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Differences in the Redistribution of Concanavalin A and Wheat Germ Agglutinin Binding Sites on Mouse Neuroblastoma Cells

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Concanavalin A (Con A), wheat germ agglutinin (WGA), and Ricinus communis agglutinin (RCA) bound with either ^{125}I, fluorescent dyes, or fluorescent polymeric microspheres were used to quantitate and visualize the distribution of lectin binding sites on mouse neuroblastoma cells. As viewed by fluorescent light and scanning electron microscopy, over 10^7 binding sites for Con A, WGA, and RCA appeared to be distributed randomly over the surface of differentiated and undifferentiated cells. An energy-dependent redistribution of labeled sites into a central spot occurred when the cells were labeled with a saturating dose of fluorescent lectin and maintained at 37°C for 60 min. Reversible labeling using appropriate saccharide inhibitors indicated that the labeled sites had undergone endocytosis by the cell. A difference in the mode of redistribution of WGA or RCA and Con A binding sites was observed in double labeling experiments. When less than 10% of the WGA or RCA lectin binding sites were labeled, only these labeled sites appeared to be removed from the cell surface. In contrast, when less than 10% of the Con A sites were labeled, both labeled and unlabeled Con A binding sites were removed from the cell surface. Cytochalasin B uncoupled the coordinate redistribution of labeled and unlabeled Con A sites, suggesting the involvement of microfilaments. Finally, double labeling experiments employing fluorescein-tagged Con A and rhodamine-tagged WGA indicate that most Con A and WGA binding sites reside on different membrane components and redistribute independenty of each other.

Key words: **lectins, binding sites, neuroblastoma cells, receptor redistribution, cell surface labeling, cytochalasin B, concanavalin A, wheat germ agglutinin, fluorescent microscopy, scanning electron microscopy**

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Light and electron microscope studies on a variety of cell types indicate that many membrane components which normally are displayed randomly over the cell surface redistribute to form small clusters or patches when labeled with multivalent ligands such as plant lectins or antibodies [1–7]. These clusters of labeled components can subsequently rearrange in an energy-dependent process to form a cap on the surface of the cell or they can become internalized by endocytosis. Indirect studies employing drugs such as cytochalasin B and colchicine [8–10] and more direct labeling studies using immunofluorescent techniques [11, 12] suggest that the cytoskeletal system may modulate the movement of certain cell surface components. The mechanism of transmembrane control of specific cell surface receptors and its importance in transmembrane signaling induced by extracellular agents, however, is not known at the present time.

In this paper we report on the organization of concanavalin A (Con A), wheat germ agglutinin (WGA), and Ricinus communis agglutinin (RCA) binding sites on mouse neuroblastoma cells. Using lectins conjugated to either fluorescent dyes or fluorescent polymeric microspheres [13] as markers for fluorescent light and scanning electron microscopy (SEM) we have observed that labeled receptors for Con A, WGA, and RCA redistribute and become internalized. However, the mode of redistribution of Con A receptors differs markedly from that of WGA or RCA receptors. Indirect evidence further indicates that microfilaments may play a role in the transmembrane control of specific membrane components.

MATERIALS AND METHODS

Reagents

Phosphate-buffered saline (PBS) and Dulbecco's phosphate-buffered saline (DPBS) containing $CaCl_2$ were prepared as previously described [14, 15]. Con A buffer was composed of 8.77 g NaCl, 0.11 g $CaCl_2$, 5.67 g HEPES and 0.02 g $MnCl_2$ per liter of distilled H_2O and adjusted to pH 7.4. Modified Con A buffer contained 2.42 g Tris base per liter instead of HEPES. N-acetyl chitobiose was prepared from chitin as described by Rupley [16]. Cytochalasin B was obtained from Aldrich Chemical Co.; and colchicine was purchased from Sigma. All other chemicals were of reagent grade.

Cell Cultures

Mouse neuroblastoma line neuro 2a cells (American Type Culture Collection) were routinely grown in Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and fungizone (1.25 μg/ml) in a humidified incubator containing 5% CO_2 at 37°C. Differentiated cells used in most labeling experiments were prepared by seeding $5 \times 10^5 - 1 \times 10^6$ cells in 60-mm Falcon tissue culture dishes containing alcohol-sterilized glass coverslips. The cells were allowed to grow for three days in MEM containing 10% fetal calf serum (FCS). The medium was then removed and FCS-free MEM was added [17]. The cells were used between 24 and 48 h later.

Lectin Preparations

Ricinus communis agglutinin (RCA, mol wt 120,000) was purified from castor beans by affinity and gel filtration chromatography according to the procedure of Nicolson and Blaustein [18]. WGA was prepared on an ovomucoid-Sepharose column following the method of Marchesi [19]. Con A was purchased from Sigma. The purity of the lectins was confirmed by SDS polyacrylamide gel electrophoresis [20, 21].

Fluorescent lectins were prepared as previously described [21]. Lectins were tagged with ^{125}I using the lactoperoxidase method [22]. Fluorescent or iodinated lectins were separated from free dye or ^{125}I and inactive lectin on the appropriate affinity column: ovomucoid-Sepharose for WGA, Sepharose 4B for RCA, and Sephadex G-200 for Con A. Lectin and dye concentrations were determined from the absorbance at 280 nm and 495 nm for fluorescein isothiocyanate (FITC) or 515 nm for tetramethyl rhodamine isothiocyanate (TRITC) [23]. Fluorescent lectin preparations typically had OD 495/280 ratios of 0.5–1.0 for FITC-lectins and OD 515/280 ratios of 0.5–0.8 for TRITC-lectins. Specific activity of ^{125}I-WGA and ^{125}I-Con A was 3.9×10^5 cpm/μg and 7.6×10^4 cpm/μg respectively. Hemagglutination titers of lectin reagents (1 mg/ml) with human red blood cells (type A) were typically 1/1,024 for RCA, 1/64 for WGA, and 1/32 for Con A.

Lectin-Microsphere Conjugates

Copolymer methacrylate microspheres approximately 80 nm in diameter were generously provided by S.P.S. Yen of the Jet Propulsion Laboratory, Pasadena, California [14, 24]. The spheres were derivatized with diaminoheptane by the CNBr method as described previously [14] and were subsequently tagged with FITC (2 mg/ml) or TRITC (0.25 mg/ml) in 0.1 M Na_2CO_3 buffer (pH 9.5) at 24°C for 12 h. For quantitative studies, FITC-microspheres were tagged with carrier-free ^{125}I by the chloramine T method [25]. Excess dye and ^{125}I was removed by either extensive dialysis or chromatography on Sepharose 6B. The spheres were then rederivatized with diaminoheptane using the carbodiimide method and were activated with glutaraldehyde [26]. Lectins were conjugated by adding 2 ml of activated spheres (12 mg/ml) in PBS containing 20 mM NaN_3 to 2 ml WGA (1 mg/ml) or 0.8 ml RCA (3.4 mg/ml). The reaction was allowed to proceed for 15 h at 24°C with stirring. For the preparation of Con A-microsphere conjugates 2 ml of activated spheres in Con A buffer was added to 2 ml of Con A (4 mg/ml) in the same buffer. The unbound lectins were separated from the lectin-microsphere conjugates on discontinuous sucrose gradients as described [14]. Finally, WGA and RCA conjugates were extensively dialyzed against PBS and Con A conjugates against modified Con A buffer. The dilutions of lectin-microsphere conjugates (initial concentration 6–8 mg/ml) which still resulted in detectable agglutination of human Type A red blood cells were 1/4,096 for RCA, 1/1,024 for WGA, and 1/64 for Con A.

Cell Labeling

Quantitation using ^{125}I-lectins or lectin ^{125}I-microspheres. Cells were harvested with 0.02% ethylenediaminetetracetic acid (EDTA) and washed in DPBS. For each assay, $(2.5) \times 10^5$ cells were incubated with ^{125}I-lectin or lectin-^{125}I microsphere conjugates in a total volume of 100 μl for 30 min at 24°C. (This allowed maximal binding with no endocytosis.) The cells were washed three times in DPBS, suspended in 200μl DPBS, transferred to scintillation vials, and counted in tritisol. In some experiments the appropriate saccharide inhibitor (0.1 M D-galactose for RCA I, 0.1 M α-methyl mannoside for Con A and 0.01 M N-acetyl chitobiose for WGA) was included in the assay mixture.

Fixed cells. Cells on coverslips were rinsed in DPBS and fixed for 10 min at 24°C in 1.25% glutaraldehyde-PBS. Glutaraldehyde was rinsed off in DPBS and the coverslips were suspended over 100 μl of lectin reagent (fluorescent lectin or lectin microsphere conjugate) in DPBS for 10–15 min at 24°C. Controls were run simultaneously in the presence of the specific inhibitory sugar.

Unfixed Cells

In discontinuous labeling studies, cells on coverslips were rinsed in DPBS and suspended over 100 μl of lectin reagent for 5 min at 37°C. Unbound lectin was rinsed off by repeated immersion of the coverslips in DPBS. The cells were either immediately examined under the light microscope or incubated in DPBS at 37° for the specified time. Reversibility of lectin binding was tested by treating labeled cells with excess lectin inhibitor (0.25 M α-methyl mannoside or D-galactose or 0.025 M N-acetyl chitobiose) for 15 min at 37°C.

In continuous labeling studies, washed cells on coverslips were immersed in the lectin reagent for the desired time (5–60 min) at 37°C.

Double Labeling

Cells were labeled with saturating levels of lectin-FITC-microspheres (1-5 mg/ml) for 5 min at 37°C. The cells were then treated with TRITC-lectin reagent for 5 min at 37°C, rinsed, and examined for both dyes under the microscope.

Studies Using Colchicine and Cytochalasin B

Cells on coverslips were suspended in 10^{-6} colchicine or in 5 μg/ml cytochalasin B containing 0.5% dimethyl sulfoxide (DMSO) for 30 min at 37°C and subsequently labeled as described above.

In parallel control experiments, cells were treated with 10^{-6} lumicolchicine or 0.5% DMSO.

Microscopy

Cells labeled with TRITC- and FITC-lectin reagents were viewed under a Leitz Dialux microscope equipped with the appropriate filters and incident light excitation. Fluorescence-stained cells were photographed with Kodak Tri-X film using exposure times of 45 sec.

For examination in the SEM, the cells were glutaraldehyde-fixed, dehydrated, and critical point-dried as previously described [13, 14].

RESULTS

Quantitation of Lectin Binding Sites

The binding of ^{125}I-WGA and ^{125}I-Con A to neuroblastoma cells is shown in Figs. 1a and 1b. Relatively high (> 240 μg/ml) concentrations of lectins were required to reach saturation with both WGA and Con A. Labeling of cells with the two lectins was reduced by greater than 90% in the presence of the appropriate saccharide inhibitor. Scatchard [27] analyses (Fig. 2a,b) indicate that there are at least two classes of binding sites for both lectins on the neuroblastoma cells. As indicated in Table I, there are over 1.5×10^7 high-affinity sites ($K_d = 2 \times 10^{-7}$ M) for Con A and WGA. The high-affinity Con A binding sites determined with ^{125}I-Con A are in close agreement with the results of Rosenberg and Charalampous [28] using ^{3}H-Con A.

When cells were labeled with WGA conjugated to ^{125}I-microspheres having an average diameter of 80 nm, the binding leveled off at 1×10^5 microspheres per cell. By coupling ^{125}I-WGA to microspheres it was found that an average of 28 lectin molecules are bound to each microsphere. Due to the geometric constraints of the microspheres, at most half

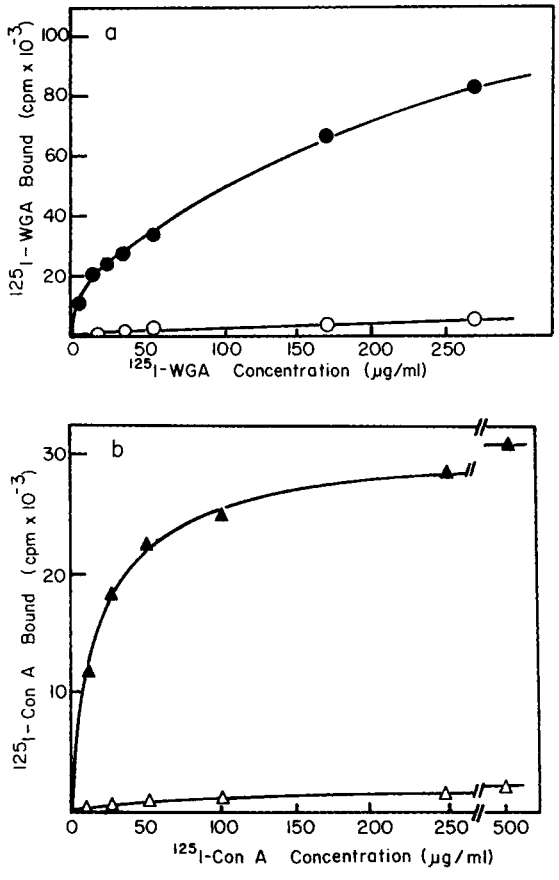

Fig. 1. Binding of ^{125}I-lectins to differentiated neuroblastoma cells. Cells (2.5) × 10^5 suspended in DPBS were incubated with increasing concentrations of lectin in a total volume of 100 μl for 30 min at 24°C. The cells were washed three times in DPBS and counted. a) ^{125}I-WGA binding in the absence (●) and presence (○) of 0.01 M N-acetyl chitobiose. b) ^{125}I-Con A binding in the absence (▲) and presence (△) of 0.1 M α-methyl mannoside. Values were corrected for nonspecific binding of Con A to the assay tubes.

of these lectin molecules would be bound to a cell at one time. Thus, when microspheres are seen to coat the surface of neuroblastoma cells, less than 2.8 × 10^6 sites, or 10% of the available high-affinity sites, are actually labeled.

Distribution of Lectin Binding Sites on Fixed Cells

When glutaraldehyde-fixed neuroblastoma cells in either the undifferentiated or differentiated state were labeled with FITC-Con A, FITC-WGA, or FITC-RCA, or the corresponding lectin-FITC-microsphere conjugates, a uniform fluorescent staining pattern was observed along the periphery of the cell body and neurite projections (Fig. 3a,b). Under the SEM, lectin-microsphere conjugates were seen to be densely packed over the entire cell surface (Fig. 3c). A similar uniform labeling pattern was observed when unfixed cells were labeled at 4°C.

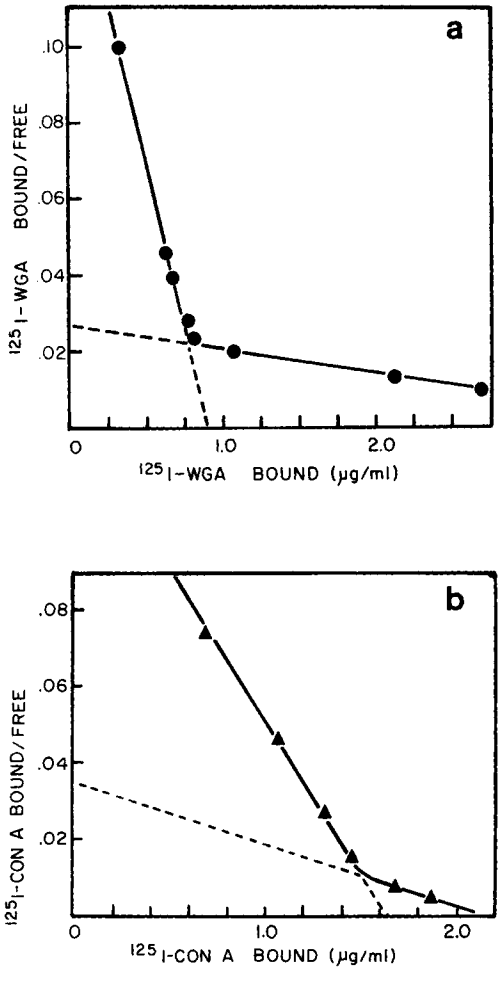

Fig. 2. Scatchard analysis of the binding curves of Fig. 1. a) ^{125}I-WGA; b) ^{125}I-Con A.

TABLE I. Lectin Binding Sites on Neuroblastoma Cells

Lectin	Sites/cell	K_{diss} (M)
^{125}I-Con A		
Class A	1.8×10^7	2.4×10^{-7}
Class B	0.5×10^6	1.2×10^{-6}
^{125}I-WGA		
Class A	2.5×10^7	2.7×10^{-7}
Class B	5.6×10^7	2.0×10^{-6}
WGA-^{125}I-microspheres	1.0×10^5	

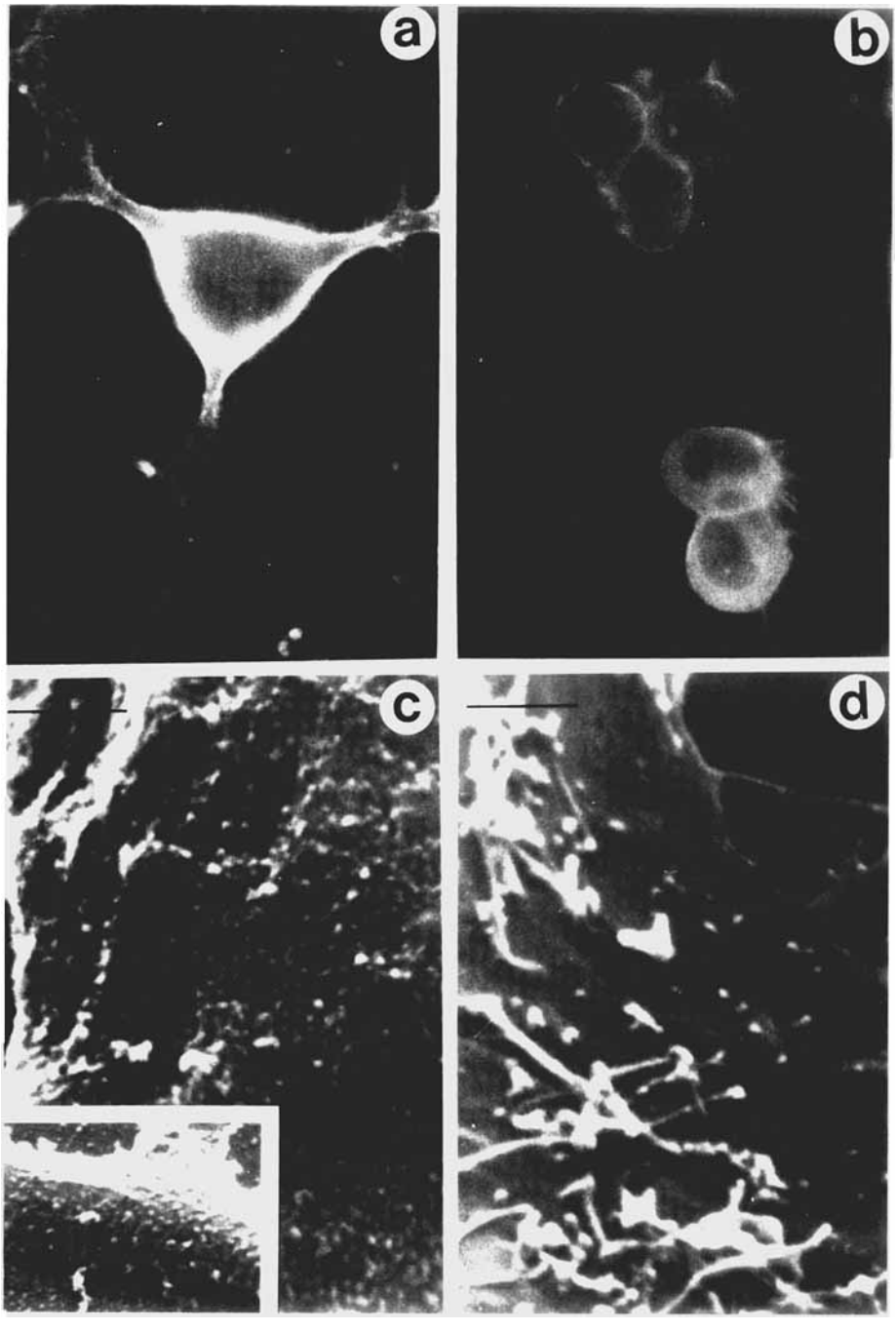

Fig. 3. a) Fluorescent micrograph of a fixed, differentiated neuroblastoma cell labeled with FITC-RCA. b) Fluorescent micrograph of fixed, undifferentiated neuroblastoma cells treated with FITC-WGA. c) SEM micrograph of a fixed cell labeled with Con A-FITC-microspheres, inset shows a neurite of a cell treated in the same way. d) SEM micrograph of a fixed cell labeled with Con A-FITC-microspheres in the presence of 0.1 M α-methyl mannoside. Bar = 1 μm in all SEM micrographs; all fluorescent micrographs = 280 × magnification and the bar = 100 μm.

In control experiments cells treated with FITC-lectins in the presence of the appropriate saccharide inhibitor showed no appreciable fluorescent staining if viewed directly after labeling. Glutaraldehyde-fixed cells, however, gradually developed some autofluorescence that was independent of labeling. The specificity of labeling was confirmed by SEM. Only a small number of lectin-microsphere conjugates were observed to bind to the cells in the presence of the lectin inhibitor (Fig. 3d).

FITC-Lectin-Induced Redistribution

Neuroblastoma cells, which were labeled for 5 min at 37°C with 50–100 μg/ml FITC-WGA and subsequently washed free of excess reagent, initially displayed a uniform ring of fluorescence (Fig. 4a). It proved possible to reverse this labeling by the subsequent addition of N-acetyl chitobiose, an inhibitor of WGA binding (Fig. 4b). If FITC-WGA-labeled cells were incubated for 60 min at 37°C, redistribution of the label into a central spot in the extranuclear region was observed on most of the cells (Fig. 4c). Some fluorescence was still visible on the cell periphery, but this staining diminished on prolonged incubation of the cells at 37°C. Addition of inhibitor resulted in a loss of the peripheral label but not the central spot (Fig. 4d). A similar redistribution of label was observed when FITC-RCA was used.

Con A binding sites also appeared to be initially distributed in a uniform pattern over the cell surface as revealed by reversible labeling studies (Fig. 5a,b). After maintaining the cells for 60 min at 37°, however, the label had redistributed into an extremely patchy arrangement (Fig. 5c). This staining was only partially removed by the addition of 0.25 M α-methyl mannoside (Fig. 5d). Spot formation was inhibited by the presence of 10 mM sodium azide.

Differences in the Redistribution of Lectin Binding Sites

Continuous labeling with lectin-FITC-microsphere conjugates. Neuroblastoma cells treated for 5 min with WGA or RCA-FITC-microspheres exhibited a characteristic ring of fluorescence (Fig. 6a). After 60 min of continuous labeling, however, an intense fluorescence was spread throughout much of the cell (Fig. 6b). Visualization by SEM indicated that WGA- and RCA-microspheres were densely packed over the cell surface throughout the duration of labeling (Fig. 6c). These results indicate that as labeled sites are internalized, additional sites become accessible for labeling with microsphere markers, thereby maintaining a heavy coating of label on the cell surface.

In contrast, ConA-FITC-microspheres which were displayed in a fluorescent ring on the cells after a 5-min labeling period (Fig. 6d) redistributed into large clusters after 60 min of continuous labeling (Fig. 6e). The sparse display of microspheres on the cell surface as visualized by SEM (Fig. 6f) after a 60-min labeling indicated that most of the Con A binding sites had been internalized under these conditions.

Double labeling. A difference in the mode of redistribution between Con A and WGA binding sites was also revealed in double-labeling experiments. Cells which were coated with WGA-FITC-microspheres could be relabeled with TRITC-WGA or WGA-TRITC-microspheres if the cells were induced to redistribute and internalize the initial label, ie, if the labeled cells were incubated at 37°C for 60–120 min prior to relabeling with TRITC lectins (Figs. 7a,b). A similar result was obtained if RCA was used in place of WGA in both labeling steps.

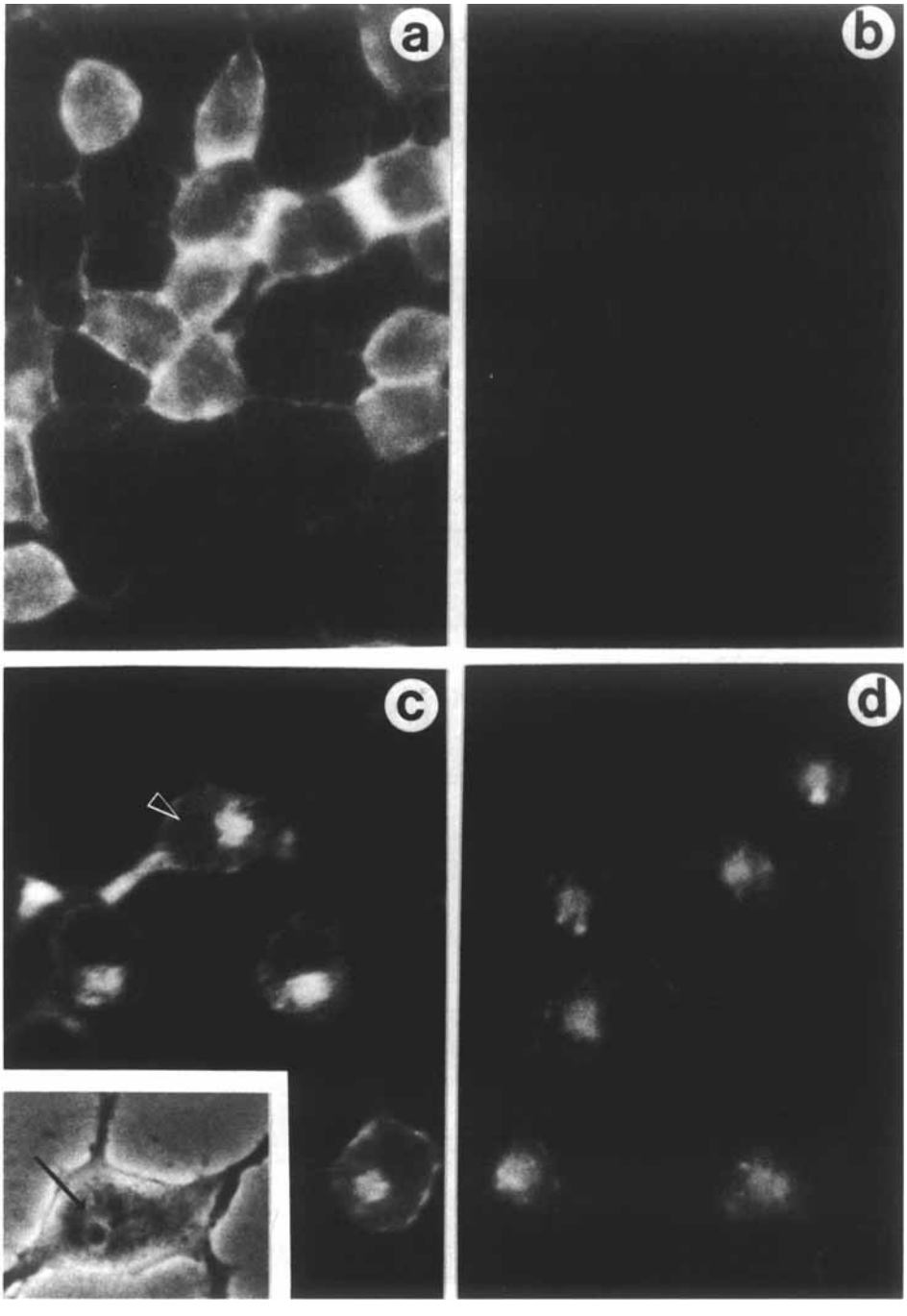

Fig. 4. Fluorescent micrographs of unfixed cells labeled with FITC-WGA. a) With 5-min label; b) 5-min label followed a 15-min incubation in 0.025 M N-acetyl chitobiose; c) 5-min label followed by rinse and 60-min incubation in buffer at 37°C; inset shows phase picture of top cell and arrows indicate the nuclear region; d) treated as in (c) and then incubated in 0.025 M N-acetyl chitobiose for 15 min.

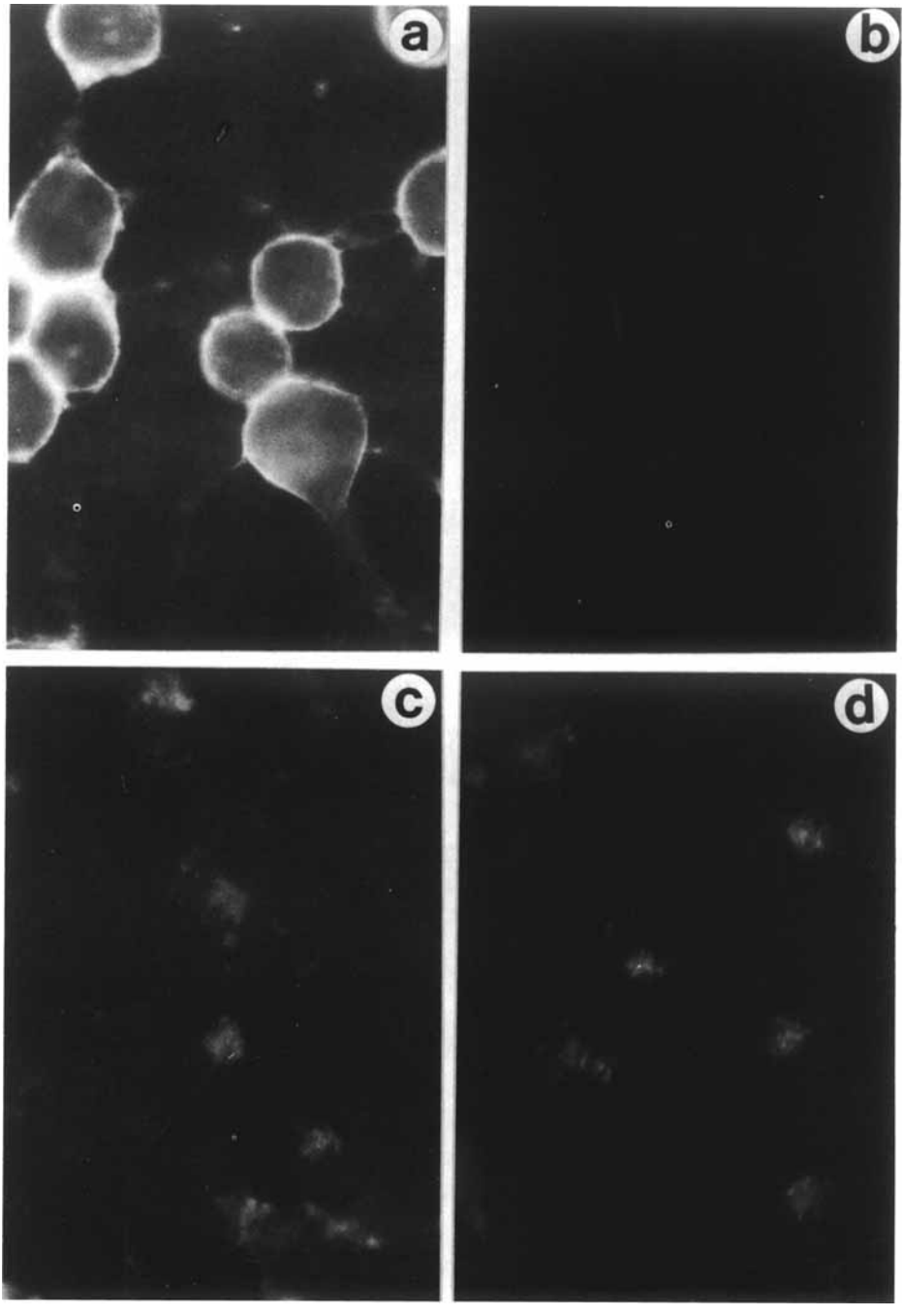

Fig. 5. Fluorescent micrographs of unfixed cells labeled with FITC-Con A. a) With 5-min label; b) 5-min label followed by a 15-min incubation in 0.25 M α-methyl mannoside; c) 5-min label followed by rinse and 60-min incubation in buffer at 37°; d) treated as in (c) and then incubated for 15 min with 0.25 M α-methyl mannoside.

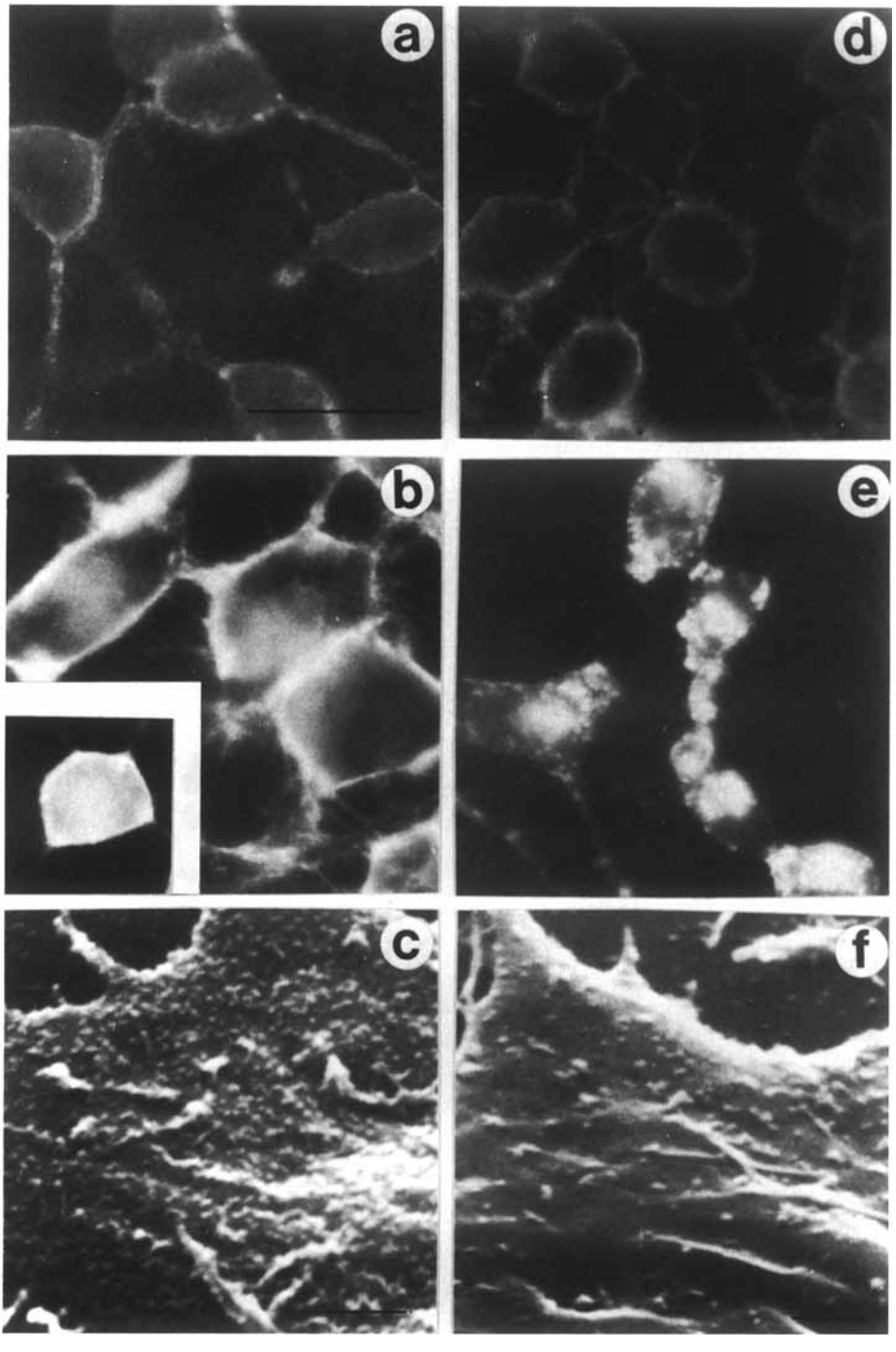

Fig. 6. Continuous labeling of cells with lectin-microsphere conjugates. Fluorescent micrographs of cells labeled 5 min (a) and 60 min (b) with WGA-FITC-microspheres. Inset shows a cell labeled for 60 min with RCA-FITC-microspheres. c) SEM micrograph of a cell treated 60 min with WGA-FITC-microspheres. Fluorescent micrographs of cells incubated 5 min (d) and 60 min (e) with Con A-FITC-microspheres. f) SEM micrograph of cell labeled 60 min with Con A-FITC-microspheres.

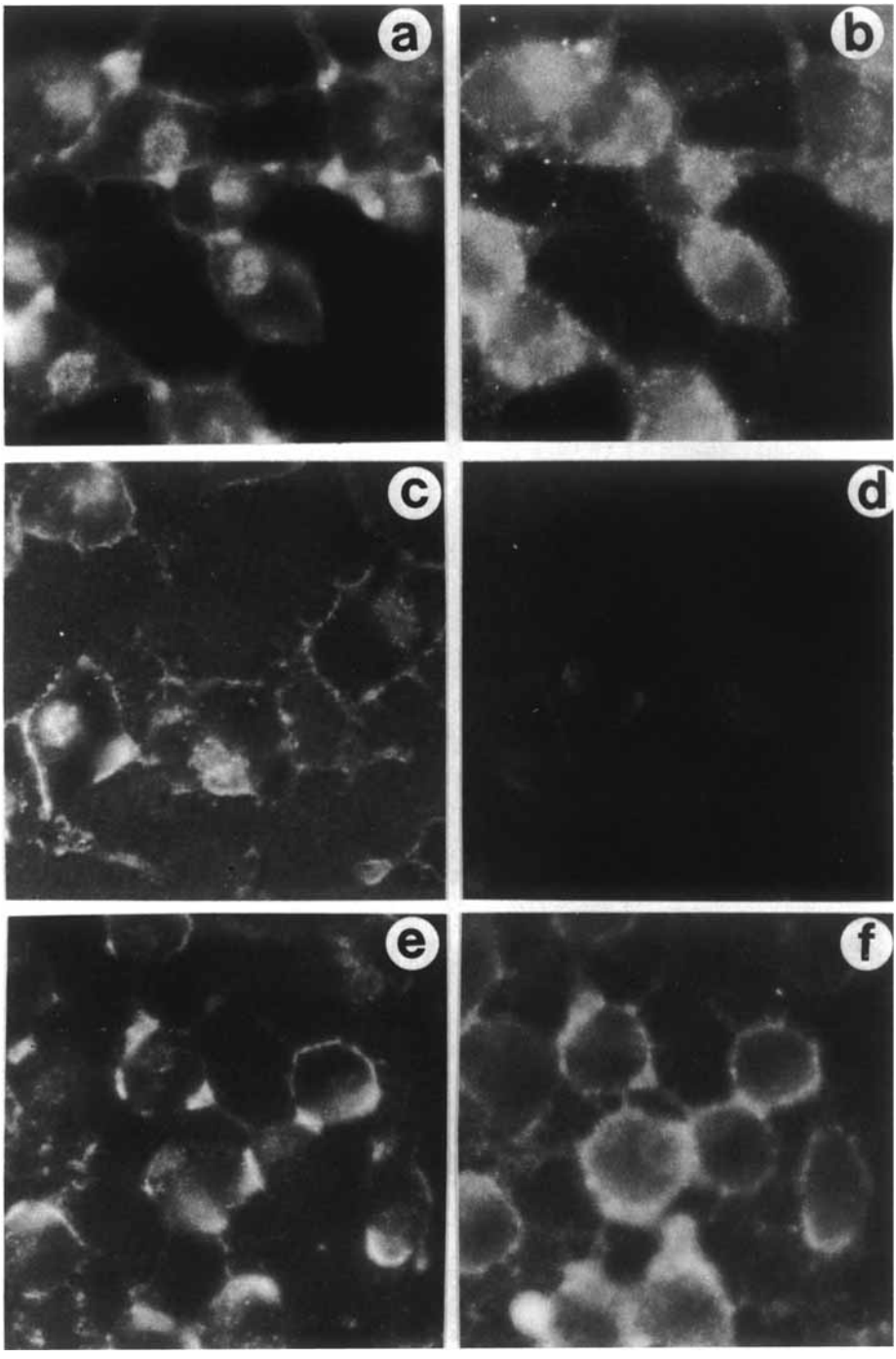

Fig. 7. Fluorescent micrographs of cells double-labeled with lectin conjugates. Cells were treated for 5 min with the fluorescein label, rinsed, incubated in buffer for 60 min, relabeled for 5 min with the rhodamine label, and subsequently examined for both FITC and TRITC staining. FITC staining (a) and TRITC staining (b) of cells treated with WGA-FITC-microspheres/WGA-TRITC-microspheres. FITC (c) and TRITC (d) staining of cells incubated with Con A-FITC-microspheres/TRITC-Con A (low degree of staining in (d) is due to FITC fluorescence (green) which is not filtered out). FITC (e) and TRITC (f) staining of cells treated with Con A-FITC-microspheres/WGA-TRITC-microspheres. (Similar results were seen if FITC Con A/TRITC WGA were used instead of their respective microsphere counterparts.)

On the other hand, cells treated with Con A-FITC-microspheres under identical conditions could not be relabeled with either TRITC-Con A or Con A-TRITC-microspheres (Figs. 7c,d) even after redistribution had occurred. Similarly, cells initially incubated with Con A at concentrations (2.5–5 μg/ml) which only labeled 10–15% of the available high-affinity sites could not be relabeled with FITC-Con A after the cells were incubated at 37° for 60–120 min. Con A-labeled cells, however, could be relabeled with TRITC-WGA, indicating that most Con A binding sites are on different membrane components than WGA sites (Fig. 7e,f). The presence of Con A and WGA receptors on different polypeptide chains has been confirmed by SDS polyacrylamide gel electrophoresis coupled with fluorescent lectin staining ([29], and unpublished results).

Effect of Cytochalasin B and Colchicine on Redistribution

Cytochalasin B altered the mode of redistribution of Con A-labeled sites and retarded the clearing of label from the cell surface. Cells which were pretreated with cytochalasin B in 0.5% DMSO and then labeled for 60–120 min with Con A-FITC-microspheres displayed a uniform ring of fluorescence around their periphery as well as a fluorescent central spot (Fig. 8a). α-Methyl mannoside was seen to displace the peripheral label, but not the central fluorescent spot (Fig. 8b). Furthermore, cytochalasin B-treated cells which were labeled briefly with Con A-FITC-microspheres, rinsed, and incubated in buffer for 120 min could be relabeled with TRITC-Con A (Fig. 8c, d). This is in contrast to untreated or DMSO-treated cells which showed a patchy surface distribution of fluorescence and could not be relabeled with TRITC-Con A after 60 or 120 min (see Figs. 7c,d). Cytochalasin B did not seem to change the pattern of redistribution for WGA receptors but did retard their clearing. Thus, cytochalasin B appears to cause Con A sites to redistribute in a manner similar to WGA- or RCA-labeled sites on untreated cells.

Cells pretreated with 10^{-6} M colchicine for 30 min rounded up. When they were subsequently labeled with FITC-Con A for 5 min, washed, and incubated in buffer for 60 min, they displayed a patchy distribution of Con A-labeled sites as seen by both fluorescent microscopy and SEM (Fig. 9a,b). Although the central spot was not formed, label could be only partially removed by addition of α-methyl mannoside. Lumicolchicine had no visible effect on the cell morphology or redistribution of lectin-labeled sites.

DISCUSSION

Quantitative studies using ^{125}I-lectins indicate that there are over 10^7 high-affinity binding sites for Con A and WGA on mouse neuroblastoma cells. These sites, as well as those for RCA, appear to be randomly distributed over the cell surface and neurites as studied by light and scanning electron microscopy. An energy-dependent redistribution of these sites occurs when the cells are labeled with FITC-lectins at 37°C. After an hour most of the label becomes displaced into a central spot. This staining pattern appears to reflect an internalization of label, since addition of a high concentration of the appropriate saccharide inhibitor cannot remove this localized staining. The view that the fluorescent spot represents internalized label is supported by results in which cells induced to redistribute lectin-FITC-microspheres into a fluorescent spot do no exhibit a localized concentration of microsphere makers on the cell surface as visualized by SEM [30]. Gonatas et al [7] have also observed the internalization of ricin-labeled sites on cultured embryonal neurons using peroxidase markers with transmission electron microscopy.

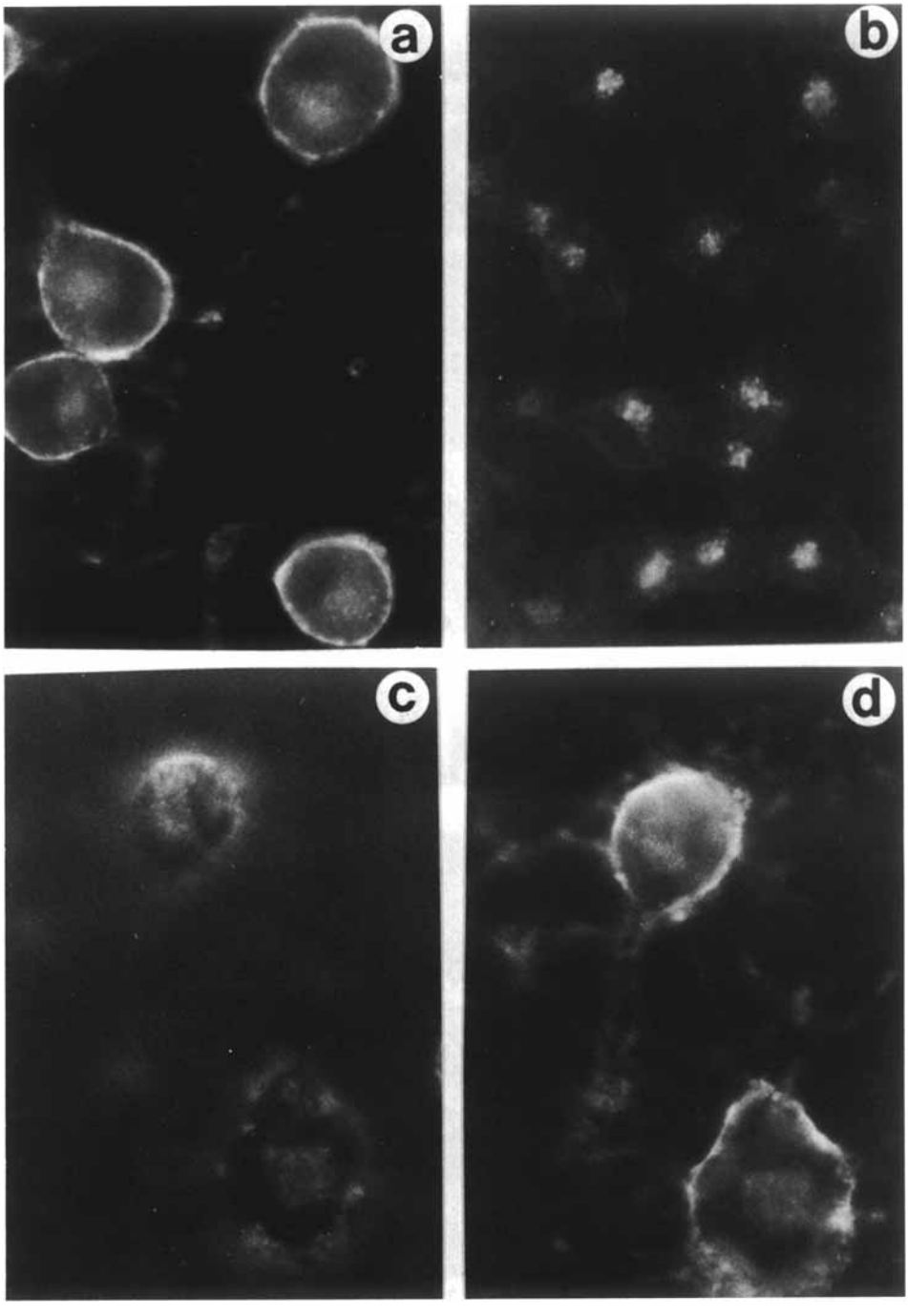

Fig. 8. Fluorescent micrographs showing the effect of cytochalasin B on the redistribution of Con A binding sites. All cells were pretreated 30 min at 37°C with 5 μg/ml cytochalasin B and labeled and washed in the presence of cytochalasin B. a) Cells continuously labeled with Con A-FITC-microspheres; b) labeled as (a) and then treated 15 min with 0.25 M α-methyl mannoside; c,d) FITC (c) and TRITC (d) staining of cells double-labeled with Con A-FITC-microspheres/TRITC-Con A. Cells were treated for 5 min with the fluorescein label, rinsed, incubated in buffer for 120 min, and relabeled for 5 min with the rhodamine label.

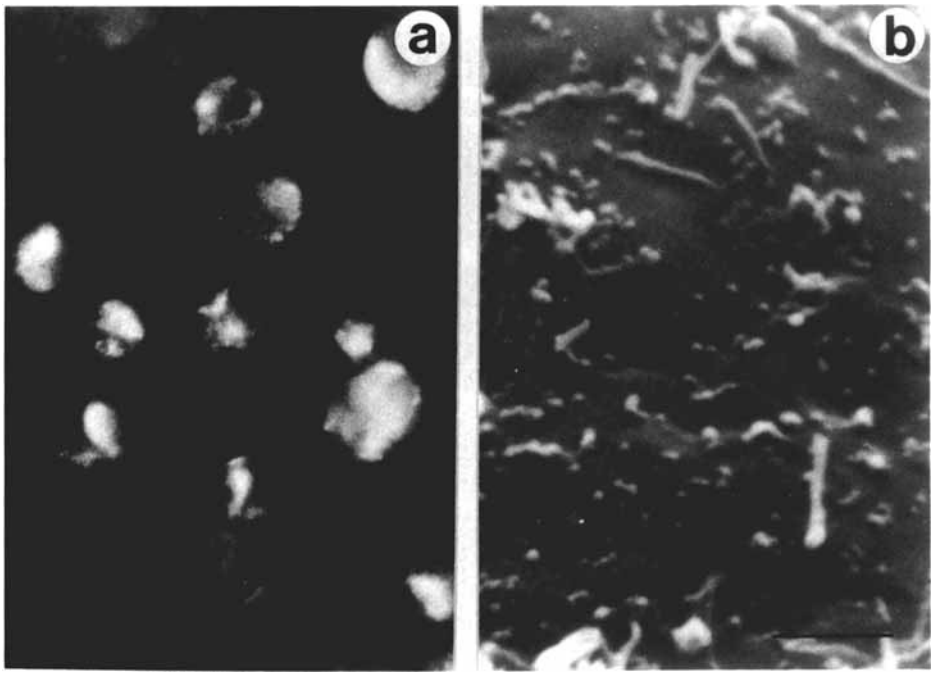

Fig. 9. The effect of colchicine on Con A redistribution. All cells were pretreated 30 min at 37°C with 10^{-6}M colchicine and labeled and washed in the presence of colchicine. a) Fluorescent micrograph of cells labeled 5 min with FITC-Con A, rinsed, and incubated in buffer 60 min; b) SEM micrograph of a cell treated 5 min with Con A-FITC-microspheres, rinsed, and maintained for 60 min in buffer.

Although Con A, WGA, and RCA cell surface binding sites can be induced to redistribute by means of labeling with lectins or lectin-microsphere conjugates, the mode of rearrangement of Con A binding sites differs markedly from that of WGA or RCA sites. Whereas labeled WGA and RCA sites redistribute independently of their unlabeled counterpart, both labeled and unlabeled Con A binding sites redistribute together. This is documented in continuous and in double-labeling experiments. When less than 10% of the total number of high-affinity binding sites are initially labeled using WGA or RCA and are induced to clear from the cell surface, additional sites become available for relabeling with the same lectin. In contrast, when cells are labeled with a subsaturating quantity of Con A under similar conditions, no relabeling of the cells with Con A is seen. Con A-labeled cells, however, can be relabeled with WGA, indicating that Con A and WGA binding sites reside on different membrane components and redistribute independently of each other.

This difference in the pattern of redistribution of Con A sites and WGA or RCA binding sites is not unique to neuroblastoma cells. When Dictyostelium discoideum cells are continuously labeled with Con A-microsphere conjugates, Con A markers redistribute into patches and caps as visualized by SEM; cells cannot be relabeled with Con A [5]. WGA-microspheres, however, are maintained in a dense uniform array on the cell surface. Brown and Revel [6] have also reported that when cultured mouse L cells were continuously labeled with ricin-hemocyanin markers, a homogeneous pattern of label was observed; cells continuously labeled with Con A-hemocyanin displayed a heterogeneous labeling pattern.

The coordinate redistribution of labeled and unlabeled Con A binding sites may reflect the presence of multiple Con A sites on the same or on tightly associated membrane components. West and McMahon [31], however, have reported that there are over 35 different Con A binding proteins in plasma membranes of Dictyostelium discoideum. It would appear unlikely that all or most of these polypeptides would be complexed together in the membrane. The result reported here that the microfilament-disrupting agent cytochalasin B uncouples the redistribution of labeled sites from unlabeled sites suggests that microfilaments may coordinate the movement of Con A sites on cell surfaces. In agreement with studies by Gonatas et al [7], 5–10 μg/ml cytochalasin B or 10^{-6} M colchicine, however, did not prevent endocytosis of lectin sites on neuroblastoma cells.

Differences in the turnover rates of the receptors for Con A and WGA could be used to explain the differences in the redistribution patterns of the receptors for these lectins. However, studies [32] have shown the mean half-life for the total proteins of plasma membranes from several cell types to be 30–60 h. Furthermore, in neuroblastoma cells, most of the membrane proteins only reach a steady-state radioactivity after 18–20 h [33], which is significantly longer than the time period for the reported experiments [1–2h].

In addition to differences in redistribution, Con A and WGA or RCA have differential effects on other cellular properties. Con A possesses mitogenic responses toward some classes of lymphocytes [34] and alters the morphology and differentiation pattern of a number of cell types. In particular, it has been reported that Con A reverses differentiation in neuroblastoma cells [35], causes a rounding up and clustering of microvilli on Dictyostelium cells [5], and inhibits ruffling activity on L cells [6]. WGA and RCA, however, have no apparent effect. It is not known whether the effect of Con A on cellular morphology is related to the coordinate redistribution of labeled and unlabeled Con A sites. One may speculate, however, that both these effects may be controlled by microfilaments [11] or related cytoskeletal structures and reflect an efficient mechanism of transmitting a signal initiated at one region on the cell surface to other parts of the cell.

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