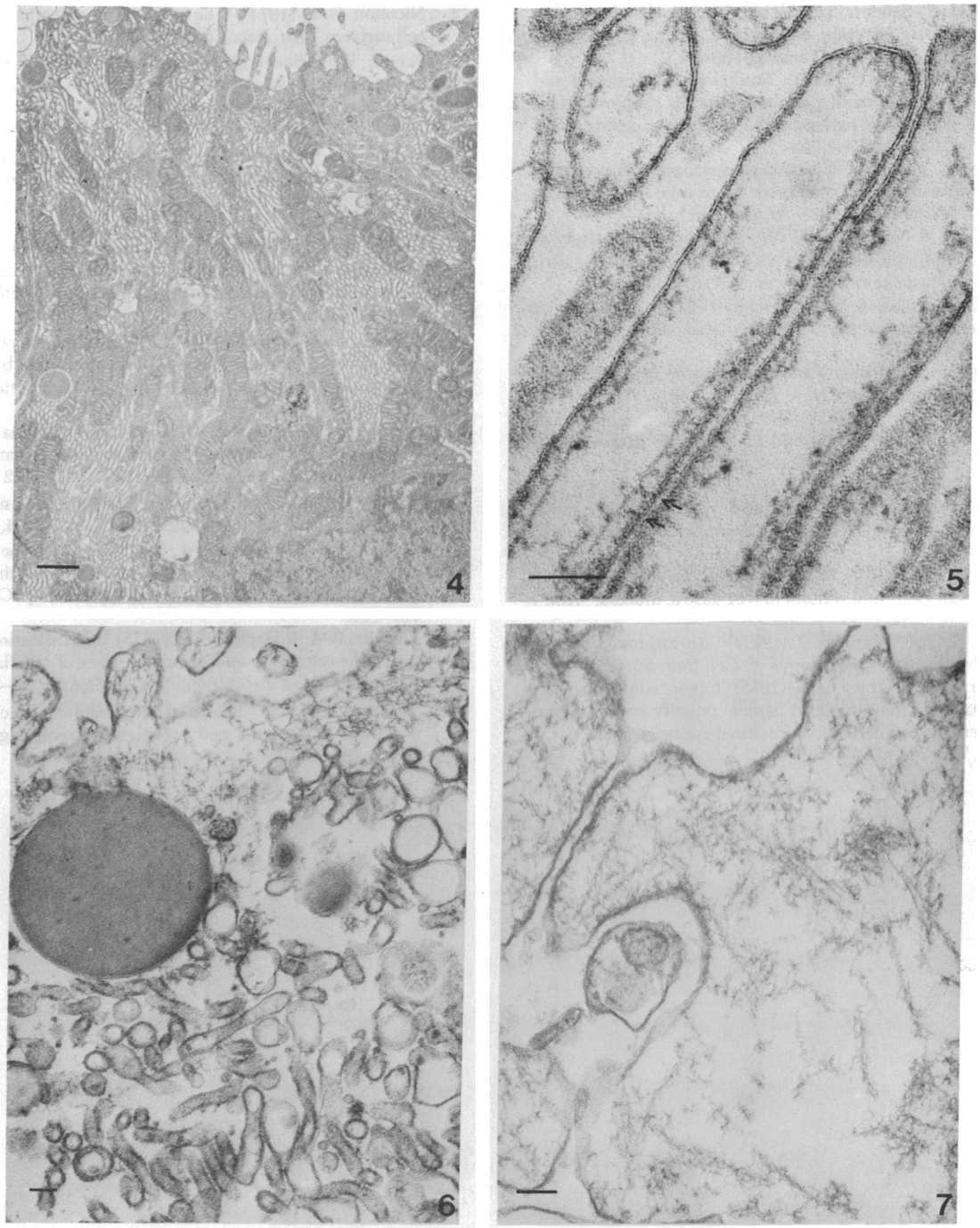
The finding in these cells of regularly spaced bridges that connect the filaments to the plasma membrane is espeially striking. These bridges may represent molecules of peripheral protein that connect the filaments to the membrane (12); they may also represent a second element in a contractile system as would be the case if these bridges were constituted by myosin-like molecules. It should be noted here that changes in filament distribution such as we have described above would preclude a permanent connection between filaments and the plasma membrane, the presence or absence of connections being related to the functional state of the membrane. Painter



FIGS. 1–3. *Fig. 1.* Isolated rat parietal cell. The intracellular canaliculus penetrates deeply into the cytoplasm; it is surrounded by elements of the tubulovesicular system. Numerous microvilli and processes project into the canalicular lumen. Bar = 1 μm. *Fig. 2.* A process inside the canaliculus of a slightly swollen parietal cell shows the arrangement of 50–80 Å filaments and their connections to the plasma membrane (arrows). Bar = 0.1 μm. *Fig. 3.* Rat parietal cell after glycerination and treatment with HMM. Bundles and networks of "decorated" filaments are seen, both inside processes and in the pericanalicular cytoplasm. Bar = 0.1 μm.

et al. (13) have shown that in WI 38 cells (a human fibroblast line) molecules that bind antimyosin antibody are present in association with the plasma membrane. They propose that these molecules may be part of a system active in controlling the mobility of protein moieties in the plane of the membrane.

In view of the generally accepted role of the tubulovesicular system as a membrane "reservoir" for activity, it is interesting to note that the elements of this system are not associated to actin-like filaments as far as conventional electron microscopy and observation of HMM-treated material can reveal. Actin-like filaments form a meshwork between the tubulovesicular system and the plasma membrane at the apical pole. The incorporation of elements of this system into the plasma membrane would require that these elements establish contact with the actin-like filaments. It may be speculated that one of the events accompanying stimulation could bring about changes in the membrane that result in association with actin and subsequent transfer of the associated elements to the free surface of the cell. It has been shown that the degree of association of actin to membranes of fibroblasts varies after transformation by Rous



FIGS. 4–7. *Fig. 4.* Toad oxyntic cell. Apical processes project into the glandular lumen, while the highly developed tubulovesicular system can be seen to occupy most of the supranuclear region of the cell. Bar = 1 μm. *Fig. 5.* Apical processes of a slightly swollen toad oxyntic cell shown at high magnification (compare with Fig. 2). Thin filaments are seen to course parallel to the membrane and their connections to it are clearly visible (arrows). Bar = 0.1 μm. *Fig. 6.* Apical region of a glycerinated toad oxyntic cell which was not treated with HMM. Thin filaments are seen to form a feltwork immediately beneath the plasma membrane. No filaments are present in association with membranes of the tubulovesicular system. Bar = 0.1 μm. *Fig. 7.* HMM-binding thin filaments in a toad oxyntic cell. Bar = 0.1 μm.

sarcoma virus (14). Studies carried out on *Dictyostelium discoideum* have shown that actin filaments become apparent after contact of the ameba with a substratum (15). Thus, two conditions which are known to affect cell surface molecular architecture (16–18) result in changes of actin distribution.

The results we report here indicate that oxyntic cell may be a favorable model for the study of the relationship between actin distribution and the functional state of the plasma membrane. Abundant evidence has been presented which relates membrane-bound organelle transport to actin-like filament systems (19–21). We propose that the regulation of organelle translocation may be related to the association and dissociation of membranes with actin.

We are grateful for the excellent technical assistance of Mrs. Teresa Socias and of Mr. Raúl Fuentes. This work was supported by funds from the Organization of American States, and from the Pacific National Defense Union/United Nations Economic, Social and Cultural Organization Program RLA 75/047.

1. Helander, H. F., Sanders, S. S., Rehm, W. S. & Hirschowitz, B. I. (1972) in *Gastric Secretion*, eds. Sachs, G., Heinz, E. & Ullrich, K. J. (Academic Press, New York), pp. 69–88.
2. Ito, S. & Schofield, G. C. (1974) "Studies on the depletion and accumulation of microvilli and changes in the tubulovesicular compartment of mouse parietal cells in relation to gastric secretion," *J. Cell Biol.* **63**, 364–382.
3. Lillibridge, C. B. (1968) "Electron microscopic measurements of the thickness of various membranes in oxyntic cells from frog stomachs," *J. Ultrastruct. Res.* **23**, 243–259.
4. Koenig, C. S. & Vial, J. D. (1970) "A histochemical study of adenosine triphosphatase in the toad (Bufo spinulosus) gastric mucosa," *J. Histochem. Cytochem.* **18**, 340–353.
5. Orrego, H., Navia, E. & Vial, J. D. (1966) "Effect of puromycin on the membrane changes of the stimulated oxyntic cell," *Exp. Cell Res.* **43**, 351–357.
6. Szent-Györgyi, A. (1951) *The Chemistry of Muscular Contraction* (Academic Press, New York), 2nd ed.
7. Lowey, S. & Holtzer, A. (1959) "The homogeneity and molecular weights of the meromyosins and their relative proportions in myosin," *Biochim. Biophys. Acta* **34**, 470–484.
8. Romrell, L. J., Coppe, M. R., Munro, D. R. & Ito, S. (1975) "Isolation and separation of highly enriched fractions of viable mouse gastric parietal cells by velocity sedimentation," *J. Cell Biol.* **65**, 428–438.
9. Villegas, L., Sananes, L. & Michelangeli, F. (1971) "Relation between water flux and content in frog gastric mucosa," *Am. J. Physiol.* **220**, 1282–1288.
10. Stempak, J. G. & Ward, R. T. (1964) "An improved staining method for electron microscopy," *J. Cell Biol.* **22**, 697–701.
11. Mooseker, M. S. & Tilney, L. G. (1975) "Organization of an actin filament-membrane complex. Filament polarity and membrane attachment in the microvilli of intestinal epithelial cells," *J. Cell Biol.* **67**, 725–743.
12. Nicolson, G. L. (1974) "The interactions of lectins with animal cell surfaces," *Int. Rev. Cytol.* **39**, 89–190.
13. Painter, R. G., Sheetz, M. & Singer, S. J. (1975) "Detection and ultrastructural localization of human smooth muscle myosin-like molecules in human non-muscle cells by specific antibodies," *Proc. Natl. Acad. Sci. USA* **72**, 1359–1363.
14. Wickus, G., Gruenstein, E. Robbins, P. W. & Rich, A. (1975) "Decrease in membrane-associated actin of fibroblasts after transformation by Rous sarcoma virus," *Proc. Natl. Acad. Sci. USA* **72**, 746–749.
15. Clarke, M., Schatten, G., Mazia, D. & Spudich, J. A. (1975) "Visualization of actin fibers associated with the cell membrane in amoebae of *Dictyostelium discoideum*," *Proc. Natl. Acad. Sci. USA* **72**, 1758–1762.
16. Kapeller, M. & Doljanski, F. (1972) "Agglutination of normal and Rous sarcoma virus-transformed chick fibroblasts by concanavalin A and wheat germ agglutinin," *Nature New Biol.* **235**, 184–185.
17. Burger, M. M. & Martin, G. S. (1972) "Agglutination of cells transformed by Rous sarcoma virus by wheat germ agglutinin and concanavalin A," *Nature New Biol.* **237**, 9–12.
18. Garrido, J. (1975) "Ultrastructural labeling of cell surface lectin receptors during the cell cycle," *Exp. Cell Res.* **94**, 159–175.
19. Murray, R. L. & Dubin, M. W. (1975) "The occurrence of actin-like filaments in association with migrating pigment granules in frog retinal pigment epithelium," *J. Cell Biol.* **64**, 705–710.
20. Palevitz, B. A. & Hepler, P. K. (1975) "Identification of actin in situ at the ectoplasm-endoplasm interface of Nitella. Microfilament-chloroplast association," *J. Cell Biol.* **65**, 29–38.
21. Rebhun, L. (1972) "Polarized intracellular particle transport. Saltatory movements and cytoplasmic streaming," *Int. Rev. Cytol.* **32**, 93–137.