Title
Reactivation of contraction in detergent-lysed teleost retinal cones.

Journal
Journal of Cell Biology, 92(1)

ISSN
0021-9525

Authors
Burnside, B
Smith, B
Nagata, M
et al.

Publication Date
1982

DOI
10.1083/jcb.92.1.199

Peer reviewed

Reactivation of Contraction in Detergent-lysed Teleost Retinal Cones

BETH BURNSIDE, BENN SMITH, MICHAEL NAGATA, and KATHRYN PORRELLO
Department of Physiology-Anatomy, University of California, Berkeley, California 94720

ABSTRACT Teleost retinal cones contract in the light and elongate in the dark. In the green sunfish, *Lepomis cyanellus*, the necklike myoid region of the cone contracts from as much as 120 μm (midnight dark-adapted) to 6 μm in the fully light-adapted state. When dark-adapted fish are exposed to light (1.4 lux), cone myoids contract with a linear rate of 1.5 ± 0.1 μm/min. We report here that detergent-lysed motile models of teleost retinal cones exhibit calcium- and ATP-dependent reactivated contraction, with morphology and rate comparable to that observed in vivo. For reactivation studies isolated dark-adapted retinas were lysed with nonionic detergent Brij-58 (0.1–1.0%). In reactivation medium containing 10^{-5} M free calcium and 4 mM ATP, the lysed cones contracted with normal morphology at in vivo rates (1.4 ± 1 μm/min). Little contraction was observed if ATP or detergent was deleted from the medium or if free calcium levels were $<10^{-8}$ M. Ultrastructural examination of cone models lysed with 1% Brij-58 revealed that, in spite of extensive extraction of the cytoplasmic matrix, cytoskeletal components (thin filaments, intermediate filaments, microtubules) were still present. Thus we have produced extensively extracted motile models of teleost retinal cones which undergo calcium- and ATP-dependent reactivated contraction with normal morphology at physiological rate.

Biochemical studies of cytoplasmic contractile proteins provide information crucial to our understanding of nonmuscle motility: i.e., the substrate and kinetics of the mechanochemical reaction and conditions which activate or inhibit it; the roster of participating protein components; and the conditions necessary for assembly of cytoskeletal elements (18, 24). Biochemical studies of cytoplasmic proteins cannot, however, provide information about the more biophysical aspects of cell contraction: i.e., about the mechanics, the thermodynamics, or the kinetics of the contractile process itself, or about the structural arrangement of the contractile machinery in vivo.

To study these aspects of contraction we need a preparation in which the organization of the contractile apparatus more closely resembles its in vivo architecture. Ideally, we would like a preparation in which the contractile machinery retains its functional organization while other complicating regulatory components of the cell are stripped away or inactivated. The glycerinated myofibril, which has played such a powerful role in muscle research, approaches closely this ideal biophysical preparation (3, 25).

Unfortunately, glycerinated or lysed-cell models of nonmuscle cells have been somewhat disappointing. Since Hoffman-Berling's first reports in 1954 (10), several attempts have been made to reactivate contraction in tissue culture cells and amoebae (cf. reviews 3, 24). When bathed with Mg/ATP, most of these cell models undergo small isodiametric contractions which seldom mimic motile events in vivo (10, 24, 25). Hoffmann-Berling (11) and Cande (6) have reactivated cytokinesis movements in lysed cells which do resemble in vivo cytokinesis; however, these reactivated movements occur so quickly over such short distances that systematic quantitation of contraction rates is hampered.

Because we feel that reactivated cell models are such important tools for studying the mechanism of contraction in nonmuscle cells, we have set out to develop a new model system in which contraction can be more easily quantified, and in which reactivated contraction more closely mimics, both in morphology and in physiological constraints, the contraction observed in vivo. We have succeeded in reactivating contraction in detergent-lysed models of teleost retinal cones and we think these models exhibit the critical characteristics that make them useful for further studies of nonmuscle contraction.

Teleost retinal cones possess several advantages which make them useful as motile models. They are long slender cells which undergo uni-axial contraction when stimulated by light. The extent of contraction is dramatic: the contractile necklike myoid

THE JOURNAL OF CELL BIOLOGY · VOLUME 92 JANUARY 1982 199–206

of the cone contracts from as long as 125 μm in the dark to 5 μm in the light. Quantitation of cone contraction is facilitated by its slow rate (1.5 μm/min in vivo), and by the parallel alignment in the retina of thousands of cones, all contracting in unison. Complications from cell-cell or cell-substrate attachments are minimized because the contractile myoid portion of the cone projects freely into the subretinal space.

We have succeeded in reactivating contraction in green sunfish cones after lysis with the nonionic detergent Brij-58 (polyoxyethylene-20-cetyl ether [7]). The detergent-lysed cone models undergo ATP- and calcium-dependent reactivated contraction with rate and morphology comparable to that observed in vivo. A preliminary report of this work has appeared in abstract (5).

MATERIALS AND METHODS

Animals

All experiments were carried out using retinas from dark-adapted green sunfish (*Lepomis cyanellus*). Fish were obtained from Funez Fish Farm in Sebastopol, Calif., and maintained either in outdoor ponds or in the lab in 30- to 75-gallon aquaria. In the lab, fish were maintained in a darkroom set with light cycle to resemble that outdoors.

Because cone length is influenced by circadian rhythms (2, 15) we were careful to carry out all experiments at the same time of day. Fish were placed in darkness at 12:30 PM and experiments begun between 5:00 and 6:00 PM. Since this time of dark adaptation falls in the subjective day of the fish, cone myoids do not achieve their maximal midnight dark-adapted length (125 μm) but instead extend only to 40–45 μm. We have carried out dark adaptation in this way for two reasons: it avoids the necessity for night experiments or time-consuming phase shifting of circadian rhythms and it avoids loss of cone ellipsoids during dissection, since the shorter cone myoids are less fragile than those of fully extended cones. Even these shorter cones exhibit extensive contraction (myoid length changes from 40 to 5 μm). In most of the experiments reported, contraction was stopped by fixation before completion so that the rates of contraction could be estimated.

Preparation of Retinas

In dim red light, the eyes were enucleated, the anterior segment and lens dissected away, and the retina was gently detached with a stream of calcium-free Hanks' buffered salt solution with 5 mM EGTA gassed with 100% O_2. The optic nerve was severed, releasing the intact retina as a hemisphere supported by the gel-like vitreous. Each retina was then bisected, yielding four half-retinas from each fish. One half-retina was immediately fixed to provide initial (t_0) cone myoid lengths and the other three were used for reactivation under various conditions. Thus, for all reactivation experiments, treated cones were compared to t_0 cones from the same fish. Half-retinas were incubated in 1.5 ml of reactivation medium in Falcon 3008 multiwell dishes (Falcon Labware); pieces were placed in the well with the gelled vitreous facing down so that the photoreceptors project into the medium like bristles on a brush. Thus photoreceptor myoids and ellipsoids were bathed directly in reactivation media. After incubation in reactivation media for specified times, half-retinas were removed to fixative.

Lysis Conditions and Reactivation Media

Two reactivation procedures were employed: (*a*) retinas were exposed to lysis detergent throughout the incubation (one-step procedure) and (*b*) retinas were exposed to lysis detergent for 3 min, then transferred to detergent-free medium (two-step procedure). In both cases, lysis was achieved with the nonionic detergents Brij-58 or Nonidet P-40 (NP-40) (7). Reactivation media were adapted from Cande and Wolniak (7) for contraction, relaxation, and rigor conditions (Table I). It is important to note that true rigor is probably not achieved under these conditions; it is likely that some endogenous ATP is still present bound to specific proteins. EGTA-calcium buffers were prepared according to Steinhardt et al. (23) (Table II). At 10^{-5} M free $CaCl_2$, as used in this report for contraction media, buffering efficiency was suboptimal; calcium electrode tests revealed some variability in free calcium concentrations in buffers made according to the 10^{-5} M formula, though the 10^{-6} M free-calcium formula was reliable (Robert Zucker, personal communication). Thus throughout this report our 10^{-5} M buffers should be understood to contain $>10^{-6}$ M free calcium. Since, in this study, we were concerned specifically with detecting a calcium requirement for reactivated contraction, we completed the study with the 10^{-5} M free-calcium buffer. Further studies into more specific calcium concentration effects are in progress and will be reported elsewhere. For all reactivation media, ATP was added with equimolar $MgSO_4$; thus all media contained 1 mM free $MgSO_4$ in excess of the ATP concentration. In the two-step procedure, lysis was achieved by adding detergent to the specific medium in which reactivation of contraction was tested. All reactivation experiments were carried out at 22°C in dim red light. This red light does activate contraction in cultured retinas; thus a light signal for contraction was unavoidably delivered during dissection. However, we have recently repeated these experiments using an infrared converter for dissection and obtained similar results; thus the light signal is not required.

At specified times retinal halves were transferred from reactivation media to 6% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0 and left overnight. From each half-retina a single block was dissected from the central fundus, carefully ensuring that comparable areas were examined from each retina. This block was removed from the retina and attached, vitreal surface down, to a square of millipore filter affixed with double stick tape to a microscope slide. The retina was then chopped into 25- to 50-μm thick slices with a razor blade attached to a manual chopper (27). Slices were immersed in 0.1 M phosphate buffer, pH 7.0, detached from the millipore filter, covered with a cover slip, and examined with Zeiss Nomarski optics at × 40 magnification (see Fig. 1). Myoid length was measured by ocular micrometer as the distance between the outer limiting membrane (olm) and the base of the ellipsoid. Twenty cones were measured for each retina, taking care that selected cones were representative of the hundreds visible in any section. For all data presented, *n* is used to represent numbers of fish examined, twenty cones being measured for each fish. Thus, means indicated are grand means from several (*n*) fish. 35-mm light micrographs served as permanent records.

Electron Microscopy

For ultrastructural studies, t_0 and reactivated retinas as well as in vivo contracting retinas were immersion-fixed in 1% glutaraldehyde (TAAB) containing 0.2% tannic acid in 0.1 M phosphate buffer, pH 7.0, with 1 mM $MgCl_2$ at room temperature. After 1 h in fixative retinas were postfixed in 1% OsO_4 in 0.1 M phosphate, pH 6.0, on ice for 1 h en bloc stained with 1% aqueous uranyl acetate for 1 h, and dehydrated in graded ethanols.

RESULTS

Cone Contraction In Vivo

When dark-adapted sunfish were exposed to fluorescent light of 1.4 lux intensity, their cones contracted at a linear rate until the fully light-adapted length was reached (Fig. 2). Contraction rate under these conditions (22°C; 1.4 lux) was 1.5 ± 0.1 μm/min (mean ± standard error (SE); $n = 11$).

Reactivation of Cone Contraction

If retinas were incubated in contraction or relaxation medium without added detergent, little or no movement was

TABLE I

Reactivation Media

Contraction	Relaxation	Rigor
0.1 M PIPES, pH 6.94	0.1 M PIPES, pH 6.94	0.1 M PIPES, pH 6.94
5 mM EGTA	5 mM EGTA	5 mM EGTA
1 mM free $MgSO_4$	1 mM free $MgSO_4$	1 mM free $MgSO_4$
10^{-5} M free $CaCl_2$	10^{-8} M free $CaCl_2$	10^{-5} M free $CaCl_2$
1–5 mM Mg-ATP	1 mM Mg-ATP	No ATP

Lysis conditions: one-step procedure, 0.1–1.0% Brij-58 present throughout; two-step procedure, 0.1–1.0% Brij-58 for 3 min, followed by detergent-free medium for 15 min.

observed (Table III). When cones were lysed (permeabilized) with the detergent Brij-58 or NP-40, cone contraction was reactivated when the bathing medium contained calcium and ATP (Fig. 2). Two procedures were used to carry out detergent lysis: in one case, the detergent was present throughout the incubation (one-step procedure); in the other, retinas were exposed to the lysis medium containing detergent for 3 min and then transferred to detergent-free media (two-step procedure). Both produced linear rates of contraction in the reactivated cones (Fig. 2).

One-step Reactivation Procedure

Initial reactivation experiments were carried out with 0.1% Brij-58 present throughout the incubation (Figs. 1 and 2*a*, Table IV). Under these conditions both calcium and ATP were required for contraction (Fig. 2*a*, Table IV). Ultrastructural examination of these reactivated retinas, however, revealed surprisingly little extraction of the cytoplasm and little detectable membrane alteration except for cone outer segments (Figs. 3, 4*a* and *b*, and 5*a* and *b*). Numerous microtubules are visible in the myoids of these reactivated cones in spite of the presence of $>10^{-6}$ M $CaCl_2$ in the bathing contraction medium (Fig. 5*b*).

A survey of effects of higher concentrations of Brij-58 revealed that reactivation of contraction is possible even when as much as 1% Brij-58 is present throughout the incubation (Figs. 4*c*, 6, 7). However, variability was greatly increased by the presence of some extremely exploded cones which fail to contract (Fig. 6*b*). To increase extraction but minimize variability we depended upon the two-step procedure for reactivation.

TABLE II

Formulae for Ca/EGTA Buffers *

EGTA	$MgSO_4$	$CaCl_2$	Calculated free Mg^{++}	Calculated free Ca^{++}
		Concentration, M		
5×10^{-3}	1.16×10^{-3}	None	10^{-3}	10^{-8}
5×10^{-3}	1.06×10^{-3}	3.20×10^{-3}	10^{-3}	10^{-6}
5×10^{-3}	1.0×10^{-3}	4.74×10^{-3}	10^{-3}	10^{-5}

* From Steinhardt et al. (23); this calculation assumes an association constant for EGTA with Ca of $10^{10.7}$.

Two-step Reactivation Procedure

When retinas were lysed with Brij-58 for 3 min and then transferred to detergent-free medium, relatively uniform contraction rates were obtained over a range of Brij-58 concentrations from 0.1% to 1.0% (Fig. 7). At concentrations above 1% Brij, contraction was more variable and rates declined. At 1.5% Brij-58, all cones looked exploded and failed to contract. With 1% Brij-58, the rates of reactivated contraction were quite reproducible and cones moved more uniformly (Figs. 2*b*, and 6*a* and *c*, Table V). Exploratory experiments using NP-40 as lysis detergent indicated that contraction also occurred in Nonidet-models, though contraction rate was somewhat slower (0.8 ± 0.1 μm/min in contraction medium with 4 mM ATP and 10^{-5} M calcium; $n = 6$). In the reactivated Brij models, outer segments were completely vesiculated, cone cytoplasm was extensively extracted, nuclei were swollen, and mitochondria were much altered by the detergent treatment (Figs. 4*d* and 8). Nonetheless, cytoskeletal elements were remarkably well preserved (Fig. 8). Microtubules were not disrupted (in spite of 10^{-5} M calcium in the contraction solution) and retained a distribution consistent with that seen in vivo (Figs. 8*b* and *d*). Intermediate filaments were commonly found associated with the microtubules (Fig. 8*b* and *d*). At early stages of contraction the myoids and their microtubules exhibit distinct helical appearance, both in models and in vivo (not shown).

As observed in normal cones (4), fixed in vivo models possessed thin filaments which originated from microvilluslike calyces at the base of the outer segment (Fig. 3) and coursed in bundles through the ellipsoid between the plasma membrane and the dense central mass of mitochondria (Fig. 8*a*). In the myoid, thin filaments were found almost exclusively in close

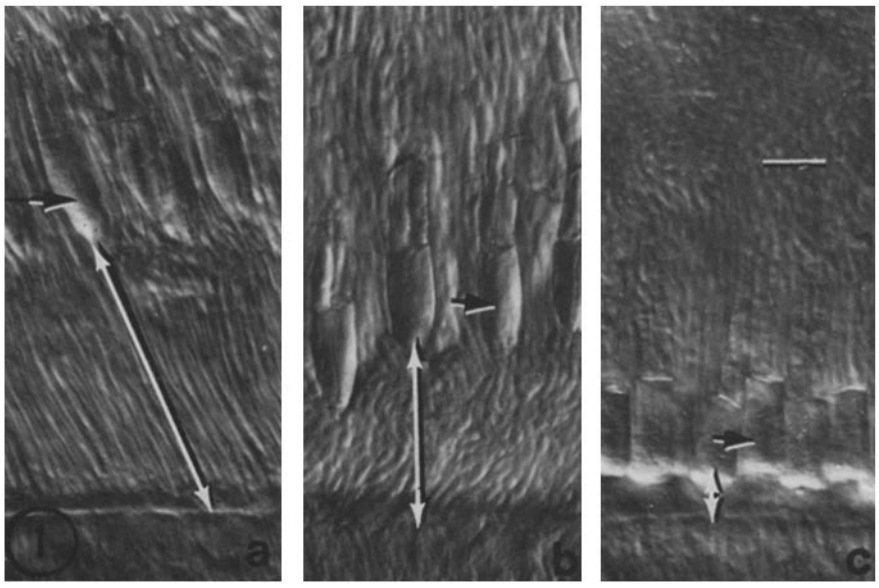


FIGURE 1 Nomarski light micrographs of chopped retinal slices showing reactivated contraction with the one-step (0.1% Brij-58) procedure. ($a = t_0$; $b = 15$ min; $c = 45$ min). Large arrowheads indicate cone ellipsoids; double-headed arrows indicate myoid lengths measured (i.e., from the base of the ellipsoid to the outer limiting membrane [olm] of the retina). Long, thin rod outer segments are interdigitated with the cone myoids. Bar, 10 μm. × 700.

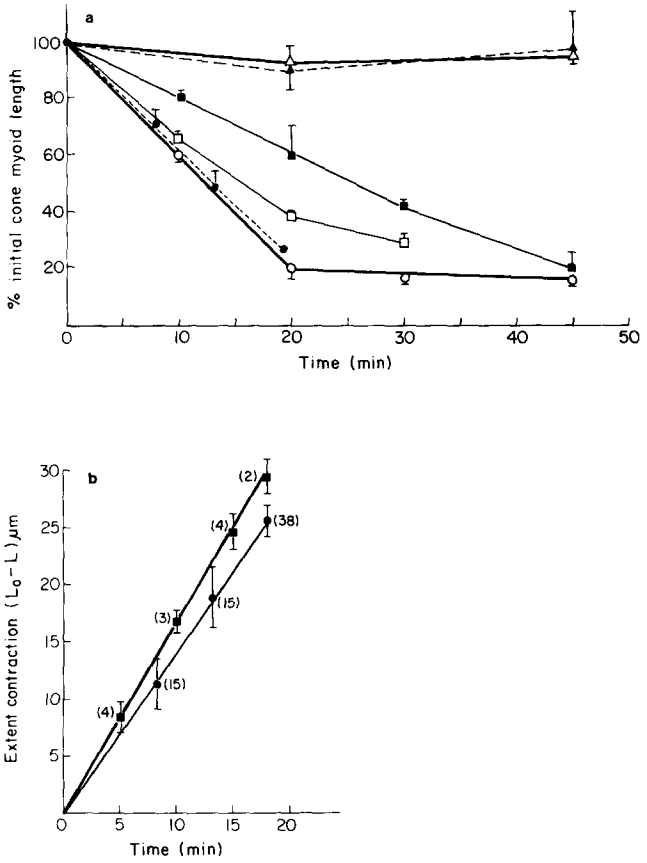


FIGURE 2 Kinetics of cone contraction in vivo and in reactivated lysed cell models. Rates and initial lengths are indicated in Tables IV and V. In *a* contraction is illustrated as percent of initial length for one-step and two-step reactivated models and for in vivo contraction (1.4 lux; 22°C). Cone contraction in vivo and in reactivated models proceeds at linear or near linear rates for at least 20 min. In vivo (○——○). Two-step: contraction medium, 4 mM ATP (●- - -●). One-step: contraction medium, 5 mM ATP (□——□); contraction medium, 1 mM ATP (■——■); relaxation medium (△——△); and rigor medium (▲- - -▲). In *b* we compare contraction in reactivated models prepared by the two-step procedure (4 mM ATP; 10^{-5} M $CaCl_2$) to in vivo contraction. Here, extent of contraction (L_o-L_t) is illustrated. Rates for these curves are 1.6 μm/min for in vivo and 1.5 μm/min for reactivated models (see also Table V). Points illustrate mean ± standard error; *n* for each point indicated in parentheses. Contraction: in vivo (■——■); and reactivated (●——●).

TABLE III

Effects of Reactivation Media in the Absence of Detergent

	L_o	ΔL in 18 min*	Contraction rate	*n*
	μm	μm	μm/min	
Contraction medium (4 mM ATP; 10^{-5} M $CaCl_2$), no detergent	39.7 ± 0.7	−0.3 ± 0.6	−0.01 ± 0.03	8
Relaxation medium (4 mM ATP; 10^{-8} M $CaCl_2$), no detergent	39.6 ± 2.0	2.1 ± 2.1	0.1 ± 0.2	3

L_o is initial length.
* ΔL, L_o-L_{final}.

association with the plasma membrane (Figs. 8*b–d*). In cone models with longer myoids (still contracting) these thin filaments were predominantly oriented parallel to the axis of contraction (Figs. 8*b* and *c*). In fully contracted models, fewer thin filaments were observed and there was no obvious favored orientation (not shown).

The plasma membrane appeared remarkably intact in the contractile models, in spite of the extensive cytoplasmic extraction (Fig. 8). Unequivocal discontinuities in the plasma membrane were not observed in the contractile models. The membranes were more obviously disrupted and the cytoplasm was almost completely extracted in those cones which failed to reactivate.

DISCUSSION

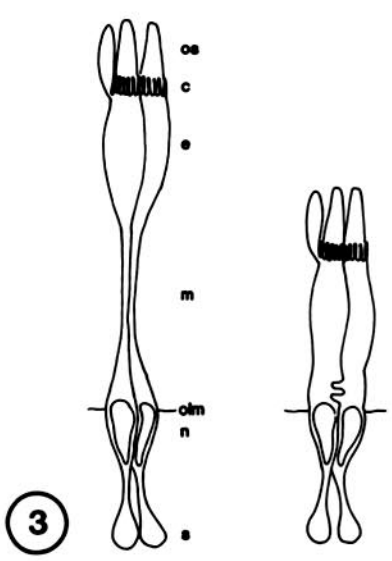
Detergent-treated teleost retinal cones undergo calcium- and ATP-dependent contraction with rate and morphology comparable to that observed in vivo. Several observations indicate that the movement we report represents *bona fide* reactivated contraction in motile cell models. Under our incubation conditions, cones did not contract unless lysed with detergent. EGTA in the reactivation medium blocked contraction unless 10^{-6} M free calcium was present, and exogenous ATP was required for full contraction. Thus we conclude that extensive exchange between the cones' cytoplasmic interior and the reactivation medium resulted from our lysis and incubation procedures. After treatment with 1% Brij-58, the cytoplasmic matrix was drastically reduced in electron density, numerous small vesicles and cisternae of smooth endoplasmic reticulum

FIGURE 3 Schematic illustration of elongated and contracted twin cones indicating landmarks considered in text and other figures: *os*, outer segment; *c*, callyces; *e*, ellipsoid; *m*, myoid; *olm*, outer limiting membrane; *n*, nucleus; and *s*, synaptic ending.

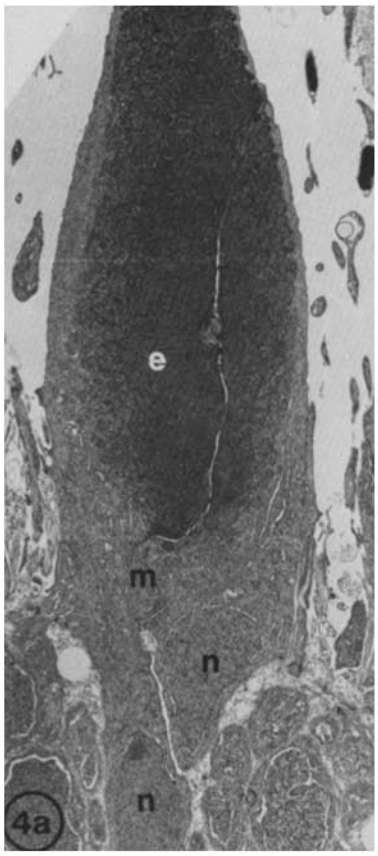
FIGURE 4 Low-magnification electron micrographs of twin cones after 18-min contraction (*a*) in vivo; (*b*) in the one-step procedure with 0.1% Brij-58; (*c*) in the one-step reactivation procedure with 1% Brij-58; and (*d*) in the two-step reactivation procedure with 1% Brij-58. Lysis with 0.1% Brij-58 (one-step) produces little cytoplasmic extraction (see also Fig. 5). Lysis with 1% Brij-58 produces considerable extraction of cone cytoplasm, yet cone landmarks remain recognizable (see also Fig. 8). *e*, ellipsoid; *m*, myoid; *n*, nucleus. Bar, 10 μm. × 3,400.

FIGURE 5 Higher-magnification electron micrographs of (*a*) a cone fixed during light-induced contraction in vivo and (*b*) a cone fixed during reactivated contraction in a cone model treated with 0.1% Brij-58 in the one-step procedure. This detergent treatment does not appear to alter membranes, microtubules (arrows), and cytoplasmic matrix in a way discernible by thin-section EM. Bar, 1 μm. × 50,000.

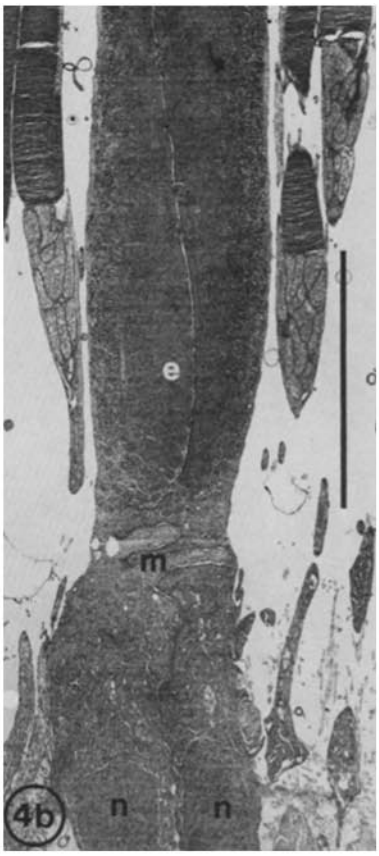
FIGURE 6 Light micrographs of plastic thick (2 μm) sections of retinas from a single fish at (*a*) t_o, and after reactivated contraction for 18 min (*b*) in the 1% Brij, one-step procedure and (*c*) in the 1% Brij, two-step procedure. Though cones contract in the 1% Brij one-step procedure, some highly exploded cones do not contract (arrow in *b*). In the two-step procedure, cones contract more uniformly. *e*, ellipsoid; *n*, nucleus. Bar, 10 μm. × 700.



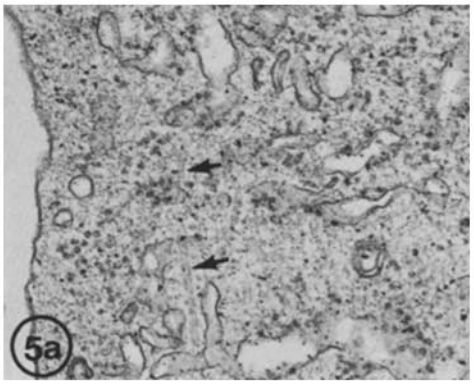
3



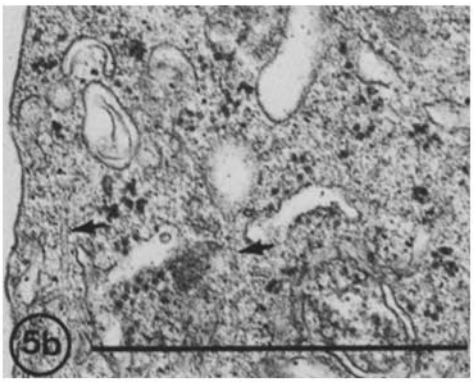
4a



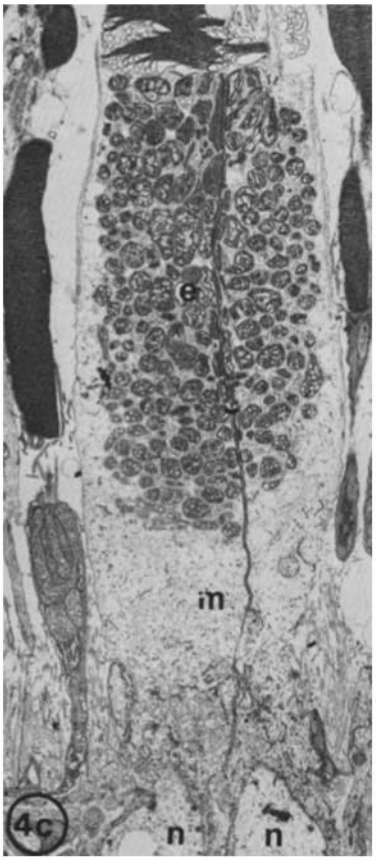
4b



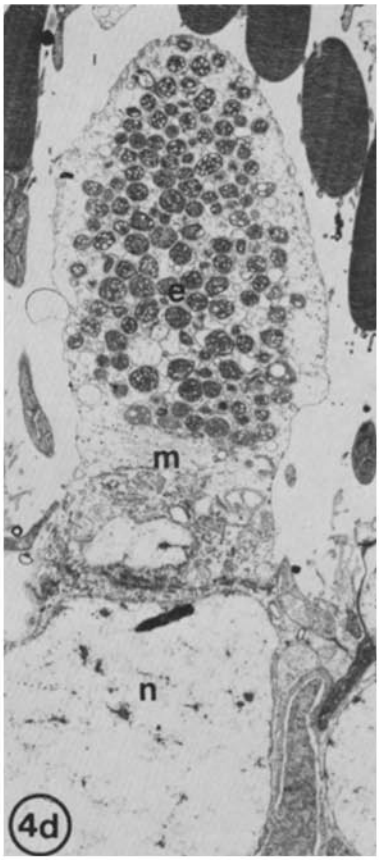
5a



5b

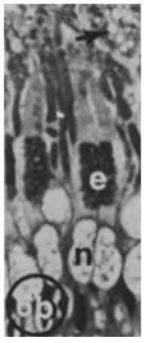


4c



4d

6a



6b

6c

TABLE IV

*One-step Reactivation Procedure**

	L_o	Rate contraction	n
	μm	μm/min	
Contraction medium I (1 mM ATP; 10^{-5} M $CaCl_2$)	40.6 ± 2.5	0.8 ± 0.1	9
Contraction medium II (5 mM ATP; 10^{-5} M $CaCl_2$)	42.1 ± 3.8	1.2 ± 0.1	4
Relaxation medium (1 mM ATP; 10^{-8} M $CaCl_2$)	45.5 ± 2.5	0.1 ± 0.1	4
Rigor medium (no ATP; 10^{-5} M $CaCl_2$)	45.5 ± 2.5	0.1 ± 0.1	4

Rates are calculated as $(L_o - L_{final})/t$ for times of 10, 20, and 30 min.
* 0.1% Brij-58 present throughout.

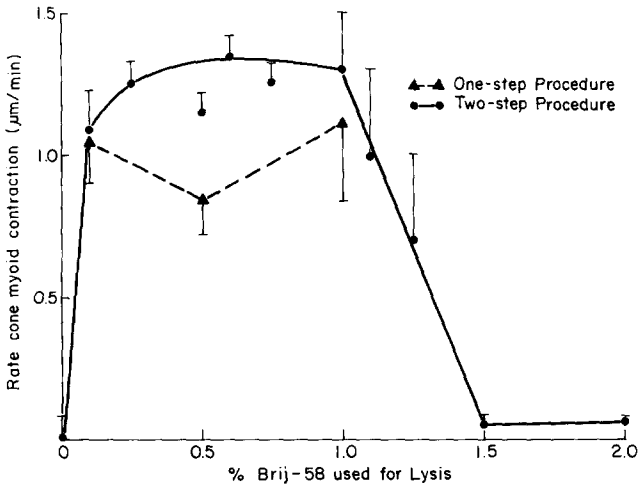


FIGURE 7 Rates of contraction of cone models after lysis with varying concentrations of Brij-58. No contraction is observed in the absence of detergent (see Table III). Rates are slower and more variable with the one-step procedure. All were incubated for 18 min. Points illustrate mean ± SEM. (▲——▲) one-step procedure; (●——●) two-step procedure.

had disappeared, and mitochondria were noticeably altered. These extensive ultrastructural changes induced by detergent treatment further confirmed that the cones had been lysed and that we were in fact working with motile cell models.

The severe cytoplasmic extraction observed in our 1% Brij-treated models enhances their value for studies of reactivated contraction. These models contract despite the fact that the myoid appears to contain little more than microtubules, thin and intermediate filaments, and a few ribosomes within the sleeve of the myoid plasma membrane. This extreme extraction facilitates morphological analysis of the contractile machinery in the cone. The association of myoid thin filaments with plasma membrane and the paraxial alignment of these filaments are emphasized in electron micrographs of extracted cone models. Close reexamination of retinas fixed while contracting in vivo reveals a similar peripheral location of myoid thin filaments in some micrographs (4); however, because of the greater cytoplasmic density of in vivo fixed cones, this peripheral location of thin filaments was not previously noticed.

The persistence of myoid microtubules in cones lysed in media containing 10^{-6} M free calcium is perhaps surprising in view of reports suggesting that microtubules are disrupted in vivo in several cell types by calcium concentrations of 10^{-6} M (12, 13, 20). It is not possible to conclude from our studies whether microtubules comprise a calcium-stable class in vivo (similar to ciliary and axostyle microtubules [20] and differing from those of the mitotic apparatus [7, 12, 19]) or whether some aspect of the lysis and reactivation procedure is conveying stability. Our observations are consistent with those of Schliwa et al. (21), who found that microtubules in Brij-58-extracted cytoskeletons of cultured monkey cells were stable in the presence of up to 10^{-4} M calcium. This result for Brij-extracted cytoskeletons was in contrast to that with Triton X-100-extracted cytoskeletons, in which 10^{-6} M calcium disrupted microtubules (21). These workers also found by immunofluorescent localization techniques that the 210,000-dalton microtubule-associated protein (MAP) was still present in Brij-extracted cytoskeletons but missing in Triton X-100-extracted cytoskeletons, therefore suggesting that this MAP may be responsible for the differential calcium sensitivity of the microtubules.

Clearly, in our Brij-extracted cone models, the microtubules are stable in the 10^{-5} M calcium of the contraction medium. Nonetheless, contraction in these cone models proceeds at a rate comparable to that in vivo. Thus contraction does not appear to require disassembly of the myoid microtubules. Both in models and in vivo, we have found numerous examples in which at early stages of contraction the myoid (along with its paraxial microtubules) appears to be assuming a helical, corkscrew configuration. As contraction proceeds, it becomes more common to see myoid microtubules oriented perpendicular to the axis of contraction. Thus it seems possible that the microtubules coil down like a spring in the contracted myoid. That such a coiled microtubule "spring" might contribute to subsequent dark-induced cone elongation is an intriguing possibility.

The calcium requirement for contraction in the teleost retinal cone models makes these models comparable to glycerinated- and detergent-lysed models of skeletal and smooth muscle (3, 22, 25). Clearly, this calcium requirement is consistent with a calcium-regulated, actomyosin-dependent mechanism for cone contraction. Nonetheless, in our model studies, some cone shortening was observed in both the relaxation and the rigor media (see Table V). There are several possible reasons why the requirements for calcium and ATP are not absolute in our cone models. The rigor conditions we use for models are not true rigor, since endogenous ATP might be expected to be present bound to intracellular sites. Also, since our dissections were done in dim red light cone contraction was activated during dissection, and therefore some calcium-dependent processes may have already been initiated before lysis and exposure of the cytoplasm to the EGTA buffer. This last possibility

TABLE V

Two-step Reactivation Procedure

	L_o	ΔL in 18 min (L_o-L_{final})	Contraction Rate	n
	μm	μm	μm/min	
Contraction medium (4 mM ATP; 10^{-5} M $CaCl_2$)	42.7 ± 1.5	25.1 ± 1.3	1.4 ± 0.1	38
Relaxation medium (4 mM ATP; 10^{-8} M $CaCl_2$)	41.1 ± 1.5	5.8 ± 1.4	0.3 ± 0.1	24
Rigor medium (no ATP; 10^{-5} M $CaCl_2$)	40.4 ± 1.8	5.5 ± 1.6	0.3 ± 0.1	23

1.0% Brij-58 for 3 min followed by detergent-free medium for 15 min.

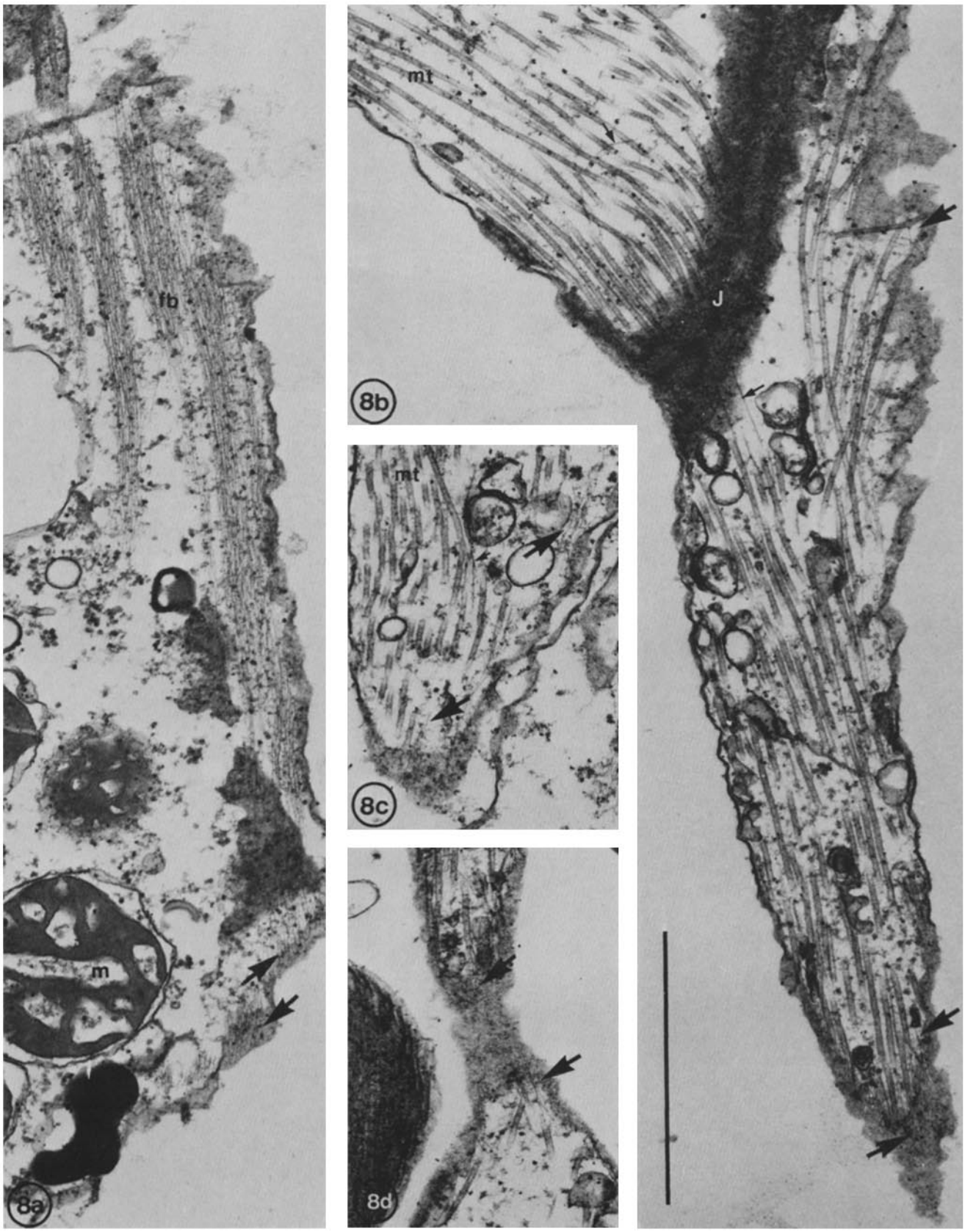


FIGURE 8 Tangential thin sections through reactivated cone models fixed during contraction in the 1% Brij, two-step reactivation procedure. Cones are illustrated at ellipsoid (*a*) and myoid (*b–d*) levels. Thin filaments (large arrows) are found in bundles (*fb*) in the ellipsoid (*a* and *b*) and associated with the plasma membrane in the myoid (*a–d*). Microtubules (*mt*) and intermediate filaments (small arrows) are preserved although cytoplasm is extensively extracted. Intermediate filaments are usually, but not always, oriented parallel to the microtubules. *J*, junction between twin cones; *m*, mitochondrion. Bar, 1 μm. × 50,000.

seems likely since in recent experiments using an infrared converter for dissection, cone shortening does not occur in relaxation medium; in fact the cones elongate slightly. Therefore, for future detailed studies of calcium effects, we will rely on infrared converters for dissection.

Numerous published observations suggest that contraction is regulated, in nonmuscle as well as muscle cells, by calcium (1, 9, 24): e.g., calcium at or above 10^{-6} M is required for contraction of isolated intestinal brush borders (17), isolated fragments of amoeba cytoplasm (24) and cytoplasmic gels formed from homogenates of several cell types (24). Furthermore, calcium-calmodulin-dependent phosphorylation of myosin light chains, similar to that observed in smooth muscle, has been described in several nonmuscle cell types (1). Since reactivated cone models exhibit calcium dependence for contraction, they should provide useful tools for investigating in more detail the mechanism of calcium regulation in cone contraction.

It is now possible to isolate physiologically active cones from dissociated teleost retinas (14). Detergent extraction of these isolated cones promises to provide models more amenable to biochemical study.

Conclusion

We have presented a new motile model for studies of nonmuscle contraction. Since the rate and morphology of contraction in reactivated cone models approximates that of light-induced cone contraction in vivo, we are encouraged to think that the mechanism of contraction in cone models faithfully reproduces the mechanism of contraction in vivo. Since the uniformity of movement and the large excursion of contraction make it possible to quantify rates and extents of reactivated movement, we believe that these models can provide useful and detailed characterizations of the effects of ions, pH, nucleotides, ionic strength, and inhibitors on nonmuscle contraction.

The authors wish to express their appreciation to W. Z. Cande for helpful discussions and suggestions throughout the course of the work and to Kent McDonald and Pat O'Connor for critical reading of the manuscript.

This study was supported by National Science Foundation grant PCM 8011792 and National Institutes of Health grant GM 23959.

Received for publication 20 January 1981, and in revised form 28 August 1981.

REFERENCES

1. Adelstein, R. S. 1980. Phosphorylation of muscle proteins. *Fed. Proc.* 39:1544–1549.
2. Ali, M. A. 1975. Retinomotor responses. *In* Vision in Fishes. M. A. Ali, editor. NATO Study Institutes Series, Life Sciences. 1:313–355.
3. Arronet, N. I. 1973. Motile muscle and cell models. Consultants Bureau, New York. 192.
4. Burnside, B. 1978. Thin (actin) and thick (myosinlike) filaments in cone contraction in the teleost retina. *J. Cell Biol.* 78:227–246.
5. Burnside, B., B. Smith, and M. Nagata. 1980. Reactivation of contraction in detergent-lysed teleost retinal cones. *J. Cell Biol.* 87(2, Pt. 2):200*a* (Abstr.).
6. Cande, W. Z. 1980. A permeabilized cell model for studying cytokinesis using mammalian tissue culture cells. *J. Cell Biol.* 87:326–335.
7. Cande, W. Z., and S. M. Wolniak. 1978. Chromosome movement in lysed mitotic cells is inhibited by vanadate. *J. Cell Biol.* 79:573–580.
8. Cheung, W. Y. 1980. Calmodulin plays a pivotal role in cellular regulation. *Science (Wash. D. C.).* 207:19–27.
9. Hitchcock, S. E. 1977. Regulation of motility in nonmuscle cells. *J. Cell Biol.* 74:1–15.
10. Hoffman-Berling, H. 1954a. Adenosintriphosphat als Betriebsstoft von Zellbewegungen. *Biochim. Biophys. Acta.* 14:182–195.
11. Hoffman-Berling, H. 1954b. Die glycerin-wasserextrahierte Teleophasezelle als Modell der Zytokinese. *Biochim. Biophys. Acta.* 15:332–339.
12. Kiehart, D. P., and Inoue, S. 1976. Local depolymerization of spindle microtubules by microinjection of calcium ions. *J. Cell Biol.* 70(2, Pt. 2):230*a* (Abstr.).
13. Kirschner, M. 1978. Microtubule assembly and nucleation. *Int. Rev. Cytol.* 54:1–71.
14. Lam, D. M. K. 1972. Biosynthesis of acetylcholine in turtle photoreceptors. *Proc. Natl. Acad. Sci. U. S. A.* 69:1987–1991.
15. Levinson, G., and B. Burnside. 1981. Circadian rhythms in teleost retinomotor movements: a comparison of the effects of circadian rhythm and light condition on cone length. *Invest. Ophthal. Visual Sci.* 20:294–303.
16. Means, A. R., and J. R. Dedman. 1980. Calmodulin—an intracellular calcium receptor. *Nature (Lond.).* 285:73–77.
17. Mooseker, M. S. 1976. Brushborder motility: microvillar contraction in Triton-treated brush borders isolated from intestinal epithelium. *J. Cell Biol.* 71:417–432.
18. Pollard, T. D., and R. R. Weihing. 1974. Actin and myosin and cell movement. *Crit. Rev. Biochem.* 2:1–65.
19. Salmon, E. D., and R. R. Segall. 1980. Calcium-labile mitotic spindles isolated from sea urchin eggs (*Lytechinus variegatus*). *J. Cell Biol.* 86:355–365.
20. Schliwa, A. 1976. The role of divalent cations in the regulation of microtubule assembly. *J. Cell. Biol.* 70:527–540.
21. Schliwa, M., U. Euteneuer, J. C. Bulinski, and J. G. Izant. 1981. Calcium lability of cytoplasmic microtubules and its modulation by microtubule-associated proteins. *Proc. Natl. Acad. Sci. U. S. A.* 78:1037–1041.
22. Small, J. V. 1977. The contractile apparatus of the smooth muscle cell. *In* The Biochemistry of the Smooth Muscle. N. L. Stephens, editor University Park, Baltimore, 379–411.
23. Steinhardt, R., R. Zucker, and G. Schatten. 1977. Intracellular calcium release at fertilization in the sea urchin egg. *Dev. Biol.* 58:185–196.
24. Taylor, D. L., and J. S. Condeelis. 1979. Cytoplasmic structure and contractility in amoeboid cells. *Int. Rev. Cytol.* 56:57–143.
25. Weber, H. H., and H. Portzehl. 1952. Muscle contraction and fibrous muscle proteins. *Adv. Protein Chem.* 7:161–252.
26. Weber, K., P. C. Rathke, M. Osborn, and W. W. Franke. 1976. Distribution of actin and tubulin in cells and in glycerinated cell models after treatment with cytochalasin B (CB). *Exp. Cell Res.* 102:285–297.
27. Werblin, F. S. 1978. Transmission along and between rods in the tiger salamander retina. *J. Physiol. (Lond.).* 280:449–470.