RESPONSE OF MYOGENIC AND FIBROGENIC CELLS TO CYTOCHALASIN B AND TO COLCEMID

I. Light Microscope Observations

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ABSTRACT

Cytochalasin B (CB) induces a biphasic retraction in some cell types. The rapid response that peaks in 30 min leads to the "dendritic" condition. Replicating myogenic and fibrogenic cells, as well as postmitotic myoblasts and myotubes, participate in this reaction. This is followed by a slower phase that requires 40 h for stabilization and leads to the fully "arborized" state. Only replicating myogenic and fibrogenic cells participate in this reaction. Postmitotic myoblasts and myotubes do not arborize but round up and float off into the medium. Pretreatment with Colcemid does not block the rapid response to CB, but does block arborization. CB-arborized cells exposed to Colcemid while in the presence of CB develop sufficient tension to pull themselves apart. If CB depolymerizes actin-like filaments, and if such filaments constitute the only contractile system in the cell, then it is difficult to visualize how cells in CB develop such tension.

Colcemid induces twisting, birefringent bands in interphase- and metaphase-arrested myogenic and fibrogenic cells, and in postmitotic myotubes. Such bands are much more evident when CB-arborized cells are removed from CB and allowed to relax in Colcemid. These birefringent bands assemble in the presence of cycloheximide, and may constitute 20% of the volume of the cell.

The conflicting literature on the pleiotropic effects of cytochalasin B (CB) has been reviewed repeatedly (6, 10, 18, 22, 32, 36, 37, 44). Reports have stated that the antibiotic (*a*) interferes with cytokinesis, (*b*) inhibits the uptake of sugars, (*c*) blocks amoeboid movement, (*d*) prevents sorting out of embryonic cells, and (*e*) disturbs endo- and exocytosis. Not all of these effects, however, are observed in all cell types (6, 13, 20, 41). There is ample, though by no means unambiguous, literature that many of these effects of CB are due to one reaction, namely the reversible disruption of microfilaments associated with the cell surface (3, 42, 44, 45, 47; see, however, references 6, 18, 24). It has been postulated that these filaments serve as a primitive contractile system, and the observation that heavy meromyosin (HMM) decorates such filaments (28) has been cited by some as evidence supporting this notion (44, 45, 47).

Sanger and Holtzer (40) and Holtzer and Sanger (23), however, have stressed that the addition of CB to muscle cultures did not interfere with either the synthesis or the polymerization of actin into thin filaments, or with its interaction with myosin required for contraction (20, 31). CB did, however, block locomotion of presumptive myoblasts and fibroblasts and did block fusion of postmitotic myoblasts into myotubes. With respect to the

localization of sites of action of CB it is interesting to note that the antibiotic induced striking changes in cell shape. CB induced replicating myogenic and fibrogenic cells to form multiple, elongated, spidery arms. Such arms were often over 50 μm in length and 2–3 μm in diameter, and a single cell could display over one-half dozen such processes. This remarkable and reversible change in shape was termed "arborization" (23, 24), and as far as is known it is a relatively unique effect of CB on *some* types of living cells; cartilage cells, HeLa cells, and Rous sarcoma virus (RSV)-transformed fibrogenic and myogenic cells do not arborize.[1]

The experiments to be reported demonstrate that: (*a*) Changes in shape and motility leading to the fully arborized state are biphasic. A rapid response requiring approximately 30 min involves a global and persistent retraction and is followed by a protracted phase of approximately 40 h. The latter, but not the former, is sensitive to Colcemid. (*b*) CB has a differential effect on presumptive myoblasts and fibroblasts as compared with postmitotic myoblasts and myotubes; it arborizes the former, but induces the latter to round up and leave the substrate. (*c*) When CB-arborized cells are allowed to relax in Colcemid, remarkable tortuous bands emerge. These winding, birefringent bands that may achieve a length of over six times the diameter of the cell consist exclusively of large numbers of 100 Å filaments (20, 21).

MATERIAL AND METHODS

Muscle cultures were prepared by trypsinizing breast muscle from 10-day-old chick embryos as described by Bischoff and Holtzer (4). The plating suspension was adjusted to 5×10^5 cells/ml, and 1.2 ml of this suspension was added to each collagen-coated 35-mm petri dish. Most observations on the CB-arborized cells were made on 4-day-old and older cultures, for it requires approximately 40 h to arborize cells fully. Most observations to be stressed in this report were made on living cells with either phase or polarization optics. A preliminary account of this material treated with fluorescein-labeled antibodies to myosin or tropomyosin, or viewed in the electron microscope (EM), has been reported (15, 20).

In all experiments the CB that was added to the cultures was dissolved in dimethyl sulfoxide (DMSO) with a final concentration in the medium of 5 μg/ml. Neither the rapid retraction nor the more slowly occurring processes leading to full arborization was induced by DMSO alone. Colcemid was always added to the cultures at a concentration of 10^{-6} M as described by Bischoff and Holtzer (5). To determine whether (*a*) early retraction, (*b*) arborization, or (*c*) recovery from either was dependent on protein synthesis or oxidative phosphorylation, cycloheximide (0.1 mM) or NaCN (1 mM) was added to the medium.

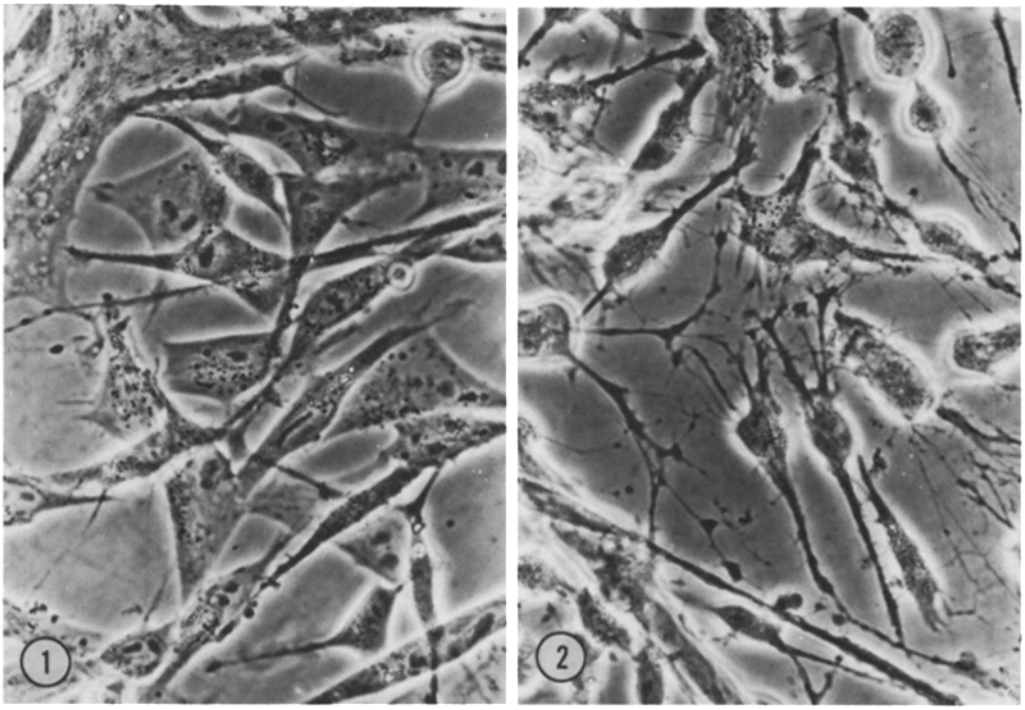
As described elsewhere (19, 23) cultures of 10-day-old breast muscle are composed of cells belonging to at least two major lineages: the myogenic and fibrogenic as well as goodly numbers of mesenchyme cells. In standard cultures, only the cells that fuse to form the multinucleated myotubes can be identified unambiguously as belonging to myogenic lineage. Contrary to the claims of Doering and Fischman (16) or of Buckley and Konigsberg (8), there are no reliable cytological means of distinguishing replicating mononucleated myogenic cells from mononucleated fibrogenic cells or from mononucleated mesenchyme cells (1, 14, 19). Accordingly, in this report the response of cells to the action of CB or Colcemid will be, for the most part, in terms of mononucleated cells or multinucleated myotubes. Postmitotic, mononucleated cells that beat spontaneously, or that display interdigitating thick and thin filaments under the EM, will be referred to as myoblasts (20, 24). This usage of the term myoblast differs from that of Konigsberg (29) and others (16).

RESULTS

Effect of CB on Mononucleated Cells and Immature Myotubes

Three-day-old muscle cultures consist of large numbers of replicating mononucleated cells, and small numbers of postmitotic mononucleated myoblasts and oligonucleated myotubes. The rapid retraction induced by CB is illustrated by comparing Figs. 1 and 2, the latter being a micrograph taken 15 min after the addition of CB. The dendritic condition of the cells after 15 min in CB is due to the fact that local regions along the original margins of the spread-out cells remain anchored to the substrate while the intervening cell membrane and cytoplasm are displaced centripetally. This results in a gradual rounding up of the central region of the cells, leaving behind arms and processes of varying shapes, lengths, and widths. After 60 min in CB, most cells exhibit an overall bipolar or tripolar configuration with a conspicuously rounded center containing the nucleus. This rounding up continues slowly, so that by the 3rd or 4th h approximately 40% of all the mononucleated cells are almost completely round and are attached to the substrate only by modest numbers of fine (circa 0.5 μm) long processes. Over the next several hours many of these processes break, and

[1] Croop, J., and H. Holtzer. manuscript submitted for publication.



FIGURES 1 and 2 Micrographs from one 3-day-old culture demonstrating the rapid retraction of cells in CB. Fig. 1 is a typical field showing the cells before the addition of CB. Fig. 2 is from the same culture 15 min after the addition of CB. The dendritic appearance is in large part due to the unequal retraction of cytoplasm and plasma membrane from the former boundaries of the spread-out cells. The tips of the dendritic processes are firmly attached to the substrate. They mark the original extent of the pretreated cell. Their uneven distribution probably reflects the fact that only at certain points do the margins of the fibroblastic cells adhere tightly to the substrate. Presumably these dendritic processes consist of a core of cytosol of varying thickness, surrounded by plasma membrane. Changes similar to these have recently been described by Miranda et al. (32) using HeLa, Vero, HEp^2 and MDBK cells and cytochalasin D.

the now completely rounded cells form loose aggregates with other similarly detached cells. In the next 48 h many, though not all, of these round cells (Fig. 3) begin to contract spontaneously, and when viewed under the EM are seen to contain large numbers of thick and thin filaments (20). These beating, round cells are postmitotic myoblasts (15) and they render untenable the claims that CB blocks the synthesis, polymerization, or activity of actin (47).

The 60% of the cells that do not detach from the substrate go on to form the fully arborized cells and cells that tend to be bipolar. The latter constitute approximately 10% of this adherent population and will not be further discussed in this paper. Figs. 4–8 illustrate the variation among the fully arborized cells. The nuclei in the central, perikaryon-like region are often difficult to see due to the thickness of that area and to the presence of vacuoles. A variety of microspikes and finer processes decorate the elongated arms. Limpid cytoplasmic extrusions, tightly adherent to the substrate, may occur anywhere along the periphery of these cells, but are found most commonly at the tips of the extended arms. Cells that have been in CB for 40 h exhibit processes that are longer in comparison to their state in the dendritic condition, indicating cytoplasmic flow in the presence of the drug. The limpid areas are exceedingly thin, have a low contrast, and sieve out all visible cell organelles and inclusions. Though simulating the morphology of a ruffled membrane, these regions do not display the active undulations characteristic of a true ruffled membrane. The nodules on the fine processes may swell or shrink and frequently the entire beaded process is severed from the cell. These bizarre cells will reacquire a fibroblast morphology within 3 h after they have been

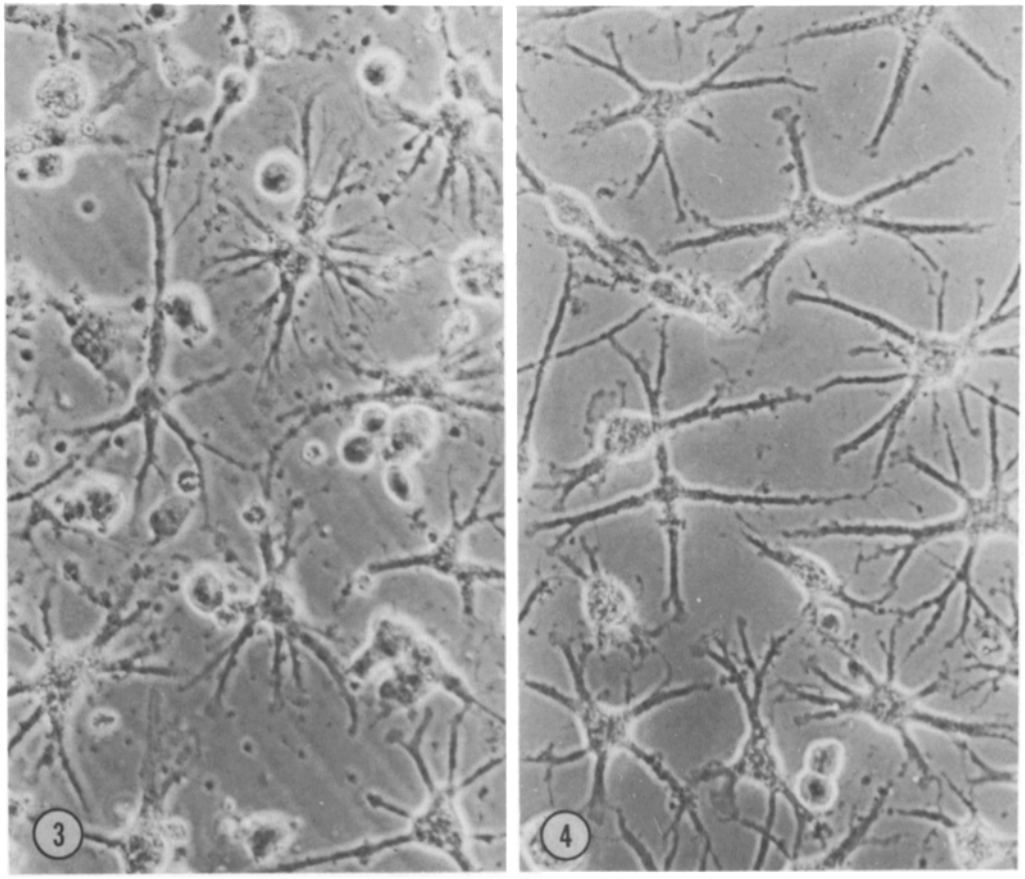


FIGURE 3 Low-power micrograph of a living 4-day-old culture under CB for 48 h. Observe the large number of round cells that are no longer attached to the substrate. In the next 24–48 h many of these round cells will begin to contract spontaneously.

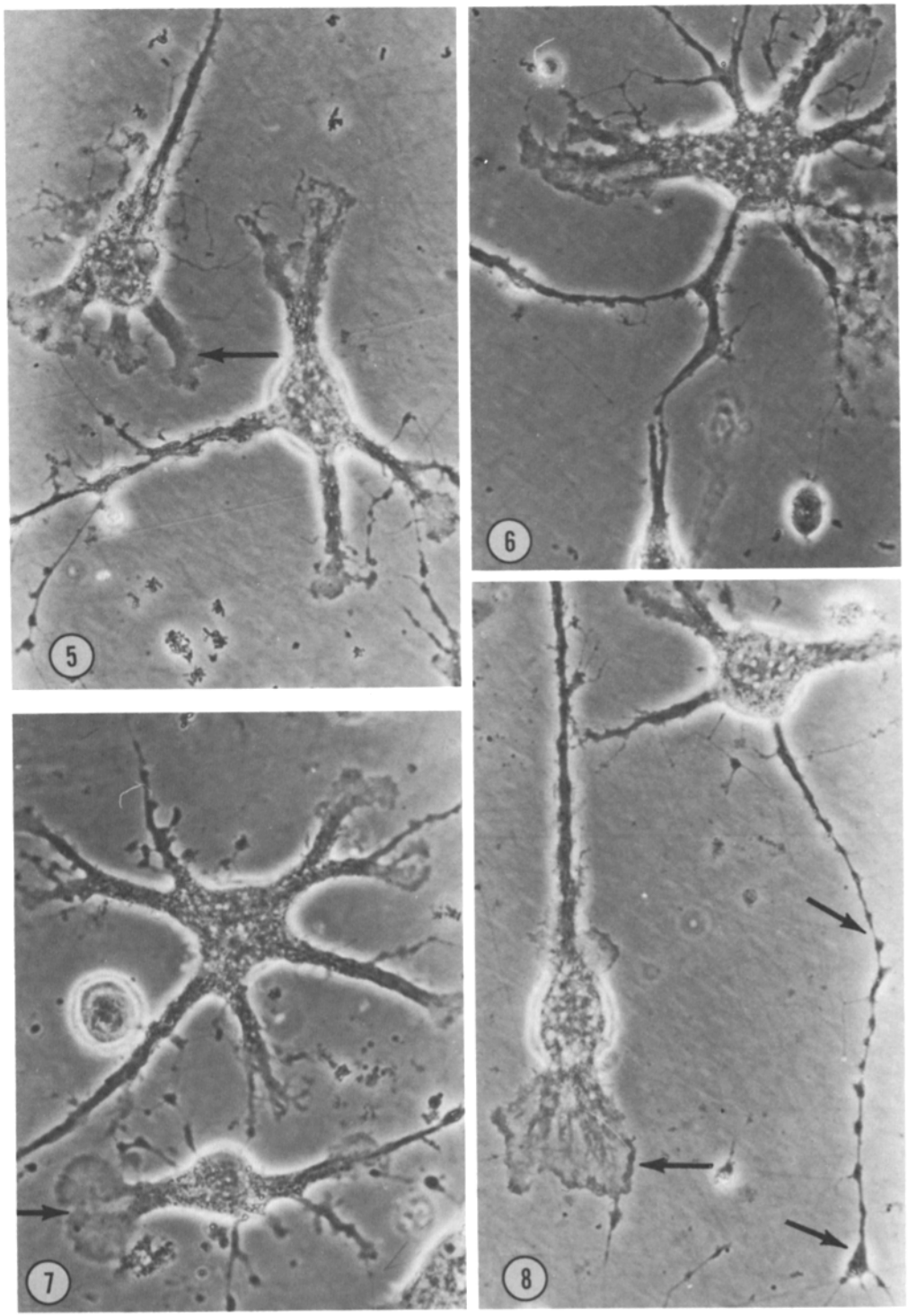
FIGURE 4 Low-power micrograph of a living 4-day-old culture under CB for 48 h. Many of the arborized cells are bi- and trinucleated. The arborized cells have never been observed to contract. Before photography the round cells present in the culture were removed with a pipette.

removed from CB. Pretreatment with cycloheximide for 1 h before the removal of CB did not delay transition to the fibroblastic state. On the other hand, in the presence of NaCN, these cells required 6–8 h for the transition to the fibroblastic condition.

Many thousands of arborized cells have been observed under the phase microscope and not once has one been observed to contract. Arborized cells do not bind fluorescein-labeled antibodies to myosin or tropomyosin, nor do they display thick or thin filaments under the EM (20). If, however, the CB is removed from the arborized cells, within 24–72 h many of these cells will replicate and fuse to form perfectly normal multinucleated myotubes. These findings differ from those reported by Sanger (38).

Since nuclear division, but not cytokinesis, occurs in many of these cells (24, 39), bi- and trinucleated myogenic and fibrogenic cells are common. The ratio of mono- to bi- to trinucleated cells varies greatly depending upon the age of the cultures at the time CB was added and upon the frequency of feeding. Evidence for the fact that arborized cells even in CB can give rise to round cells has been presented elsewhere (15).

4-day-old muscle cultures, in contrast to 3-day-old cultures, consist of a dense mat of branched, immature, multinucleated myotubes interspersed with large numbers of replicating mononucleated



FIGURES 5–8 Micrographs of four fully arborized cells. These cells may be mono-, bi-, or trinucleated. The black horizontal arrows point to the limpid, extruded areas that often assume a fanlike contour and may best be described as immobile, ruffled membranes. The slanted arrows indicate the branched, nervelike processes; often such processes fragment and form small spheres. One such small sphere is shown in the lower right of Fig. 6. A round, mononucleated myoblast is shown in Fig. 7.

cells. Modest numbers of cross-striated myofibrils are present in most myotubes. The collagen substrate is obscured by this dense sheet of cells (Fig. 9 *a*). The cells are attached to each other and to the collagen. Cell-to-cell adhesion is indicated by the observation that the cells can be lifted with forceps as an intact sheet from the petri dish. 15 min after CB is added to these cultures, sizeable areas of the collagen substrate are exposed, owing to the fact that myotubes and mononucleated cells retract and separate from one another and from the substrate as they round up. In contrast to the many mononucleated cells that go on to arborize, all of the myotubes completely retract from the substrate, round up, and after 10 h are suspended in the medium as grapelike clusters, anchored here and there by fine, long processes that eventually break.

In summary, all cells respond to CB by retraction within the first 15 min. Thereafter, however, there is a differential response in the mononucleated population so that by 10 h approximately 40% of the cells are quite rounded and loosely attached to the substrate, and 60% are dendritic and firmly attached to the substrate. The round mononucleated cells are postmitotic myoblasts. Those cells that remain adherent to the substrate go on to form fully arborized cells and include both replicating myogenic and fibrogenic cells. Within 24 h all the immature myotubes will also be dislodged from the substrate and found floating in the ambient medium. It will be interesting to learn why CB arborizes replicating myogenic and fibrogenic cells, but induces postmitotic myogenic cells to round up.

Rapid Effect of Colcemid Alone, and of Colcemid Combined with CB, on Mononucleated Cells and Myotubes

The effects of Colcemid on the mononucleated cells and the myotubes are quite different from those of CB. Colcemid induces the elongated, spindle-shaped mononucleated cells to assume a more flattened, disklike configuration: this change is first noticeable in about 2 h and is complete in about 15 h. After 5 or 6 h, round detached cells begin to accumulate in Colcemid-treated cultures, but these are metaphase-arrested cells and are readily distinguished from the round cells induced by CB. Fig. 10 is a micrograph taken with a polarizing microscope of a 5-day-old culture in Colcemid for the previous 24 h. The flattened interphase cells adherent to the substrate are not apparent in this micrograph. Note the birefringent bands in the round, metaphase-arrested cells. These birefringent bands may assume the simple shape of a circle, or, equally common, a more complex dumbbell or figure-eight configuration. By careful focusing, a negative image of these birefringent bands can be observed in the phase microscope, owing to the fact that all inclusions are excluded from their domain. Ishikawa et al. (see Fig. 8 in reference 27) demonstrated that these birefringent bands are bundles of 100-Å filaments. These metaphase-arrested cells do not bind fluorescein-labeled antibodies against myosin or tropomyosin and do not display thick or thin filaments under the EM (19, 24, 27, 34). It therefore follows that neither thick nor thin filaments contributed to the birefringence illustrated in Fig. 10.

Clear bands are also induced by Colcemid in replicating, interphase myogenic and fibrogenic cells (Fig. 11 *a–c*). These bands are easier to observe under the phase microscope in interphase than in metaphase cells, but this may be due only to the flattened condition of the interphase cell.

The effect of Colcemid on myotubes is also to be contrasted with the effect of CB. Colcemid collapses the elongated myotubes (17, 24). The transformation of anisodiametric myotubes into isodiametric myosacs is associated with the depolymerization of microtubules (27). The myosacs remain adherent to the collagen substrate in contradistinction to being dislodged from the substrate in CB. Clear areas develop between the nuclei in these myosacs. The clear areas are positively birefringent with respect to their long axis and correspond to the lakes of 100-Å filaments previously described (24, 27). More detailed observations regarding the birefringence of these clear bands in myosacs are complicated by the fact that some of the birefringence is probably due to the myofibrillar material also present in these regions.

The response of 3-day-old muscle cultures to CB and Colcemid applied *together* is different from the response to either alone. Retraction of both mononucleated cells and myotubes was observed, but the retraction was less vigorous and the cells never appeared as dendritic as in CB alone. The most novel feature of the action of the two agents together was the rapid accumulation of cell debris between the contracting cells. This debris results from the amputation of the fine dendritic processes induced by CB. These sloughed-off cytoplasmic processes form opaque droplets ranging up to 3

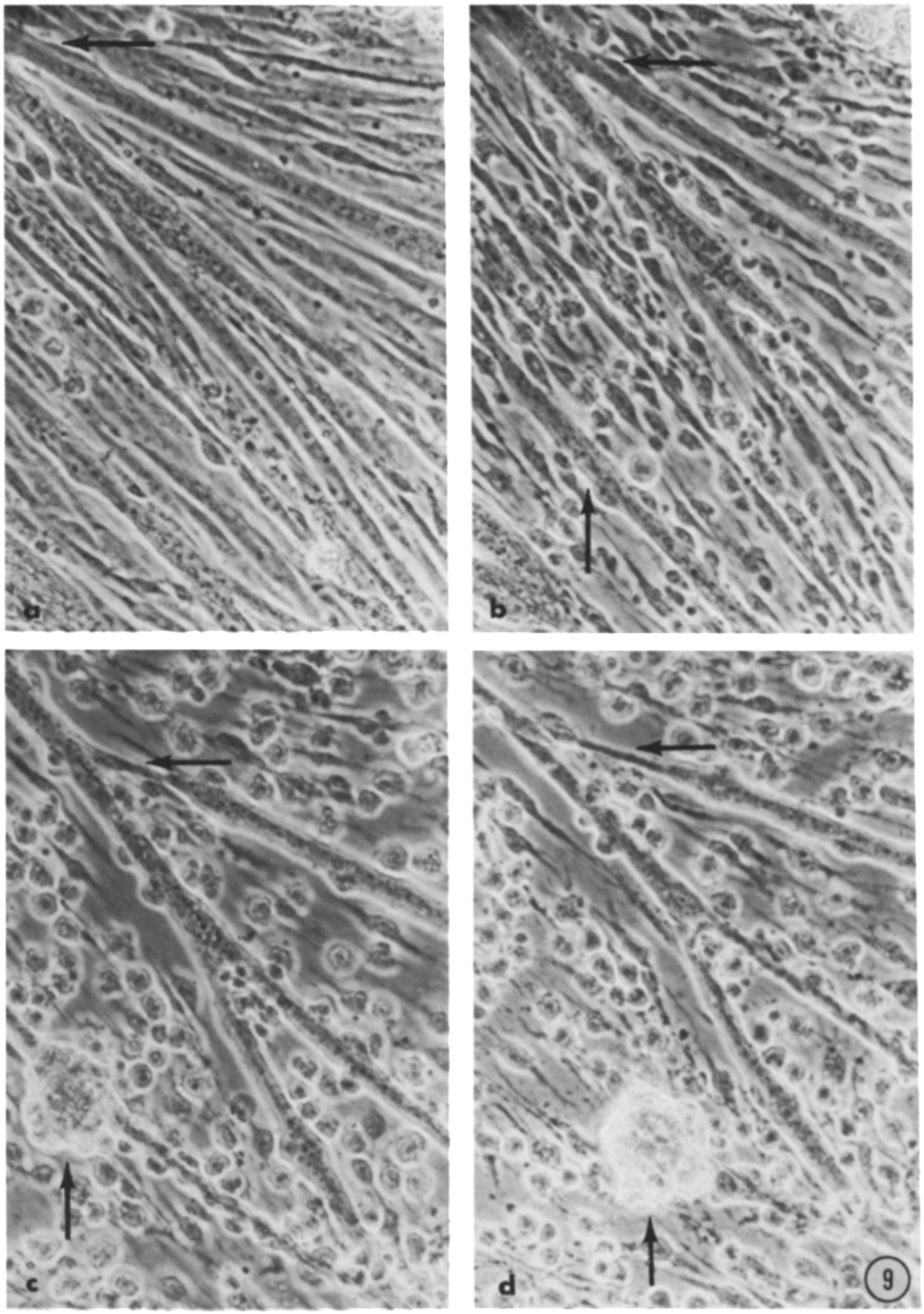


FIGURE 9 *a–d* Low-power micrograph of a living 4-day-old culture which demonstrates the relatively rapid retraction of mononucleated fibroblastic cells and of multinucleated myotubes induced by CB. Fig. 9 *a*: note the several elongated, branched, multinucleated myotubes and how the flattened fibroblastic cells carpet the collagen substrate. Fig. 9 *b*: 15 min after the addition of CB, virtually all the fibroblastic cells have retracted, exposing large areas of the substrate. Fig. 9 *c* and *d*: 5 and 8 h, respectively, after CB was added. Arrows indicate the retraction of myotubes. The vertical arrows in Fig. 9 *b–d* illustrate how a single myotube gradually retracts, balls up, and, within 8 h, detaches from the substrate. Note in Fig. 9 *c* that not all of the myotube has contracted into the grapelike cluster indicated by the vertical arrow. Within the next 3 h, as shown in Fig. 9 *d*, the entire myotube has completely retracted from the substrate. By 10 h all myotubes in this culture have retracted and formed detached clusters similar to that indicated in Fig. 9 *d*.

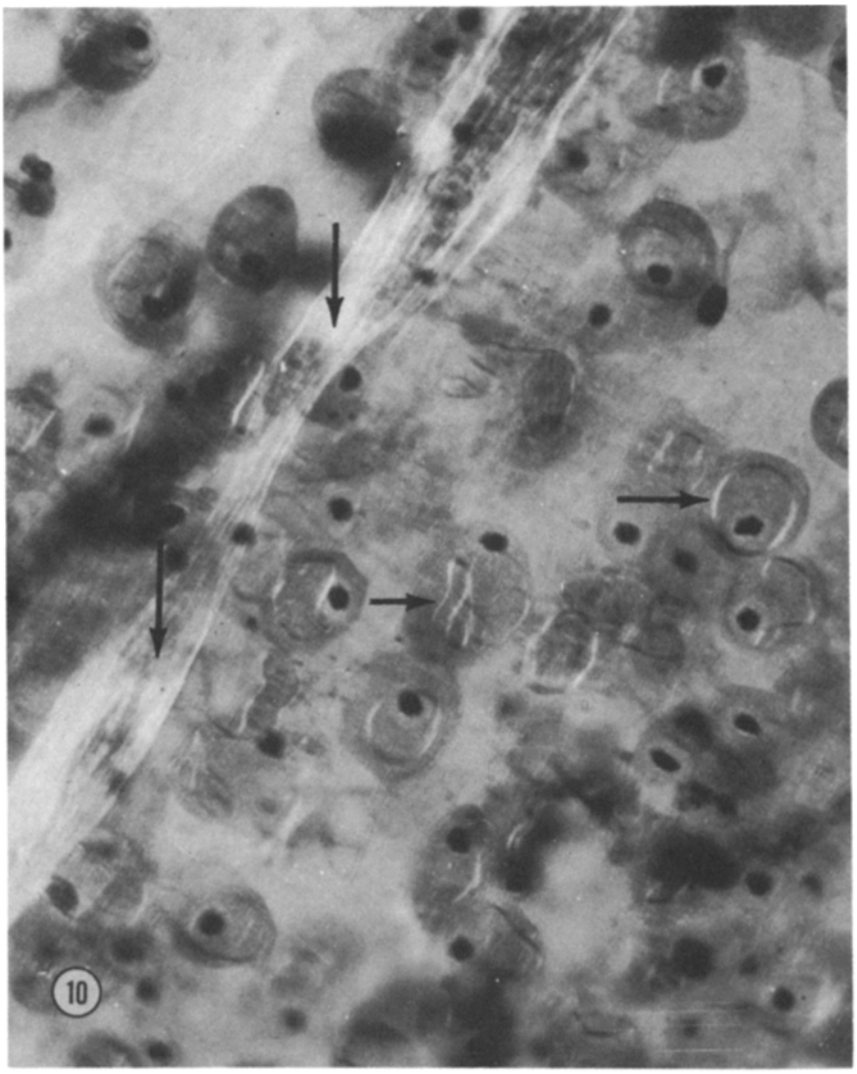


FIGURE 10 A 5-day-old muscle culture in Colcemid for the last 24 h viewed through the polarizing microscope. Each of the metaphase-arrested cells is readily identified by its dark pycnotic nucleus. Observe that each of these cells displays a conspicuous, generally continuous, positively birefringent band (see horizontal arrows). Under the EM these metaphase-arrested cells do not exhibit any cytoplasmic thick myosin or thin actin filaments, and they have been shown to consist exclusively of 100-Å filaments (Figs. 8–10 in reference 27). Vertical arrows point to a myotube that is transforming into a myosac.

μm. Often within 30 min a single cell will shed a string of three or four droplets. These droplets eventually separate as the connecting thread of cytoplasm between them thins and breaks. After 30 h in the combined drugs most cells are moribund and only a minority survive if placed in normal medium.

Response of CB-arborized Cells to the Addition of Colcemid

Fig. 12 *a–d* illustrates the sequence of changes induced by the addition of Colcemid to fully arborized cells. It is to be stressed that in these experiments the Colcemid is added to medium

already containing CB. Within 1 h after adding the Colcemid, the arborized cells begin to fragment. The long arms of the arborized cells transform into beaded structures. With time the nodes enlarge, presumably by the displacement and accumulation of cytoplasm contained in the intervening connecting regions, until the attenuated processes break. Cells that have lost a considerable portion of their cytoplasm by this shedding are still viable. If CB and Colcemid are removed after 15 h these cells will reacquire their fibroblastic morphology.

Many investigators have reported that changes in cell shape are correlated with the presence of intact microtubules and that changes induced by Colcemid may be mimicked by cold (26, 33, 35, 46). Accordingly, arborized cells were placed in the cold (4°C). Essentially the same sequence of events depicted in Fig. 12 *a–d* occurred in the cold, except that approximately 48 h rather than 2 h were required to fragment the arborized cells to the same degree.

The effect of Colcemid on arborized cells and the finding that cold approximated this effect led to the testing of the proposition that the acquisition of the arborized state is dependent on the integrity of microtubules. Accordingly, cells were pretreated with Colcemid for 1 h before they were exposed to CB. Though the early phase of retraction was observed, cells pretreated with Colcemid never arborized. There is, however, a deleterious effect when both agents act together, and after 30 h many of these cells are moribund.

From these experiments we conclude that a

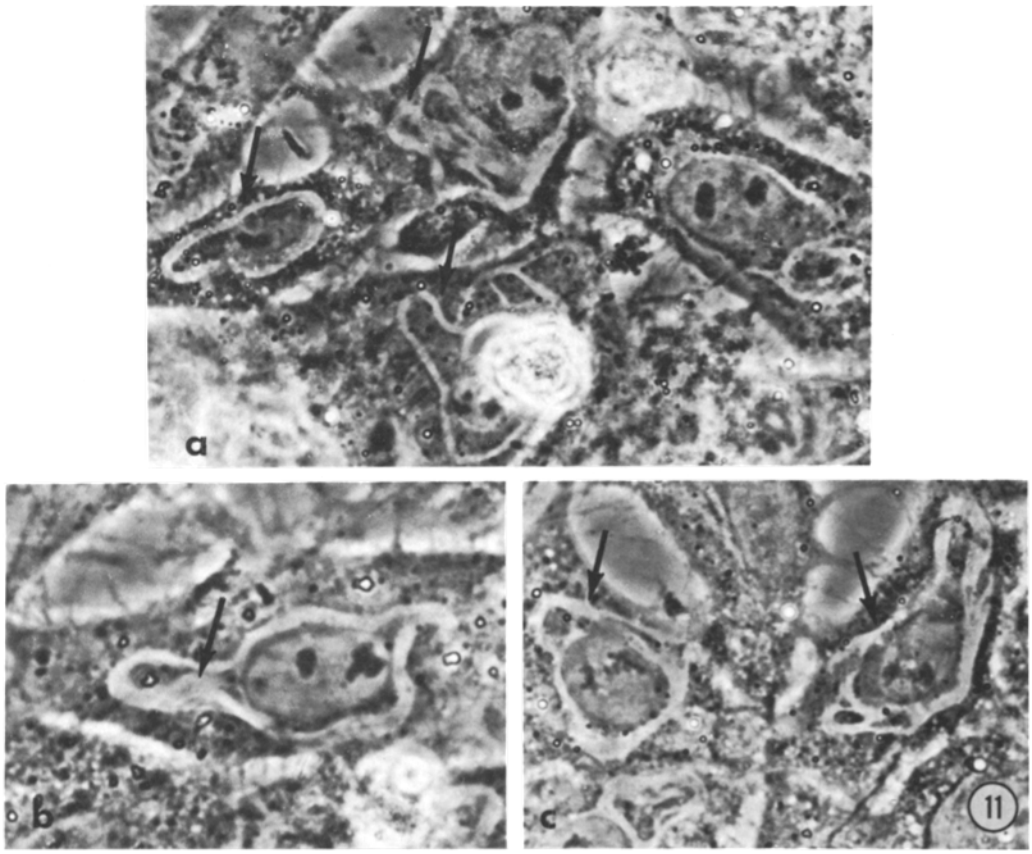


FIGURE 11 *a–c* Micrographs of clusters of living fibroblastic cells from a 4-day-old culture that has been in Colcemid for the last 20 h. Note the clear, channel-like regions that generally encircle the nuclei (arrows). These clear regions are positively birefringent and prove to be rich in 100 Å filaments (21). Observe how the diameter of these bands varies.

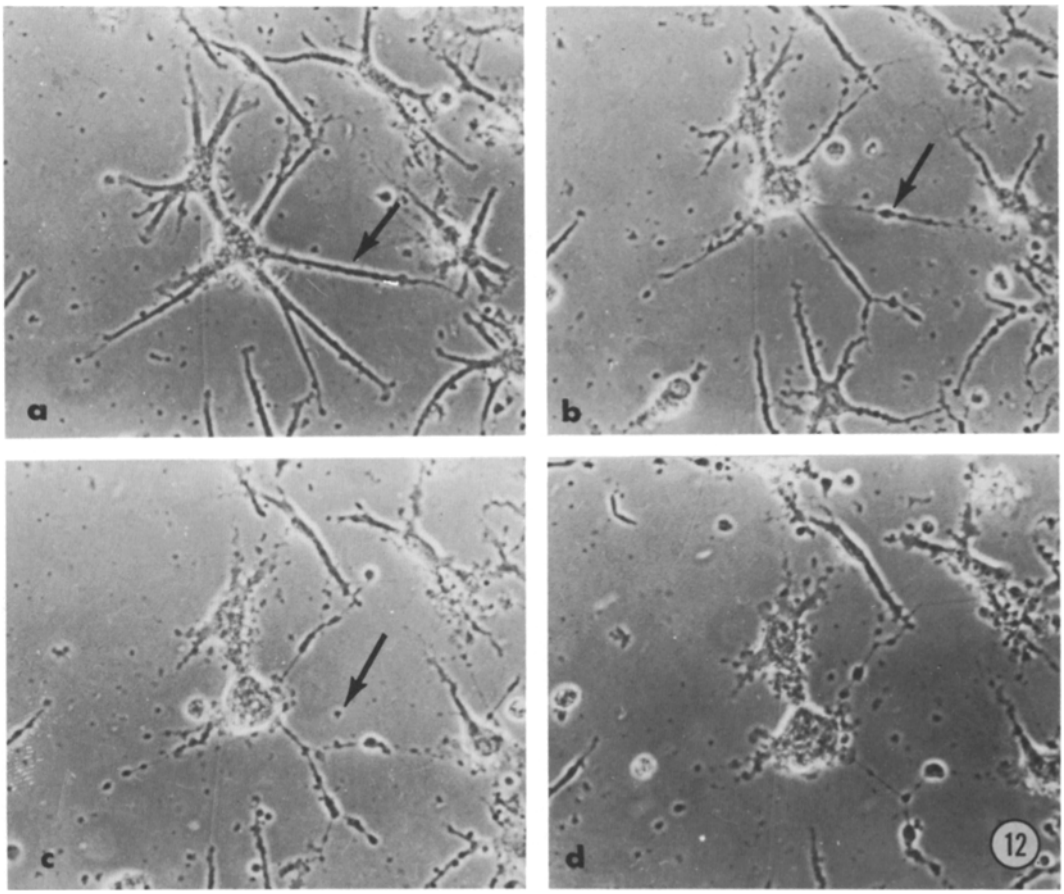


FIGURE 12 *a–d* Micrographs demonstrating the response of a fully arborized cell to Colcemid. Fig. 12 *a* illustrates an arborized cell before Colcemid was added to the medium. This particular arborized cell happened to be trinucleated, two nuclei located in the lower, one in the upper, hublike, region. Figs. 12 *b*, 12 *c*, and 12 *d* were taken 45 min, 80 min, and 130 min, respectively, after the addition of Colcemid. CB was present at all times. Observe how the arms radiating from the upper hub tended to shrivel before fragmenting into globules, whereas the lower arms converted into beaded structures that have a striking resemblance to nerve processes seen in vitro. Note that the process indicated by the arrow in Fig. 12 *b* disappeared by the time Fig. 12 *c* was taken. Virtually all the processes connecting the nodes shown in 12 *d* broke within the next 60 min. It will be interesting to determine whether these processes always contain a cytosol core, or whether they may be rods or droplets of pure "plasma membrane."

Colcemid-sensitive molecule is required to arborize cells and to maintain them in the arborized state.

Induction by Colcemid of Birefringent Bands in Cells Recovering from the Arborized State

In this series of experiments CB was removed from fully arborized cells and Colcemid added. These experiments differ from those described in the previous section in that the CB was removed when the Colcemid was added. Under these conditions the arborized cells did not rapidly "relax" to a fibroblastic morphology, but rather gradually assumed a disklike shape over a 5-h period. The periphery of these cells was rich in microspikes, but ruffled membranes never appeared. This disklike shape was not achieved by withdrawal or retraction of the arms of the arborized cells as much as by a general centrifugal redistribution of the cytoplasm of the cells. Viewed under the phase microscope after 20 h in Colcemid, the formerly arborized cells displayed conspicuous, tortuous,

clear bands. By 40 h these clear bands dominated the appearance of these cells as shown in Fig. 13 *a* and *b*. These bands, which at first may be confused with intracellular channels, were optically evident by exclusion of all cytoplasmic organelles. No Brownian movement was observed in these bands. Fig. 14 *a* and *b* demonstrates that these bands consist of positively birefringent material. It is worth stressing that virtually every cell in the culture displayed these conspicuous bands. In most cells the clear band forms one continuous, twisted, linear structure. Table I shows the total length of

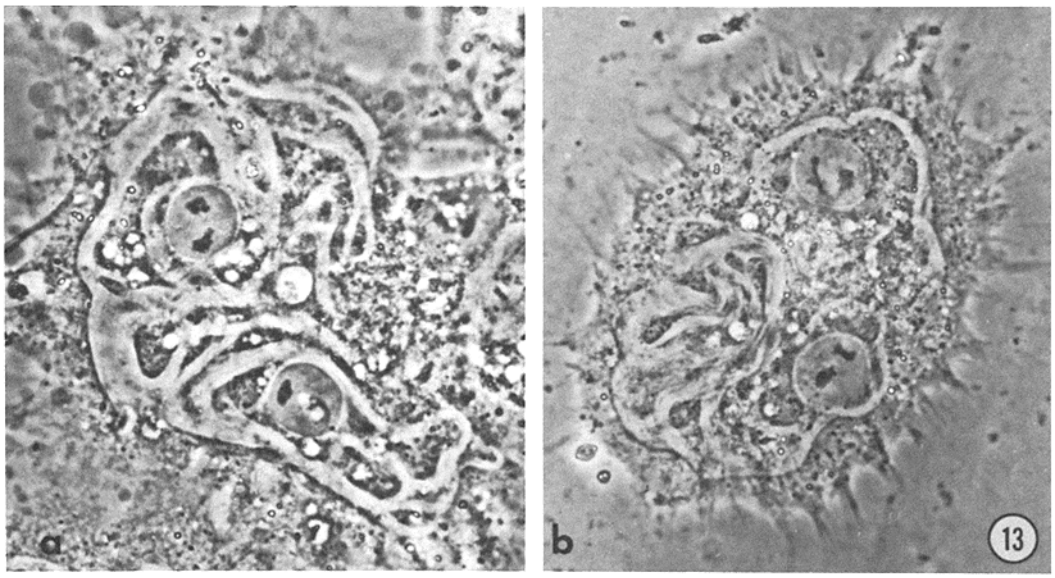


FIGURE 13 *a* and *b* Two formerly arborized cells that have been removed from CB and allowed to relax in Colcemid for 40 h. Both cells are binucleated. The twisting, continuous clear bands are quite prominent. It is to be stressed that virtually every cell in the dish exhibited these convoluted clear bands.

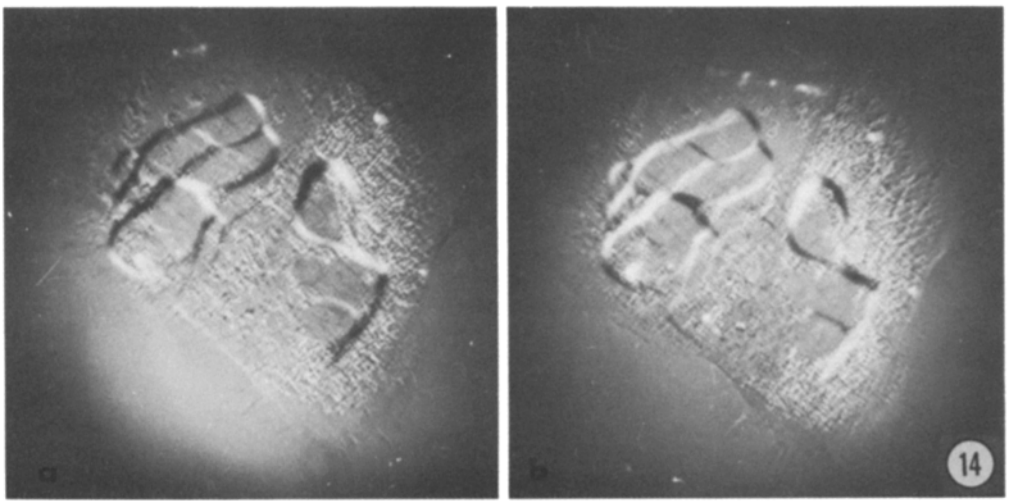


FIGURE 14 *a* and *b* Micrograph taken under the polarizing microscope showing that the clear bands are birefringent. Electron micrographs of these bands demonstrate that they are made up exclusively of 100 Å filaments (21). (We are indebted to Dr. H. Sato, Department of Biology, University of Pennsylvania, Philadelphia, Pa., for taking these photographs.)

TABLE I

Clear-Band Lengths of Cells Which Have Been Arborized and Allowed to Relax in Colcemid

Cell no.	Cell size	Length of clear bands
	cm	*cm*
1	7 × 4.9	39
2	5.25 × 5.25	44
3	8 × 5	67

The long and short diameters and the length of the intracellular clear band were measured in three different cells. The lengths were determined by running a road map calculator over standard micrographs. Lengths in centimeters were taken directly from calculator.

the clear-band in three different cells that had been arborized and then allowed to relax in Colcemid. Accurate estimates of the volume of the clear-band are yet to be made; nevertheless a conservative approximation of ±20% will not be far off.

In another series of experiments CB-arborized cells were placed in normal medium along with cycloheximide. The emergence of the clear bands occurred at the same rate and to the same extent as that in cells grown in the absence of the inhibitor of protein synthesis. Elsewhere we have shown that these clear bands consist of massive cables of 100-Å filaments (20, 21). In most cells the clear band forms one continuous, twisted, linear structure.

DISCUSSION

Any useful theory regarding the target(s) of CB must account for the early tonic-like retraction of the mononucleated cells and of the immature myotubes. Such a theory must also account for the finding that after 2 days in CB some cells have arborized and are immobilized, whereas others are round, postmitotic, have organized hexagonal arrays of thick and thin filaments, and contract spontaneously.

The notion that the microfilaments subtending the cell surface have a function analogous to that of the filaments in skeletal muscle is an attractive one. The finding that CB inhibits some kinds of "contractile" activities and concurrently dismantles the microfilaments in some kinds of cells suggests that there may be some correlation (43, 47). Nevertheless, there is much to be worked out regarding the composition, the shifting localization of the microfilaments, and the manner in which they might function in different physiological states. For example, it is by no means certain that the arrowhead complexes which appear after treatment with HMM are, in fact, (*a*) all, or (*b*) only a small fraction of, the *pre-existing* 60-Å microfilaments. As originally stressed by Ishikawa et al. (28) and Holtzer et al. (24), and recently confirmed by Chang and Goldman (11), after treatment with HMM the cell appears richer in total numbers of filaments than do controls not treated with HMM. This finding raises the possibility that HMM may be polymerizing actin monomers not normally part of the microfilament network, and that to this degree many of the *in situ* observed decorated filaments may be "artifacts." That more actin monomers may be present in a variety of cells than is indicated by microscopic inspection of the numbers of microfilaments is consistent with the findings that in embryonic nerve (7) and cartilage (25) as much as 10% of the total protein in the cell may be actin or an actin-like protein.

But even more perplexing is the manner in which an agent that supposedly dismantles microfilaments induces cell *retraction*. The most simple analogy with skeletal thick and thin filaments, and the one most discussed in the literature, leads to the prediction that CB, by dispersing contractile filaments, should produce cell relaxation or extension or cell immobility: this indeed is, in part, the effect of CB on cell cleavage (see, however, 24, 30, 39). On the other hand, the action of CB on mononucleated cells or on myotubes appears to be the converse of what is anticipated. CB should induce cell relaxation, the response which is in fact elicited by Colcemid, an agent not known to react with microfilaments or with actin in myofibrils. Given our current information, it is difficult to propose a sliding filament model which links the dispersion of microfilaments to the early retraction that is observed with CB. Indeed the data presented in this paper are consistent with the notion that some of the microfilaments may function as *skeletal* elements.

Some of the contradictory reports on the effects of CB almost certainly are due to the concentration used, to the duration of the exposure, and, most importantly, to the kinds of cells being monitored. This was clearly demonstrated in the present work. There are three major categories of cells in our cultures: (*a*) replicating fibrogenic cells, (*b*) replicating myogenic cells, and (*c*) postmitotic

myoblasts and myotubes. It is clear that all three types exhibit the rapid response to CB. However, we believe it most interesting that postmitotic cells organizing thick myosin and thin actin filaments into hexagonally-stacked arrays prove to be resistant to the arborizing effects of CB, whereas both replicating fibrogenic and myogenic cells, which lack thick and thin filaments, do arborize (15, 20, 21, 23). There is much circumstantial evidence suggesting that terminally differentiating myogenic cells have cell surfaces with properties different from those of cells in earlier compartments of the myogenic lineage (2, 19, 24). It will be important to learn if this differential response is in fact due to the absence of CB-sensitive molecules in the cell surface of terminal myogenic cells (e.g., the sugar transport system discussed in references 12, 33, 49).

Many kinds of cells tend to assume a more isodiametric configuration in Colcemid. This is consistent with the notion that microtubules function as skeletal elements (46). That CB-arborized cells treated with Colcemid "pull themselves apart" suggests that the arborized cells are under considerable tension. Whether this tension is generated by the microtubules acting to extend the cells internally, or whether tension is a surface phenomenon generated by the growth tips of the plasma membrane pulling the cells centrifugally, is not clear. Irrespective of how tension is generated and whether it resides in an "elastic" cell surface, or in the cytoplasm, or both, the fragmentation produced by the synergistic action of CB and Colcemid on normal cells, or by Colcemid on CB-arborized cells is not an event that would be predicted from what is known about the action of CB or Colcemid.

It has been known for some time that fibrogenic and myogenic cells arrested in metaphase by Colcemid display cables of 100-Å filaments (27). Inoué and Sato (26) have described birefringent bands composed of 100-Å filaments in Balb/c$^+$ renal tumor cells similar to the bands described in this paper. Goldman and Knipe (18) reported that BHK21 cells in Colcemid formed a juxtanuclear birefringent zone consisting of 100-Å filaments (see also 9, 48). Clearly, in many cells there are unexpectedly large quantities of a Colcemid-sensitive molecule that can be ordered into microscopically visible bands of 100-Å filaments even in the absence of protein synthesis (20, 21). Pending experiments quantitating the material in these bands, it is not yet possible to prove whether these bundles constitute another stable form of tubulin, intermediate between microtubules and its monomers. Similarly, without such quantitation it is also impossible to assess the basis for the greater prominence of the bands in cells that had been arborized and then exposed to Colcemid vs. bands in cells exposed only to Colcemid. The optically more evident bands in cells pretreated with CB might reflect the fact that often CB-treated cells are flattened and binucleated and hence have more cytoplasm, rather than indicate that such cells contain more 100-Å filaments per total DNA. In any case it will be worthwhile to learn of the way in which Colcemid-sensitive molecules function in maintaining the shape of cells and whether they participate in cytoplasmic motility, particularly whether the 100-Å filaments interact with the cytoplasmic actin that has been found in these and most other eukaryotic cells.

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