Proc. Nat. Acad. Sci. USA
Vol. 69, No. 1, pp. 253–257, January 1972

Cytochalasin B: Effects on Cell Morphology, Cell Adhesion, and Mucopolysaccharide Synthesis

(cultured cells/contractile microfilaments/glycoproteins/embryonic cells/sorting-out)

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Communicated by Daniel Mazia, November 4, 1971

ABSTRACT **Cytochalasin B reversibly causes extensive branching of myoblasts, fibroblasts, and nonencapsulated chondroblasts; it does not induce the formation of similar processes in myotubes, erythrocytes, amnion cells, encapsulated chondroblasts, or HeLa cells. The drug has no effect on the spontaneous contractions of isolated skeletal, cardiac, or smooth-muscle cells. Within 60 min, it depresses the incorporation of [^{14}C]glucosamine into total mucopolysaccharide and glycoproteins by over 50%. The drug interferes with adhesion and sorting-out of dissociated embryonic cells. Cytochalasin B is likely to produce changes in components of the cell surface whose function is not readily or solely related to a system of "primitive contractile microfilaments."**

Carter's (1) report that cytochalasin B interferes with cytokinesis, but not with nuclear division, and that the antibiotic diminished cell motility, has been confirmed (2–4). Schroeder (5, 6), working with cleaving sea-urchin eggs and HeLa cells, demonstrated changes in microfilaments in the contractile ring after exposure to cytochalasin B; this effect was rapid and reversible (see, however, refs. 7 and 8). Since cytochalasin B blocked tail resorption in ascidian tadpoles (9, 10), an activity also attributed to microfilaments, this added further support to the notion that the antibiotic interfered with a "primitive contractile" system of microfilaments present in virtually all cells. Wessels and coworkers (11–14) have elaborated on this theme and presented electron microscopic observations to the effect that alterations of cortical microfilaments induced by cytochalasin B not only interfered with cell motility, but with blood clotting, cytoplasmic streaming, and embryonic morphogenesis. The site of action of cytochalasin B in the cell is not known. However, it has been suggested that if a property is affected by the drug, then this may be evidence for the presence of microfilaments (11). This view suggests that the effect of cytochalasin B on the cytological integrity of these microfilaments may be analogous to the action of colcimide on microtubules.

Our experiments demonstrate that cytochalasin B has a rapid, but reversible, effect on total synthesis of mucopolysaccharides and glycoproteins, which are important constituents of the cell surface. This report also stresses the striking, but reversible, effects of the drug on the morphology of some cells. Furthermore, while cytochalasin B does not inhibit the spontaneous contractile activity of isolated skeletal, cardiac, or smooth-muscle cells, it does interfere with cell aggregation and cell sorting-out. These results suggest that at least one effect of the drug is due to its blockage of a component of the cell surface that rapidly turns over.

MATERIALS AND METHODS

Amnion and smooth-muscle cells were prepared from 10-day chick embryos and grown as described in ref. (15,22). Cultures of myogenic, fibrogenic, and chondrogenic cells were prepared and grown as described (16–18). HeLa cells (Microbiological Associates, Bethesda, Md.) were grown in the medium used for myogenic cells. Replicating erythrocytes from 3- and 4-day-old embryos were prepared as described (19).

D-[1-^{14}C]Glucosamine hydrochloride (1.0 μCi/ml, 42 Ci/mol, New England Nuclear Co.) was added to cultures for various times to follow the synthesis of mucopolysaccharides. As shown (15), most of the isotope is incorporated into glucosamine and galactosamine. After exposure to the isotope, the cells and the medium were each treated for the isolation of mucopolysaccharides (15). Aliquots were counted in a scintillation counter. [^{3}H]Leucine (1 μCi/ml, 5 Ci/mmol, New England Nuclear) was used to follow incorporation into material insoluble in trichloroacetic acid.

Cytochalasin B (Imperial Chemicals, Ltd.) was made up as a 0.1% stock solution in dimethyl sulphoxide, divided into 1-ml portions, and stored frozen. Except where noted, the concentration of cytochalasin B used was 5 μg/ml of medium. 0.1 mM Cyclohexamide was used to inhibit protein synthesis and 1 mM NaCN was used to inhibit electron transport and phosphorylation.

RESULTS

Effect of Cytochalasin B on Cell Morphology. After 48 hr in cytochalasin B, presumptive myoblasts, fibroblasts, and stellate chondroblasts assume a markedly branched or arborized morphology (Fig. 1). About 24 hr is required for a cell to become fully arborized. The number of nuclei in arborized cells varies from 1 to 5, and the length of the processes radiating from the nucleated center may exceed 80 μm. It is not uncommon for an arborized cell to display 10 or more processes. The degree of arborization varies with the nature of the substrate (e.g., collagen or plastic), with composition of the medium, and with cell density.

When cytochalasin B is removed, the cells rapidly revert to a more-normal morphology. The rapid loss of the arborized condition can be appreciated by comparison of Figs. 1 and 2. These photomicrographs are of the same cells; Fig. 2 was taken 30 min after removal of cytochalasin B. If cytochalasin B is added back to the medium, the cells will arborize a second time. This treatment was repeated five times during

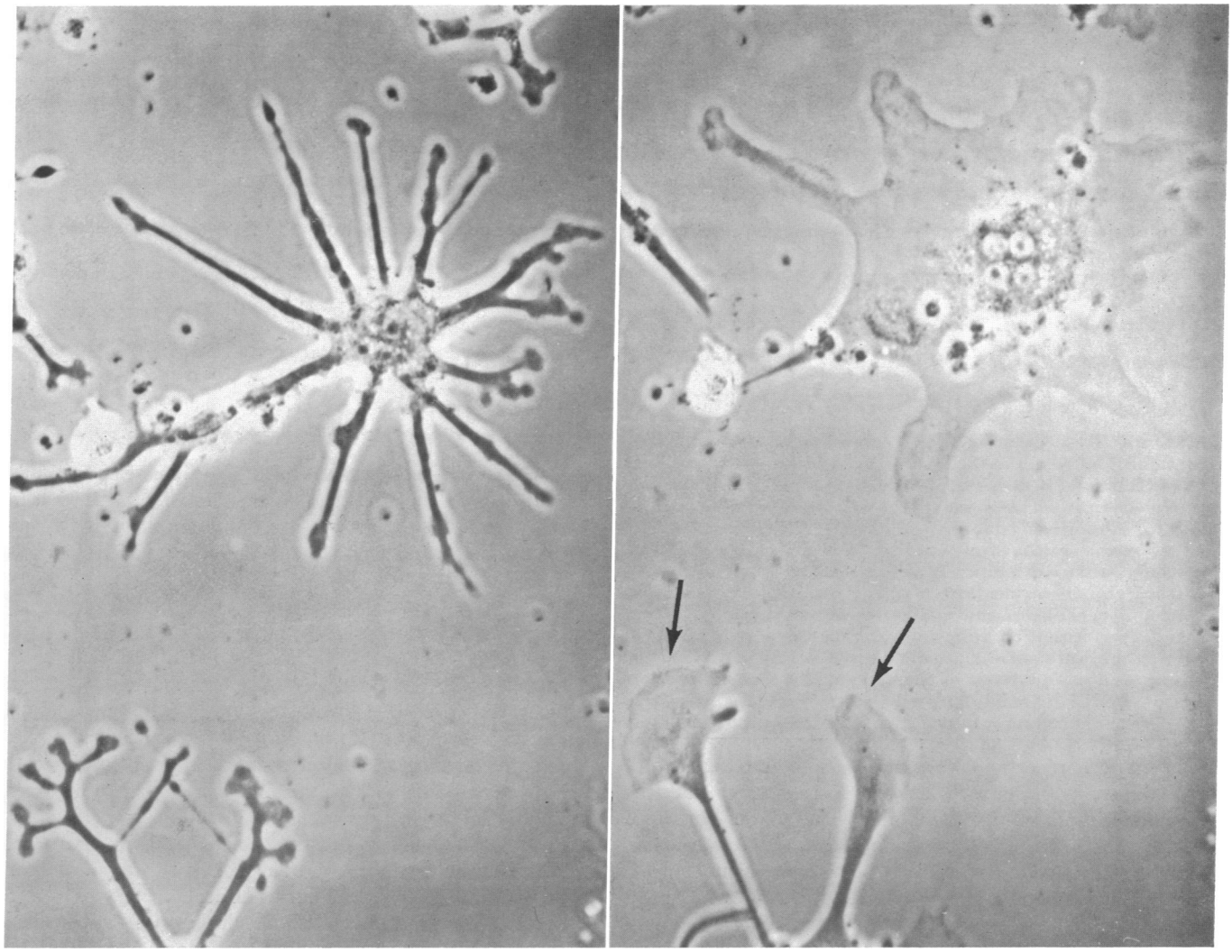

FIG. 1. (*left*) One arborized cell and a few processes of an adjacent arborized cell treated with cytochalasin B after 2 days. Under the phase-contrast microscope, myoblasts could not be distinguished from fibroblasts or stellate chondroblasts. ×1000.

FIG. 2. (*right*) Same cells 30 min after cytochalasin B was replaced by normal medium. Observe expanding and coalescing lobopodia at the tips of the processes of the lower cell (*arrows*). Also note that the long diameters of the cell have not significantly "contracted". Most microscopic cell organelles are confined to the nucleated center of the new exceedingly flattened cell. It is doubtful that the cell in this figure is "larger" in volume than in Fig. 1, but it is flatter. ×1000.

a 3-day period, with the same results: arborization and reversibility. The loss of the arborized state does not involve retraction or contraction of the extended processes. The dimensions of the long processes do not change significantly in Figs. 1 and 2; the cells lose their branches when numerous small lobopodia gradually fill the area *between* the processes. The outflow of the lobopodia is indistinguishable in form from the advancing tip of amoeba, and no ruffled membrane is involved.

The motile activity responsible for the loss of the arborized state does not require protein synthesis, but is blocked by inhibition of phosphorylation. Arborized cells treated with cyclohexamide and cytochalasin B for 30 min, then transferred to normal medium containing cyclohexamide, undergo the same reversal in shape as do cultures not treated with cyclohexamide. Arborized cells treated with NaCN remain arborized when the cytochalasin is removed. When the NaCN is also removed, the arborized cells undergo a reversal of shape.

As indicated in Table 1, replicating erythrocytes undergo nuclear divisions in cytochalasin B, but do not undergo cytokinesis or arborize. Multinucleated myotubes, the fusion product of myoblasts, do not arborize. Amnion cells synthesizing hyaluronic acid (15,22) and differentiated chondrogenic cells depositing chondroitin sulfate do not arborize, whereas dedifferentiated chondrogenic cells (17, 18) do arborize.

Shortly after immersion in cytochalasin B, HeLa cells are studded with optically dense droplets, which vary in diameter from 2 to 5 μm. After prolonged exposure, these opaque bodies are no longer present. Often the surfaces of HeLa cells are corregated, and the tips of the stellate processes often terminate in clusters of fine (1-μm) vesicles. The drug has striking effects when HeLa cells enter anaphase or telephase. The normal blebbing occurring in these cells during division is accelerated.

All of the above cell types reared in cytochalasin B are virtually immobile. Cell cultures treated with cytochalasin B for several days show a reduction in cell numbers and an

increase in cellular debris which indicates that the drug is mildly cytotoxic. Nevertheless, muscle, amnion, and HeLa cells have been maintained as multinucleated cells for more than a week.

Lack of Effect of Cytochalasin B on Contracting Embryonic-Muscle Cells. If the primary site of action of cytochalasin B is on a "primitive contractile system", it might be expected to interfere with the initiation or maintenance of the spontaneous contractions of different kinds of muscle cells. Accordingly, the antibiotic was added to cultures of skeletal myogenic cells 2-, 4-, 6-, and 8-days-old. Though it depressed cell migration and cell fusion, cytochalasin B did not block the initiation or maintenance of the spontaneous contraction of the binucleated or multinucleated myotubes (20, 21).

Sizeable numbers of single, contracting smooth-muscle myoblasts are present in cultures from 10-day chick amnions (15,22). The presence of 0.5-10 μg/ml cytochalasin B did not block the slow peristaltic contraction of these single, isolated muscle cells.

Trypsinized, isolated cardiac myoblasts from 4-day-old chick hearts exposed for 24 hr to cytochalasin B beat as vigorously as did controls. After a 3-day exposure to the drug, several cells, while contracting, were observed to undergo mitosis to form contracting binucleated cells.

Effect of Cytochalasin B on the Synthesis of Mucopolysaccharides. While cytochalasin B did not inhibit the contraction of isolated cardiac myoblasts, it did attenuate the coordinated contraction of whole hearts. Hearts treated with the antibiotic, and then mechanically agitated, were more readily dissociated into component cells than were untreated controls. These observations led us to test the proposition that cytochalasin B acted on the surface mucopolysaccharides or glycoproteins that mediate cell adhesion.

Amnion cells, HeLa cells, and skeletal myogenic cells were incubated in the presence of [^{14}C]glucosamine, and the synthesis of total, nondialyzable mucopolysaccharides was monitored. The extent of inhibition by 0.5 μg/ml of cytochalasin B in amnion and 1 μg/ml in HeLa cells is 80% and 55%, respectively (Figs. 3 and 4). Similar results were obtained with muscle cultures (0.5 μg/ml), though the percent of inhibition within the first hour did not exceed 50%. The rate of total protein synthesis, as indicated by the incorporation of [^{3}H]leucine into acid-precipitable material did not markedly diminish in these cultures over a 5-hr period. Presumably, the glycoproteins or mucopolysaccharides that might be associated with the cell surface would constitute a very small percentage of the total protein synthesized by the cell in this period. If such a small percentage of the total protein synthesized was blocked by cytochalasin B, our measurements would not be sensitive enough to detect it. The type or types of mucopolysaccharides and glycoproteins whose synthesis is inhibited by cytochalasin B is being investigated.

The rapid recovery of polysaccharide synthesis after the

TABLE 1. *Effects of cytochalasin B on various cell types after 36 hr.*

Cell Type	Multi-nucleated	Reduced motility	Arborized
Erythrocytes	+		−
Presumptive skeletal myoblasts	+	+	+
Myotubes			−
Cardiac myoblasts	+	+	±
Fibroblasts	+	+	+
Polygonal chondroblasts	+		−
Stellate chondroblasts	+	+	+
HeLa	+	+	−
Amnion	+	+	−

As the myotubes contain postmitotic nuclei, the cytochalasin B has no effect on the nuclei within the myotubes. Also, it is difficult to assess "motility" of myotubes in the drug; they do, however, elongate in the presence of the drug. Polygonal chondroblasts are encapsulated in a shell of metachromatic chondroitin sulfate (17, 18), and they do not migrate. This shell of mucopolysaccharide is absent from the stellate chondroblasts.

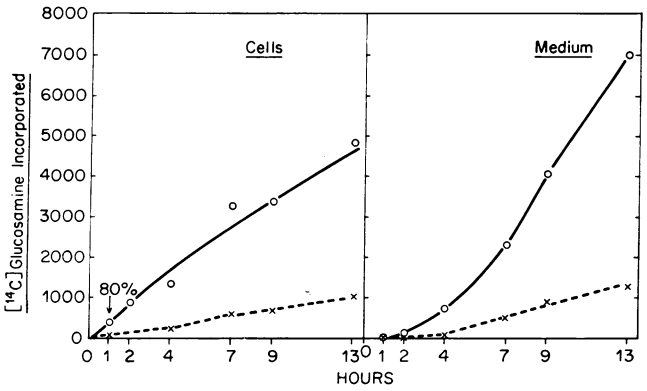

FIG. 3. Incorporation of [^{14}C]glucosamine into the mucopolysaccharide fraction of amnion cultures over 1–13 hr. Equal numbers of cells were plated out in normal media. The cells were permitted to grow for 1 day. The medium was removed and fresh medium containing [^{14}C]glucosamine, with and without 0.5 μg/ml of cytochalasin B added. At the indicated time periods, the medium was removed, the cells were washed and collected and each fraction was counted. O——O, control; ×——×, plus cytocholasin B.

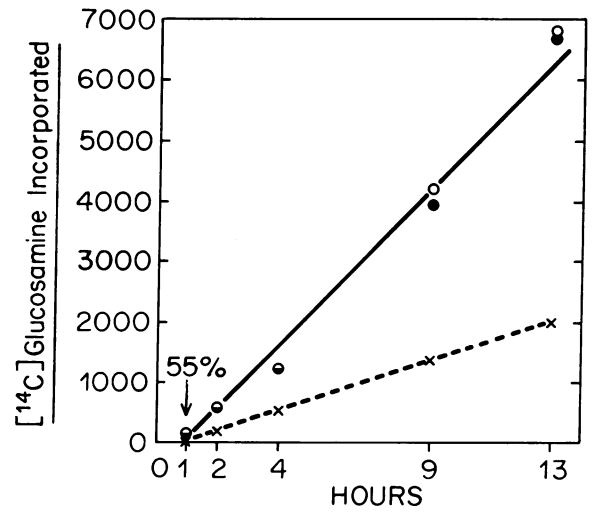

FIG. 4. Effect of 1.0 μg of cytochalasin B per ml on the incorporation of [^{14}C]glucosamine into the mucopolysaccharide fraction of HeLa cells. The lack of effect of dimethylsulfoxide (13 mM) used to dissolve the cytochalasin B is also indicated. O——O, control; ×——×, cytochalasin B; ●——●, dimethylsulfoxide.

removal of cytochalasin B was investigated. After 4 hr in cytochalasin B, amnion cells were washed and grown in normal medium. Within 2 hr the rate of mucopolysaccharide synthesis was comparable to that of the controls.

Effect of Cytochalasin B on Cell Adhesion and Cell Sorting-Out. The following experiments with 3-day-old chick embryos, dissociated 2-day-old eyes, and mixtures of myogenic and chondrogenic cells demonstrate that cytochalasin B does interfere with adhesive interactions between various cells.

Aliquots of cell suspensions prepared by trypsinizing 3-day-old chick embryos were placed in 3 ml of medium in sterilized conical test-tubes, with or without cytochalasin B. The cells were centrifuged down and cultured for 24 hr. In the control tubes, the cells formed a single cohesive pellet that could be dislodged and transferred to another tube. In contrast, the cells reared in cytochalasin B did not form a pellet. When dislodged from the bottom of the tube, they dispersed into separate cells. When the cytochalasin B was removed, and the cells were reincubated in normal medium, the cells formed a pellet.

Comparable experiments have been performed with dissociated 2-day eyes. After normal dissociated cells were cultured for 24 hr in the test tube, and another 24 hr in organ culture, they formed characteristic rosettes of neural epithelium and masses of lens cells. The bi- and trinucleated cells grown in cytochalasin B do not associate to form such structures.

Mixtures of 2×10^6 chondrogenic and 2×10^6 myogenic cells were cultured in conical test tubes for 48 hr. Histological sections prepared from the control pellets revealed masses of metachromatic cartilage and swirls of multinucleated myotubes. Histological sections prepared from the cells cultured in cytochalasin B did not reveal aggregates of chondrocytes, and myotube formation was drastically inhibited.

DISCUSSION

Cytochalasin B interferes with a number of motile processes (11). Wessels and coworkers have stated (11, 12) that cytochalasin-B interferes with the spontaneous contraction of cardiac and smooth-muscle cells. These findings could not be confirmed in this study. The failure of cytochalasin B to affect the contractile activity of differentiating or differentiated skeletal, cardiac, or smooth-muscle cells suggests that the antibiotic has no direct effect on these kinds of contractile systems (20). The paradox is that actin, clearly implicated in muscle contraction and cell motility in amoeba, is not the target for cytochalasin B. Though there is evidence linking actin-like filaments to amoeboid movement (23–26), there is only circumstantial evidence linking microfilaments in the cell *cortex* to such movement. The finding (27) that actin, or actin-like, molecules are in the cortex of many kinds of cells, while it is consistent with this notion, does not however, prove it. For example, incorporation of 5-bromodeoxyuridine (BrdU) into the DNA of replicating presumptive myoblasts suppresses the synthesis of actin, as well as myosin and tropomyosin in their progeny (28–31). Nevertheless, these BrdU-suppressed cells continue to synthesize the actin-like molecules that are associated with the cell cortex, and that are capable of binding heavy meromyosin. In spite of the presence of these actin-like cortical filaments, however, BrdU-treated cells are immobile. Actin-like molecules are also found associated with membranes of mammalian erythrocytes (32, 33). The actin-like molecules in the cortex of erythrocytes are not likely to be associated with motility. These observations should caution one against making any simple correlation between microfilaments in the cell cortex and cell motility modeled on the interaction between actin and myosin filaments.

The finding that cytochalasin B promptly depresses the synthesis of mucopolysaccharides and glycoproteins raises possibilities of relating cell mobility to cell adhesion and cell sorting-out. Glycoproteins or mucopolysaccharides are likely to be involved in such cell–cell interactions. That cytochalasin B inhibits mucopolysaccharide synthesis and prevents such cell–cell interactions supports this view. There is other evidence that agents that alter mucopolysaccharide synthesis alter the properties of the cell surface, and also block cell motility. BrdU-suppressed myogenic, chondrogenic, fibrogenic, and amnion cells are all immobile (15, 34). The kinds and quantities of mucopolysaccharides synthesized by BrdU-suppressed cells differ considerably from their respective controls (15, 17, 22). Lastly BrdU-suppressed chondrogenic cells mixed with BrdU-suppressed myogenic cells do not sort-out (unpublished results).

While various agents, such as actinomycin-D, FdU, BrdU, cycloheximide, uncouplers of phosphorylation, colchicine, green light, mica substrates, and EDTA, distort the morphology of myogenic, fibrogenic, and chondrogenic cells in culture (35), *only cytochalasin B arborizes* these cells. The arborizing effects of cytochalasin B on some cell types, but not on others, may reflect fundamental differences in their cell surfaces. Consistent with this assumption is the finding that, in cytochalasin B, presumptive myoblasts arborize, whereas the fusion product of myoblasts, the myotube, does not. There is evidence that the surface of myoblasts undergo considerable change after they fuse to form multinucleated myotubes (35–37). Likewise, the fully functional chondroblast encapsulated within a shell of chondroitin sulfate does not arborize, whereas the nonfunctional chondrocyte, which lacks a capsule, does arborize. The mucopolysaccharide may cover the site on the surface sensitive to the drug, or it may confine the cell mechanically.

It is commonplace to ascribe to the cell surface a central role in cell division, cell movement, and cell adhesion. Nevertheless, critical evidence for such correlations is modest, and often it is difficult to demonstrate whether changes in properties of the cell surface are the cause or the result of the biological activity being examined. Clearly, further work is required to determine whether the multiple effects of cytochalasin B are due to action on one, or several, cellular sites. Cytochalasin B appears to be a compound that can be used to probe changes in the cell surface.

This work was supported by grants from the Muscular Dystrophy Associations of America, the National Science Foundation, the American Cancer Society, and the University of Pennsylvania Plan to Develop Medical Scientists.

1. Carter, S. B. (1967) *Nature* **213**, 261–264.
2. Ridler, M. A. C. & Smith, G. F. (1968) *J. Cell Sci.* **3**, 595–602.
3. Krishan, A. & Ray-Chaudhuri, R. (1970) *J. Cell Biol.* **43**, 618–621.
4. Krishan, A. (1971) *J. Ultrastruct. Res.* **36**, 191–204.
5. Schroeder, T. E. (1969) *Biol. Bull.* **137**, 413–414.
6. Schroeder, T. E. (1970) *Z. Zellforsch. Mikrosk. Anat.* **109**, 431–449.
7. Bluemink, J. G. (1971) *Cytobiologie* **3**, 176–187.

8. Estensen, R. D., Rosenberg, M. & Sheridan, J. D. (1971) *Science* **173,** 356–358.
9. Cloney, R. A. (1969) *Z. Zellforsch. Mikrosk. Anat.* **100,** 31–53.
10. Lash, J., Cloney, R. A. & Minor, R. R. (1970) *Biol. Bull.* **139,** 427–428.
11. Wessells, N. K., Spooner, B. S., Ash, J. F., Bradley, M. O., Luduena, M. A., Taylor, E., Wrenn, J. T. & Yamada, K. M. (1971) *Science* **171,** 135–143.
12. Spooner, B. S., Yamada, K. M. & Wessells, N. K. (1971) *J. Cell Biol.* **49,** 595–613.
13. Yamada, K. M., Spooner, B. S. & Wessells, N. K. (1971) *J. Cell Biol.* **49,** 614–635.
14. Wessells, N. K., Spooner, B. S., Ash, J. G., Luduena, M. A. & Wrenn, J. T. (1971) *Science* **173,** 358–359.
15. Mayne, R. M., Sanger, J. W. & Holtzer, H. (1971) *Develop. Biol.* **25,** 547–567.
16. Bischoff, R. & Holtzer, H. (1968) *J. Cell Biol.* **36,** 111–127.
17. Chacko, S., Holtzer, S. & Holtzer, H. (1969) *Biochem. Biophys. Res. Commun.* **34,** 183–189.
18. Chacko, S., Abbott, J., Holtzer, S. & Holtzer, H. (1969) *J. Exp. Med.* **130,** 417–442.
19. Weintraub, H., Campbell, G. & Holtzer, H. (1971) *J. Cell Biol.* **50,** 652–668.
20. Sanger, J. W., Holtzer S. & Holtzer, H. (1971) *Nature New Biol.* **229,** 121–123.
21. Sanger, J. W. & Holtzer, H. (1970) *J. Cell Biol.* **47,** 178a.
22. Bischoff, R. (1971) *Exp. Cell Res.* **67,** 224–236.
23. Hatano, S., Tsuyoshi, T. & Oosawa, F. (1968) *Biochim. Biophys. Acta.* **140,** 109–122.
24. Wolpert, L., Thompson, C. M. & O'Neill, C. H. (1964) *Primitive Motile Systems,* ed. Allen, R. & Kamiya, N. (Academic Press, New York).
25. Nachmias, V. T., Huxley, H. E. & Kessler, D. (1970) *J. Mol. Biol.* **50,** 83–90.
26. Pollard, T. D., Sherton, E., Weiking, R. R. & Korn, E. D. (1970) *J. Mol. Biol.* **50,** 91–97.
27. Ishikawa, H., Bischoff, R. & Holtzer, H. (1969) *J. Cell Biol.* **43,** 312–328.
28. Stockdale, F., Okazaki, K., Nameroff, M. & Holtzer, H. (1964) *Science* **146,** 533–535.
29. Okazaki, K. & Holtzer, H. (1965) *J. Histochem. Cytochem.* **13,** 726–739.
30. Bischoff, R. & Holtzer, H. (1970) *J. Cell Biol.* **44,** 134–150.
31. Holtzer, H. & Bischoff, R. (1970) In *Physiology and Biochemistry of Muscle as a Food,* ed. Briskey, E. & Cassens, R. (Univ. of Wisconsin Press, Madison, Wis.), 29–51.
32. Marchesi, V. & Steers, S. (1968) *Science* **159,** 203–204.
33. Mazia, D. & Ruby, A. (1968) *Proc. Nat. Acad. Sci. USA* **61,** 1005–1012.
34. Abbott, J. & Holtzer, H. (1968) *Proc. Nat. Acad. Sci. USA* **59,** 1144–1151.
35. Holtzer, H. (1970) in *Cell Differentiation,* ed. Schjeide, O. & de Vellis, J. (Van Nostrand Reinhold Co., Princeton, N.J.), p. 476–503.
36. Okazaki, K. & Holtzer, H. (1966) *Proc. Nat. Acad. Sci. USA* **56,** 1484–1490.
37. Bischoff, R. & Holtzer, H. (1968) *J. Cell Biol.* **41,** 188–203.