EFFECTS OF COLCHICINE, CYTOCHALASIN B, AND 2-DEOXYGLUCOSE ON THE TOPOGRAPHICAL ORGANIZATION OF SURFACE-BOUND CONCANAVALIN A IN NORMAL AND TRANSFORMED FIBROBLASTS

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ABSTRACT

The distribution of surface-bound concanavalin A on the membranes of 3T3, and simian virus 40-transformed 3T3 cultured mouse fibroblasts was examined using a shadow-cast replica technique with a hemocyanin marker.

When cells were prefixed in paraformaldehyde, the binding site distribution was always random on both cell types. On the other hand, labeling of transformed cells with concanavalin A (Con A) and hemocyanin at 37°C resulted in the organization of Con A binding sites (CABS) into clusters (primary organization) which were not present on the pseudopodia and other peripheral areas of the membrane (secondary organization). Treatment of transformed cells with colchicine, cytochalasin B, or 2-deoxyglucose did not alter the inherent random distribution of binding sites as determined by fixation before labeling. However, these drugs produced marked changes in the secondary (but not the primary) organization of CABS on transformed cells labeled at 37°C. Colchicine treatment resulted in the formation of a caplike aggregation of binding site clusters near the center of the cell, whereas cytochalasin B and 2-deoxyglucose led to the formation of patches of CABS over the entire membrane, eliminating the inward displacement of patches observed on untreated cells. The distribution of bound Con A on normal cells (3T3) at 37°C was always random, in both control and drug-treated preparations.

Pretreatment of cells with Con A enhanced the effect of colchicine on cell morphology, but inhibited the morphological effects of cytochalasin B. The mechanisms that determine receptor movement and disposition are discussed.

INTRODUCTION

Many physiological changes which arise when cells are transformed from a normal to a malignant state can be ascribed to alterations of membrane function. Thus, physiological manifestations of transformation include changes in contact inhibition of growth (1), cell shape (2), membrane transport (3–5), and surface-related phenomena such as agglutination by plant lectins (6–11). Trans-

formation is usually accompanied by increased agglutinability of cells by the lectin concanavalin A (Con A)[1] (9). Although it was originally hypothesized that the greater agglutinability of transformed cells might be associated with increased numbers or unmasking of "cryptic" Con A binding sites (CABS) (8, 12), the bulk of the available evidence suggests that this is not the case (13–16).

The relative agglutinability of cells by Con A and other plant lectins has, however, been correlated with the topographical (surface) distribution of bound Con A (17). Thus the CABS[2] on normal ("contact inhibited") fibroblasts are randomly dispersed, while virus-transformed cells have CABS gathered into clusters (17, 18). The density of CABS in these clusters apparently favors cross-linking of adjacent cells by the tetravalent lectin molecule, leading to cell agglutination. Recently it has been shown that the clustering of CABS on transformed cells is induced by the multivalent Con A itself, whereas the inherent binding site distribution on the cell surface is dispersed and random (18, 19). The ability of Con A to induce clustering of its binding sites is one characteristic of the transformed state.

In the course of our experiments we have observed that the CABS clusters themselves assume a characteristic distribution on the surface of the cell membrane, since they are not usually observed in the distal areas of the pseudopodia or on the peripheral area of the cell membrane. Thus two types of organization contribute to the induced topography of CABS on the SV3T3 cell membrane. We define a primary organization which corresponds to the clustering of CABS, and a secondary organization which refers to the spatial distribution of the clusters on the cell membrane. In transformed cells this secondary organization of CABS is stringently correlated with the clustering phenomenon, hence its relative contribution to physiologic properties is still uncertain.

[1] *Abbreviations used in this paper*: CABS, concanavalin A binding sites; Con A, concanavalin A; Con A/H, sequential treatment of cells with Con A and hemocyanin; DMEM, Dulbecco's modified Eagle's medium; PBS, Dulbecco's phosphate-buffered saline; PFA, paraformaldehyde; SV3T3, 3T3 cells transformed by SV40 virus.

[2] Since these surface elements are observed only after they have bound Con A, it is to be understood that CABS as used here refers to the Con A receptor complex on the cell surface.

We report here alterations of the secondary organization of CABS effected by colchicine, 2-deoxyglucose, and cytochalasin B which permit, to some extent, assessment of the contribution of primary as opposed to secondary organization to the physiological properties of cells treated with Con A. These experiments also provide evidence that the secondary organization of surface-bound Con A is modified by colchicine and other drugs that depolymerize microtubules and suggest possible mechanisms involved in the movements of CABS across the cell surface.

MATERIALS AND METHODS

3T3, and 3T3 cells transformed by SV40 virus (SV3T3) (generous gifts from Dr. Howard Green of the Massachusetts Institute of Technology, Cambridge, Mass.), were grown to confluency in plastic tissue culture flasks (Falcon Plastics, Division of Bioquest, Oxnard, Calif.). Cultures were incubated in 10% CO_2 in air. The culture medium was Dulbecco's modified Eagle's medium (DMEM) (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% heat-inactivated (56°C, 1 h) calf serum and 10% tryptose phosphate broth (Microbiological Associates, Inc., Bethesda, Md.), and containing 75 U/ml penicillin and 75 U/ml streptomycin. Cultures were examined periodically for mycoplasma contamination. Cells were removed from culture flasks with 0.25% trypsin, plated onto 15-mm glass cover slips contained in 35- × 10-mm Petri dishes, and allowed to grow for 36 h before use in any experiment. The cells were plated at low density, so that there was little cell-to-cell contact on the monolayers.

Solutions of colchicine (10^{-6} M, Sigma Chemical Co., St. Louis, Mo.), vinblastine sulfate (10^{-6} M, Eli Lilly and Co., Indianapolis, Ind.), Colcemid (10^{-6} M, Ciba Pharmaceutical Co., Summit, N. J.), 2-deoxyglucose (10 mM, Sigma Chemical Co.), and alpha-methyl-D-mannoside (50 mM, Pfanstiehl Labs., Inc., Waukegan, Ill.), were prepared in DMEM buffered with Tricine (Sigma Chemical Co.) to pH 7.4. Lumicolchicine was prepared as described by Wilson and Friedkin (20). Cytochalasin B (ICI Research Laboratories, Cheshire, England), (1 mg/ml), was dissolved as a stock solution in dimethyl sulfoxide and diluted to 10 μg/ml in DMEM for use in experiments. In control experiments it was shown that the low (1.0%) concentrations of dimethyl sulfoxide present in the final dilution had no apparent effect on the cells.

To mark the surface distribution of Con A, we employed the technique of Smith and Revel (21), using hemocyanin (*Busycon canaliculatum*) which binds to Con A molecules and is readily visible in conven-

tional shadow-cast replicas. The details of this technique have been described previously (18). Solutions of Con A (twice crystallized, Miles-Yeda Ltd., Miles Laboratories, Inc., Elkhart, Ind.), and hemocyanin were prepared in Dulbecco's phosphate-buffered saline (PBS) at pH 7.4.

In most experiments the cover slips were first incubated for 60 min in DMEM with Tricine, with or without drugs. After three serial rinses in PBS (20°C), the cover slips were then incubated in Con A (100 μg/ml at 37°C or 200 μg/ml at 4°C) for 10 min. The cover slips were again rinsed serially three times and incubated a further 10 min in hemocyanin (500 μg/ml at 37°C or 1.0 mg/ml at 4°C). In experiments where cells were treated with drugs, the same compound was present in the labeling solutions. In some cases the cells were fixed in 1% paraformaldehyde (PFA) (MC & B Manufacturing Chemists, Norwood, Ohio) in PBS (pH 7.4) for 10 min at 37°C after the drug treatment. In one type of experiment the cells were first labeled with Con A and hemocyanin (Con A/H) and then incubated for 60 min in DMEM with or without the addition of drugs.

Cells were fixed and prepared for electron microscopy as described previously (18). Replicas were examined in an AEI EM 801 electron microscope with an accelerating voltage of 60–80 kV.

[^{125}I]Con A was prepared by the method of Helmkamp et al. (22) and purified as described previously (18). Binding of [^{125}I]Con A was studied using the same conditions and concentrations employed in the morphological studies. Cover slips treated with [^{125}I]Con A were rinsed in PBS and the attached cells were dissolved in Lowry's solution A (23). Samples were taken for determination of protein (Lowry et al. [23]) and radioactivity by liquid scintillation.

ATP was extracted from the cells in 5% perchloric acid at 4°C. The extract was neutralized and ATP was measured employing a luciferin-luciferase preparation (Sigma), using the method of Addanki et al. (24).

RESULTS

Distribution of CABS on Normal and Transformed Mouse Fibroblasts

SV3T3: Monolayer cultures of SV3T3 cells contain a pleomorphic population of both rounded and fibroblast-like cells showing surface filopodia over much of the cell surface, but particularly concentrated over the central area of the cell, presumably the nuclear region. Large pseudopodia project from the lateral cell borders. As reported previously, the inherent distribution of CABS on these cells, seen when the cells are fixed in 1% PFA before Con A/H labeling, or when labeling is performed entirely at 4°C, is random (18). When clustering of CABS is induced by Con A, hemocyanin clusters cover much of the cell surface, but are absent from the distal aspects of pseudopodia and usually from the edges of the cells as well[3] (Fig. 1). To test whether this localization of the patches represented CABS *in transit* to another distribution, or whether it constituted the final distribution, we incubated the cells in DMEM for various periods of time after Con A/H treatment (before fixation). After 60 min of incubation in DMEM, there was no change in the pattern of CABS relative to controls (labeled and fixed immediately). Observation of the cells by phase microscopy showed that over a 20-min period at 37°C, with or without Con A, there was no noticeable movement of the cells nor any change in the shape of the pseudopodia. Since exposure of lymphocytes to low temperatures has been shown to affect the final distribution of CABS on lymphocytes (25), perhaps by causing depolymerization of microtubular protein (26), we studied CABS distribution as a function of temperature. When cells were treated with Con A and hemocyanin at 4°C and then warmed to 37°C for 10 or 60 min, the distribution of CABS was identical to that of cells labeled at 37°C (never having beeen cooled).

3T3: 3T3 cells treated with Con A/H with or without prior fixation show hemocyanin distributed in a random pattern over the entire cell membrane, including pseudopodia (18).

Effect of Colchicine on the Distribution of CABS

SV3T3: Colchicine (10^{-6} M, 60 min) had a striking morphological effect on the cells. They became rounded-up, and numerous long, stiff microspikes originated from a region of membrane replete with filopodia near the center of the cell (presumably the nuclear region). These microspikes protrude the plasma membrane and extend to the cell borders. When cells were treated with colchicine and fixed in 1% PFA before Con A/H treatment, the hemocyanin was dispersed. Thus colchicine did not change the inherent distribution of CABS. Monolayers that were pretreated with colchicine and labeled with Con A/H

[3] We have recently observed a similar distribution of Con A/H on *spontaneously* transformed baby hamster fibroblasts.

FIG. 1 Shadow-cast replica of an SV3T3 cell surface showing clusters of hemocyanin (arrows) after Con A/H treatment at 37°C. Clusters are *absent* from edges of cell (*E*) and pseudopodia (*P*). Direction of shadow is from bottom to top in all replicas. Bar = 1 μm. × 9,500.

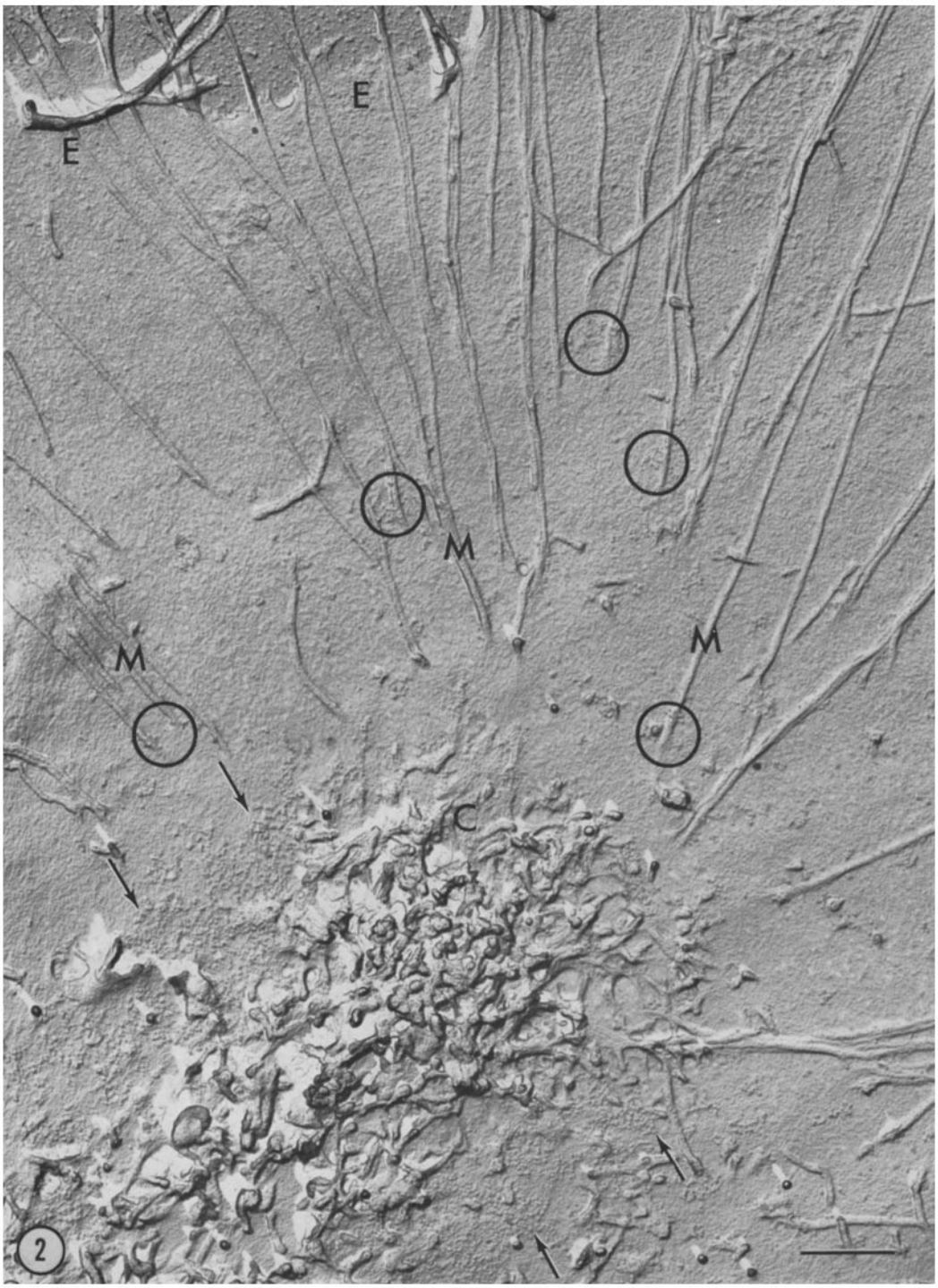

FIG. 2 SV3T3 cell treated with 10^{-6} M colchicine before Con A/H labeling. Microspikes (*M*) extend from the central area of the cell (*C*) past the cell edges (*E*). Large aggregates of hemocyanin (arrows) are seen in the center of the cell, and small clumps (circles) are often associated with the points of origin of the microspikes. Bar = 1 μm. × 13,000.

in the presence of the alkaloid showed no change in the primary organization of CABS (cluster formation), but the distribution of the clusters over the surface of the cell (secondary organization) was very different from that in control preparations (Fig. 2). Patches were found predominantly in the central area of the cell, often in large aggregates, and, in addition, often at the points of origin of the microspikes. Clusters were also preferentially associated with areas of presumed membrane activity such as filopodia. Overall, the CABS had moved centripetally, but the persistence of independent clusters distinguishes their organization from the tight "caps" of receptors formed on lymphocytes under similar conditions (27). Thus colchicine did not change the inherent distribution of CABS nor their primary organization (patches) but rather the ordering of patches on the membrane which we refer to as a second level of CABS organization.

Identical effects were seen when monolayers were treated with vinblastine sulfate (10^{-6} M), or Colcemid (10^{-6} M). However, SV3T3 cells treated with lumicolchicine (10^{-6} M), a photochemical derivative of colchicine that does not bind to microtubular proteins (28), showed normal morphology and the control distribution of hemocyanin patches.

Thin sections of SV3T3 cells revealed a network of microfilaments and microtubules subjacent to the plasma membrane (Fig. 3 *a*). Treatment of these cells with 10^{-6} M vinblastine for 60 min at 37°C resulted in the disappearance of most of the microtubules, while the microfilament network remained apparently intact (Fig. 3 *b*). In addition, profiles of the microspikes revealed them to be straight tubular projections of membrane above the cell surface which were filled with microfilaments.

Colchicine did not affect the time-course or kinetics of binding of [^{125}I]Con A to SV3T3 or 3T3 cells as determined on monolayers. The design of the labeling experiments did not permit comparison of the relative amounts of Con A bound to normal vs. transformed cells. The same amount (90%) of bound [^{125}I]Con A was removed from control and colchicine-treated monolayers by alpha-methyl-D-mannoside (50 mM), indicating that the alkaloid had neither induced nor depressed internalization of bound Con A, which does not occur to any great extent with these cells under any conditions. Also, colchicine does not affect the state of aggregation of the Con A subunits or Con A-saccharide interactions (25).

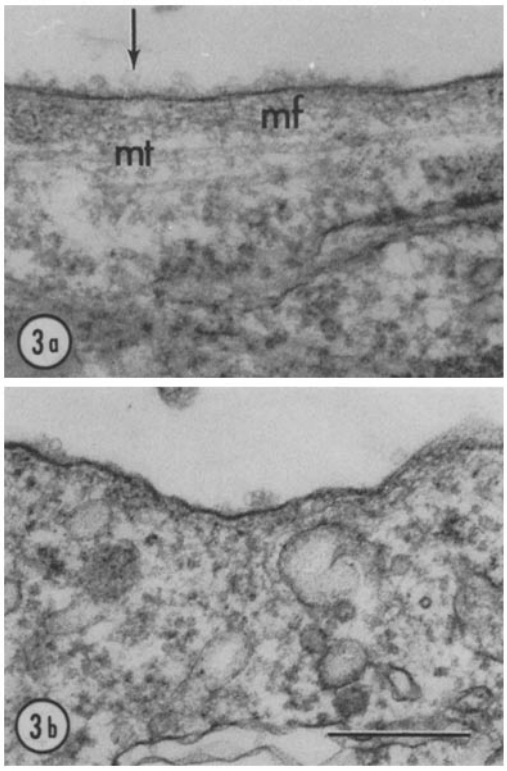

FIGURE 3 (*a*) Thin section of Con A/H-treated SV3T3 cell revealing a network of microfilaments (*Mf*) and microtubules (*Mt*) subjacent to the plasma membrane. Hemocyanin (arrow) is present on the cell surface. × 75,000. (*b*) SV3T3 cell treated with 10^{-6} M vinblastine before Con A/H labeling. Microtubules are absent. Bar = 0.25 μm. × 75,000.

3T3: The morphological effects of colchicine, vinblastine, and Colcemid in these cells were similar to those seen with SV3T3 cells. However, the hemocyanin was present in a random, dispersed pattern, indistinguishable from control monolayers.

Effect of Cytochalasin B on the Distribution of CABS

SV3T3: Incubation of SV3T3 cells in cytochalasin B (10 μg/ml) led to changes in the morphological appearance of the cells that were consistent with previous observations (29). Long pseudopodia terminating in stellate arborizations radiated from severely contracted cell bodies. In contrast to the centripetal distribution of

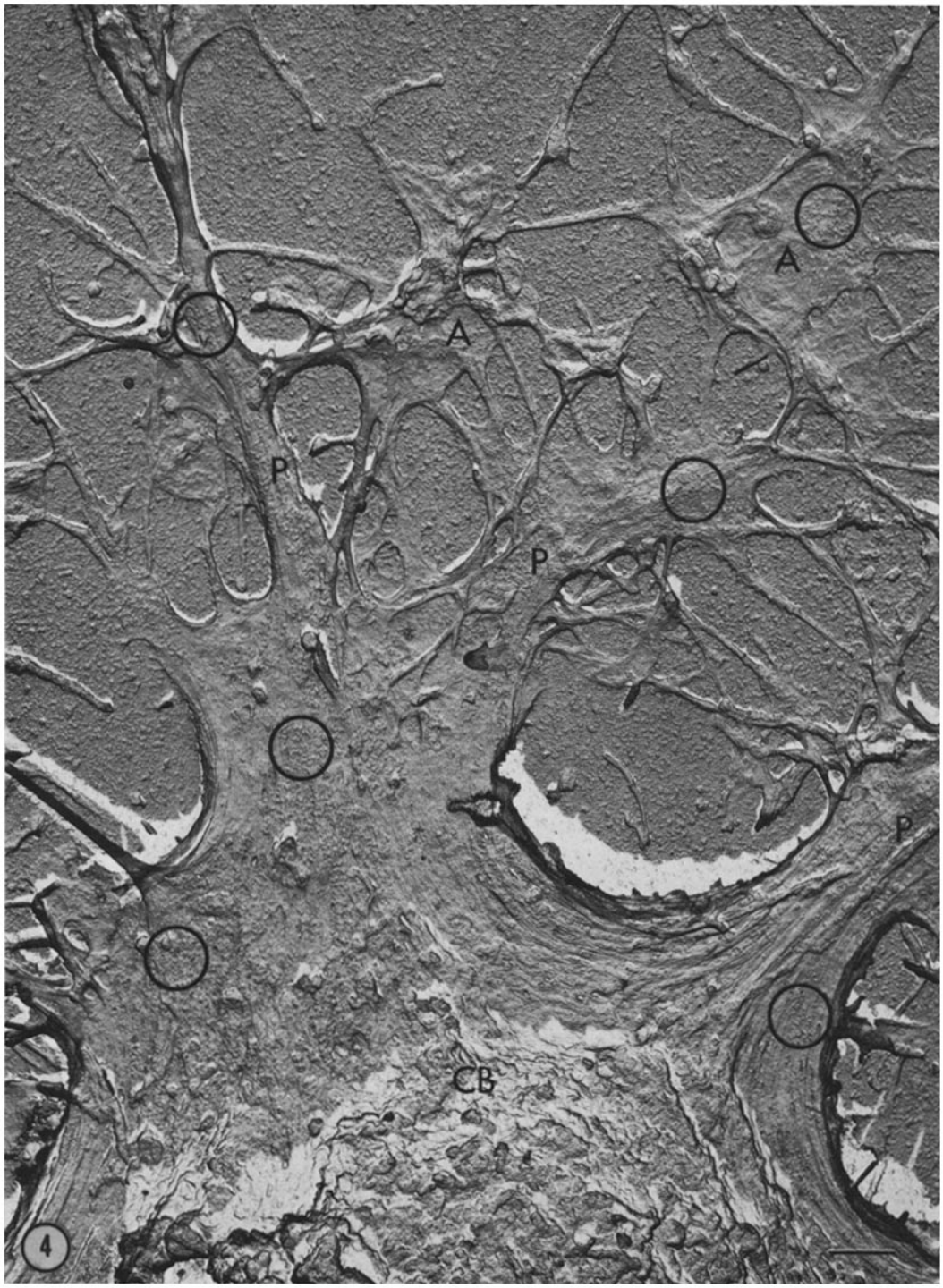

FIG. 4 SV3T3 cell treated with 10 μg/ml cytochalasin B before Con A/H labeling. Long pseudopodia (*P*) terminate in stellate arborizations (*A*) which radiate from a contracted cell body (*CB*). Clusters of hemocyanin (circles) are present near cell edges as well as within pseudopodia and arborizations. Bar = 1 μm. × 9,500.

patches on control cells, cytochalasin B-treated cells showed patches distributed over the entire cell surface including the long, stellate pseudopodia (Fig. 4). This effect was readily reversible. When cytochalasin-treated monolayers were washed and incubated in DMEM for 60 min before Con A/H treatment, the morphology and hemocyanin pattern was the same as in control SV3T3 monolayers. When cytochalasin B-treated cells were fixed before Con A/H labeling, the hemocyanin pattern was random. Cytochalasin has been shown to inhibit the membrane transport of various substances such as glucose (30–33). To test the possibility that the cytochalasin effect was mediated indirectly through inhibition of transport, monolayers were treated with cytochalasin in PBS (which contains no nonelectrolytes) instead of DMEM. The results of this experiment were identical with the previous one, for both control and cytochalasin-treated cells. Furthermore, an 80-min incubation in cytochalasin B caused no change in the intracellular levels of ATP, as previously reported (34).

3T3: Cytochalasin B produced the same changes in morphology seen in SV3T3 cells, without any effect on the distribution of hemocyanin on Con A/H-treated 3T3 cells.

Effect of 2-Deoxyglucose on CABS Distribution

To test the requirement for intracellular energy stores in determining the organization of CABS on the cell surface, we treated SV3T3 cells with 10 mM 2-deoxyglucose for 60 min before Con A/H. This procedure depleted the levels of ATP by 80%. The morphological appearance of 2-deoxyglucose-treated cells was very similar to that of cytochalasin-treated cells, and, as with cytochalasin-treated cells, the hemocyanin marker was found in patches distributed over the entire cell surface. When 2-deoxyglucose-treated cells were fixed before Con A/H labeling, the hemocyanin pattern was random.

Colchicine and Cytochalasin after Con A/H

COLCHICINE: Con A/H pretreatment did not noticeably alter the morphology of SV3T3 cells incubated for 60 min in DMEM buffered with Tricine. However, pretreatment of SV3T3 cells with Con A or Con A/H *enhances* the effect of colchicine on cell morphology. Con A/H-treated cells were incubated for various periods in colchicine (10^{-6} M) and compared with controls (PBS, colchicine). Pretreatment with Con A/H or Con A alone greatly accelerated the rounding-up of the cells caused by colchicine. Although a sufficiently long incubation (120 min) in colchicine resulted in rounding-up of virtually all the cells, Con A pretreated or not, the cells not pretreated with Con A took considerably longer to reach the final characteristic colchicine-induced morphology. At 60 min only 25% of the control cells were completely rounded-up, while 100% of the Con A-treated cells were rounded. This effect was related to the dose of Con A employed; 5 μg/ml had little effect and 20 μg/ml had an intermediate effect. When the experiment was repeated with 10^{-5} M colchicine, the morphological changes occurred more quickly in both control and Con A-treated cells, but the Con A-treated cells still rounded-up more rapidly than PBS controls. The final binding site distribution of Con A/H-colchicine-treated cells was similar to that seen on cells pretreated with colchicine.

CYTOCHALASIN B: In contrast, treatment of cells with Con A or Con A/H before incubation in cytochalasin B prevented much of the rounding-up of the cell body that is usually observed in cells treated with cytochalasin B. This inhibitory effect was also Con A dose dependent. The CABS distribution on Con A-cytochalasin B-treated cells was similar to that of control (Con A only) cells. Thus cytochalasin B does not cause the patches to move back to the peripheral areas of the membrane after they have migrated centrally as a result of prior Con A treatment.

DISCUSSION

Colchicine, cytochalasin B, and 2-deoxyglucose have been used to investigate the mechanisms involved in determining the surface topography of receptor-Con A complexes on normal and transformed fibroblasts. We have shown that: (*a*) none of the drugs affected the inherent random distribution of CABS; (*b*) none of the drugs affected the capacity of Con A to induce clustering of CABS (primary organization) on SV3T3 cells. However, all three drugs affected the topographical distribution of the induced clusters on transformed cells (secondary organization). The results of these experiments are summarized in Table I.

TABLE I

*Distribution of CABS on Fibroblasts at 37°C after Con A/H and Drug Treatment**

Drug	SV3T3 Cells	3T3 Cells
None	Clusters, withdrawn from periphery	Dispersed
None-prefix	Dispersed	Dispersed
Colchicine	Central cap	Dispersed
Colchicine-prefix	Dispersed	Dispersed
Cytochalasin B	Random clusters	Dispersed
Cytochalasin B-prefix	Dispersed	Dispersed
2-Deoxyglucose	Random clusters	Dispersed
2-Deoxyglucose-prefix	Dispersed	Dispersed

* Cells were treated with drugs and labeled with Con A/H in the presence of the same drug, as described in the text. In "prefix" experiments cells were treated with drugs and fixed in 1% paraformaldehyde before ConA/H labeling.

The manner by which CABS patches reach their final distribution on the membrane is unknown, and is obviously a complex process. Data from several laboratories have suggested that the distribution and mobility of surface receptors may be controlled by cytoplasmic structures. Berlin and Ukena (35–37) and more recently Edelman et al. (25) have proposed that the mobility of cell surface receptors is regulated by a colchicine-sensitive assembly somewhere in the vicinity of the plasma membrane. The distribution of receptors, therefore, is presumably related to the state of a "colchicine-binding protein" which may correspond to the microtubule system of the cell (38).

The effects of colchicine on the secondary organization of CABS in our system are consistent with, but do not constitute proof of, a role for microtubules in regulating mobility of surface receptors. In addition, variables such as cell motility, the microfilament network, local membrane movements, and the state of fluidity of the membrane per se must be considered in any model of receptor disposition, yet their respective roles have not been fully clarified at present.

Diffusion and Membrane "Activity" as Determinants of CABS Topography

SV3T3 cells observed by phase-contrast microscopy do not appear to move or change their general shape during the Con A/H labeling period. Therefore, the observed secondary CABS topography must result from a redistribution of CABS on the cell surface that is not related to gross cell movement. In this regard Ryan et al. have recently shown that cell movement does not affect the formation of a single compact aggregate of bound Con A (capping) on human polymorphonuclear leukocytes, but rather the final location of the capped Con A (39). Caps were found at the trailing edge of actively moving cells, but over the central area of nonmotile cells (39), the latter in a fashion similar to that observed on colchicine-treated SV3T3 cells shown here.

Mouse fibroblasts treated with antibodies to surface H-2 antigens showed a labeling pattern similar to the CABS topography on SV3T3 cells at 37°C (40). This organization of surface components was also prevented by metabolic inhibitors and low temperature, but, in contrast to our results with CABS, Colcemid treatment did not lead to cap formation in this system (40).

The translocation of CABS in control and alkaloid-treated SV3T3 cells may represent independent movement of CABS across the cell surface, perhaps accompanied by further cross-linking of CABS, or, alternatively, it could be caused by a bulk membrane flow (41) that carries CABS along. In the former case, the movement could be simply the result of diffusion, influenced by the mobility of CABS, and the size and density of clumps, as well as membrane activity such as "ruffling" or extension and retraction of filopodia. Of relevance to this latter possibility are the microcinematographic studies of Vasiliev et al. showing that Colcemid and vinblastine induce movements of normally stable areas of fibroblast cell membranes (42). In colchicine-treated SV3T3 cells, clumps were often found at the centrally located bases of the microspikes, suggesting that membrane activity influences their distribution.

Relation of Primary to Secondary Organization

Our results do not define whether cluster formation and migration of CABS occur separately or simultaneously. However, treatment of SV3T3 cells with cytochalasin B or 2-deoxyglucose prevents the usual migration of CABS clusters away from the cell periphery and out of pseudopodia, showing that CABS can be clustered at any point on the membrane. These data suggest that cluster

formation can be accounted for by local diffusion and the cross-linking properties of the multivalent Con A molecule. On the other hand, centripetal migration of CABS has not been observed in the absence of patch formation. For example, the ligand receptor complex on the 3T3 cell membrane is always dispersed and distributed over the entire membrane, even after colchicine treatment. Thus, cluster formation might trigger or facilitate centripetal movement of CABS, or at least be indicative of a membrane state which allows this movement of CABS.

Energy Requirement for CABS Movement – Possible Role of Microfilaments

The action of 2-deoxyglucose in causing retraction of the SV3T3 cell body and preventing the secondary topographical redistribution of CABS indicates an energy requirement for the centripetal movement of CABS. The similar effect produced by cytochalasin B suggests that this energy requirement may be mediated through the microfilament system (29). However, in preliminary experiments we were unable to observe a morphological effect of cytochalasin B on the network of microfilaments closely apposed to the membrane. Although the effect of cytochalasin B on CABS was shown to be unrelated to membrane transport, it is, of course, possible that this action represents another direct effect on the membrane, a conclusion that is supported by the rapid reversal of the cytochalasin B effect. Metabolic inhibitors have also been shown to inhibit cap formation in lymphocytes (43) and polymorphonuclear leukocytes (39). These observations can be taken to favor the involvement of membrane flow or active translational forces on CABS clumps, but 2-deoxyglucose and cytochalasin B may influence other membrane properties.

Restricted Mobility of the Con A Receptor Complex

When cells were treated with Con A/H in the cold, rinsed, and then warmed to 37°C, the secondary organization of CABS was attained in 10 min (the shortest time tested). This topography was stable over at least a 1-h period. Thus the interaction of Con A with its surface receptors is followed rapidly by formation of CABS clusters and movement of CABS toward the center of the cell. However, the process of aggregation and translation slows or stops when the characteristic secondary topography is attained. Yahara and Edelman (44) have recently reported that Con A in doses greater than 5 μg/ml inhibits the formation of caps on lymphocytes by Con A itself and also by antibodies directed against surface immunoglobulins. The stable secondary topography of CABS on SV3T3 cells may represent a similar inhibition of receptor mobility. Although Con A does not inhibit the initial movements of CABS on SV3T3 cells, the clusters may reach a limiting size or density as they move in from the edges of the cells, resulting in restriction of their mobility, perhaps due to increased inertia of larger clumps or to induced changes in structures regulating receptor mobility, as proposed by Edelman et al. (25).

Our observations of the effects of colchicine and cytochalasin B on cells pretreated with Con A also suggest that cross-linking of membrane proteins by Con A has significant effects on membrane properties. Both of these compounds cause cells to condense into relatively spherical forms. When the CABS clusters remain dispersed over much of the membrane surface, as on cells treated with Con A/H and then cytochalasin B, the rounding-up of the cells is greatly inhibited. In the Con A-colchicine-treated cells where CABS clusters aggregate in a small central area of the membrane, rounding-up occurs much more rapidly than in cells treated with colchicine alone. Thus, cross-linking of CABS on the peripheral areas of the membrane appears to stabilize the cell against the morphological effects of cytochalasin B and redistribution of these receptors into a small area of the cell surface accelerates the rounding-up induced by colchicine.

The temperature at which the cells are treated with Con A has no effect on the final distribution of hemocyanin after the cells have been warmed to 37°C. This result is in contrast to recent evidence (25) showing that treatment of lymphocytes with Con A at 4°C followed by warming resulted in cap formation, a result attributed to slow reassembly of cold-sensitive microtubular protein. The discrepancy may be attributed to the different types of cells used in the two experiments, or could imply the involvement of other factors in determining the final CABS topography.

Mechanism of Colchicine Effect on CABS Topography

When SV3T3 cells are treated with colchicine or related alkaloids, the CABS move to the center

of the cell forming an aggregate of clusters similar to a cap. Similarly, Edelman et al. report that colchicine antagonizes the inhibition of lymphocyte cap formation by Con A (25). The pharmacology of this effect parallels the actions of these drugs on microtubular protein, since (*a*) colchicine is effective at low doses (10^{-6} M), (*b*) vinblastine sulfate and Colcemid have identical effects, and (*c*) lumicolchicine has no effect on the topography of CABS. These actions of colchicine may involve nonmembrane structures such as subsurface microtubules, which are shown to be absent after the addition of vinblastine or, alternatively, colchicine may have direct actions on drug-sensitive structures within the cell membrane. Our results do not distinguish between these alternatives, but in any case, the result of colchicine treatment is to allow or promote free movement of CABS, leading to their aggregation and redistribution.

Agglutination by Con A of SV3T3 Cells

It is reasonable to suppose that the increased agglutinability of fibroblasts that accompanies transformation is determined by the induction of CABS clusters, allowing multiple Con A bridges to form between cells at a single point of contact, as suggested by Nicolson (45). However, the effect of the concomitant secondary CABS organization on agglutinability has not been determined, since primary and secondary CABS organization cannot normally be analyzed separately. It has been previously reported that colchicine inhibits the Con A-mediated agglutination of erythrocytes to SV3T3 cells (37). It was proposed that this resulted from an inhibition by colchicine of the primary organization of CABS (clustering). Our experiments show that the effect of colchicine on agglutination is most probably related to a change in the secondary organization of CABS (distribution of patches), rather than an effect on cluster formation. If all clusters are withdrawn into a relatively small area after colchicine treatment, the probability that cell-to-cell contact will occur in areas containing CABS is reduced and less agglutination is observed. Also, the proximity of CABS on colchicine-treated cells to the central area of filopodial activity may result in decreased mechanical accessibility of the bound Con A to an apposing red blood cell membrane, preventing cross-links from forming between the cells.

In summary, two degrees of binding site organization have been described: (*a*) the rearrangement of CABS into clusters (primary), and (*b*) the ordering of these clusters over the cell surface (secondary). Both types of binding site organization must be considered in any analysis of the properties of Con A-treated cells. Pharmacological evidence suggests that microfilaments and microtubular proteins may influence CABS distribution on the SV3T3 cell surface.

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