Journal of Supramolecular Structure 2:629–645 (1974)

EFFECT OF MEMBRANE LIPID COMPOSITION AND MICROTUBULE STRUCTURE ON LECTIN INTERACTIONS OF MOUSE LM CELLS

Harry G. Rittenhouse, Robert E. Williams, and C. Fred Fox

Molecular Biology Institute and Department of Bacteriology, University of California, Los Angeles, California 90024

Concanavalin A (Con A) binding and Con A-mediated hemadsorption to LM cells were found to decrease significantly at both 5–7°C and 15–19°C. The higher of these critical temperatures responds to a change in state of the membrane lipids and can be increased or decreased in cells where the membrane phospholipids contain less or more double bonds, respectively. The lower critical temperature for Con A binding or Con A-mediated hemadsorption does not respond to these changes in membrane lipid composition. Though the amount of Con A bound to the cell surface is a determinant of Con A-mediated agglutinability, the major components of the decreases in Con A-mediated hemadsorption which occur at both these critical temperatures do not have their origin in the decreases in Con A binding which occur over these same temperature ranges – that is 5–7°C and 15–19°C.

Con A-mediated hemadsorption measured at 22°C was dramatically inhibited when LM cells were first incubated at 7°C or less. Reversal of this inhibition required 20–30 min of subsequent incubation at 22°C, indicating that factors other than membrane lipid "fluidity" are determinants of agglutinability. LM cells treated with the microtubule-disrupting alkaloids colchicine, colcemid, or vinblastine at concentrations as low as 10^{-6} M were as much as fourfold more agglutinable with Con A. By contrast, lumicolchicine, an inactive derivative of colchicine, had a slight inhibitory effect on Con A-mediated hemadsorption. Colchicine, vinblastine, or lumicolchicine treatment of LM cells did not alter the quantitative binding of labeled lectin. The results suggest that membrane lipid "fluidity" and the cell cytoskeleton (microtubule/microfilament system) are important determinants of lectin interactions with cell surfaces. The results are interpreted in terms of a model of cell–cell and cell–lectin interactions which assigns a central role to the Con A receptor.

INTRODUCTION

Since the initial studies of Aub et al. (1), plant lectins have been used extensively as probes of animal cell surface structure (2–4). The plant lectins concanavalin A (Con A), wheat germ agglutinin (WGA), and soy bean agglutinin (SBA) have been shown to interact differentially with several transformed cell lines and their normal cell counterparts (2–4). At low concentrations of lectin the transformed cells are agglutinated and their normal

cell counterparts are not. Transformed cells are more sensitive to the toxic effect of Con A than are normal cells, a finding which has allowed for the selection of revertants from the transformed state (5, 6).

Recent studies of lectin binding to cells have revealed equal numbers of lectin receptors (WGA and Con A) present on the cell surface of both transformed cells and their normal cell counterparts (7–9). The differential agglutinating properties of the lectins cannot then be due to unequal amounts of bound lectin. Nicolson (10) has proposed that transformed cell surface proteins are more mobile, allowing for the clustering of receptor sites by the multivalent lectin. The formation of clusters of lectin receptors would favor the agglutination of cells. Fluorescent, ferritin, and hemocyanin labeling of lectin receptors have revealed a clustered arrangement of receptors in transformed cells induced by lectin treatment, while the control cell counterparts show a random arrangement of receptors (11–14). Low temperature (4°C) inhibits the clustering of Con A receptors in transformed cells, demonstrating that the lectin induces changes in cell surface topography resulting in a clustered receptor distribution. The apparent difference in cell surface properties between transformed and normal cells may therefore be a consequence of an increased mobility of surface receptors in transformed cells and not an inherent difference in cell surface topography. The inhibition of lectin receptor mobility in transformed cells by low temperature indicates the importance of fluid membrane lipids for the process of lectin-induced clustering of receptors.

Both Con A binding and Con A-mediated agglutination of cells are highly temperature-dependent processes (15–18). A critical temperature at approximately 15°C was demonstrated for these two Con A-related phenomena with 3T3 and viral transformed 3T3 cells (15, 16). Using a transformed mouse fibroblast cell line (LM), we demonstrated two critical temperatures (5–7°C and 15–19°C) for these two Con A phenomena (17, 18). In the present paper we report the influence of membrane lipid composition and microtubule-disrupting drugs on the Con A binding and Con A-mediated hemadsorption of LM cells.

MATERIALS AND METHODS

Mouse LM cells derived from NCTC clone 929 (L cells) were maintained on Eagle's minimal essential medium (MEM) with Hank's salts plus 0.5% Difco bacto-peptone (MEM + P) as described previously (19). The procedure used to modify the fatty acid composition of LM cells has been described (20). Confluent cells were detached from Falcon tissue flasks by treatment with 0.005% trypsin (Sigma: type 2, pancreatin; 1,500 BAEE units per mg) and seeded at an approximate 20-fold lower cell density on Linbro multidish trays (35 mm diameter). The cells were incubated for 24 hr in MEM modified to contain twice the normal glutamine and vitamin concentrations and 2 mg of the biotin antagonist dl-desthiobiotin per liter (MEM + GVdB). The medium was then replaced with MEM + GVdB containing a fatty acid covalently attached to a detergent (Tween). Tween-nonadecanoic acid (Tween-19:0) at 8 μg/ml of fatty acid equivalent (fae) was used to increase the proportion of saturated fatty acids in phospholipid, and Tween-linolenic acid (Tween-18:3) at 8 μg/ml (fae) was added to increase the double bond content of fatty acids in phospholipid.

Con A (3X crystallized, Miles Laboratories) was labeled with ^{3}H-acetic acid anhydride (500 mCi/mmole, Amersham/Searle) by the method of Agrawal et al. (21). A specific activity of 5.8×10^{6} cpm per mg of protein demonstrated two to three residues of labeled acetate bound per tetramer of Con A (MW approximately 120,000).

For studies of the effects of various alkaloids on Con A binding and Con A-mediated hemadsorption, cultures were used at 80–100% confluency. Vinblastine, colcemid, colchicine, and lumicolchicine were each dissolved in 0.85% (w/v) NaCl immediately before use at a concentration of 1 μg/ml and added to LM cells in culture medium (MEM + P). The cells were incubated at 37°C for the desired time before assaying for Con A binding and Con A-mediated hemadsorption. Lumicolchicine was prepared from a 10^{-5} M colchicine solution in absolute ethanol using ultraviolet irradiation as described by Mizel and Wilson (22). The formation of lumicolchicine was followed by a decrease in absorbance at 350 nm spectrophotometrically.

RESULTS

Effects of Lipid Composition on Cell–Lectin Interactions

Fatty acid analysis of the combined phosphatidylcholine (PC) and phosphatidylethanolamine (PE) fraction derived from cells grown in medium containing Tween-19:0 at 8 μg/ml (fae) revealed a saturated fatty acid content of approximately 45% (20). In contrast, cells grown in MEM + P (control cells) contained about 30% saturated fatty acid in PC and PE. The saturated fatty acid content (PE and PC) of cells cultivated with the polyunsaturated fatty acid supplement Tween-18:3 did not differ significantly from control cells (approximately 30%). However, the double bond content of fatty acids in cells grown with Tween-18:3 is greater than that of control cells since approximately 11% of the total fatty acid was identified as linolenic acid. Polyunsaturated fatty acids were not detectable in control cells (20).

The temperature dependence of both Con A binding and Con A-mediated hemadsorption to LM cells is significantly affected by changes in fatty acid composition (Figs. 1 and 2). The upper critical temperature for these Con A processes in control cells (15–19°C) is shifted 7° upward in cells with a higher saturated fatty acid content (Fig. 1B vs 1A and Fig. 2B vs 2A). The lower critical temperature of Con A binding was not altered significantly. The upper critical temperature for both Con A-related phenomena was shifted downwards to 7–11°C for cells enriched with linolenic acid (Fig. 1B vs 1C and Fig. 2B vs 2C). The lower critical temperature of Con A binding was unchanged for cells containing linolenic acid (Fig. 1B vs 1C). The lower critical temperature for Con A-mediated hemadsorption is more apparent at 200 μg/ml of Con A than at 100 μg/ml of Con A (Fig. 2) and is essentially the same for cells enriched for saturated fatty acids (5–7°C) as for control cells (6–9°C). The lower critical temperature for Con A-mediated hemadsorption for cells enriched with unsaturated fatty acids is obscured by the upper critical temperature at 7–11°C (Fig. 2C). We considered the possibility that the temperature dependence of Con A-mediated hemadsorption of LM cells (Fig. 2) was due to the decreased binding of Con A at lower temperatures (Fig. 1). To test this possibility we incubated cells at 22°C with Con A for all samples and then proceeded with the

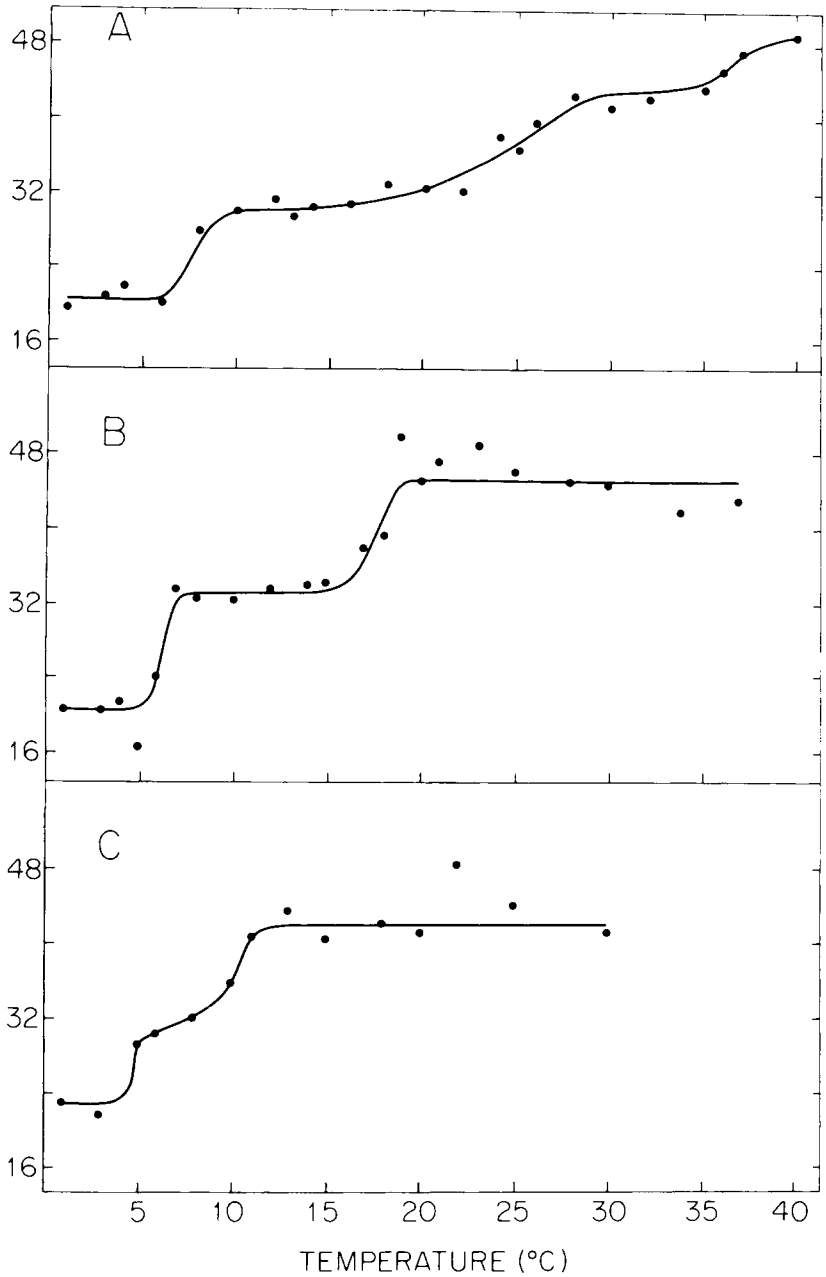

Fig. 1. Effect of fatty acid composition and temperature on the binding of radioactively labeled Con A to LM cells. Cells grown to 80–100% of confluency in 35 mm diameter wells (Materials and Methods) were incubated for 10 min at the test temperature (all washings and incubations were at the test temperature), washed twice with 0.85% NaCl (saline), and incubated for 5 min with ^{3}H-Con A (100 μg/ml) in a 0.1 M Na_2HPO_4 buffer, pH 7.2, containing 0.85% NaCl and 0.001 M $MgCl_2$ (PBS). After five washes with saline the cells were suspended by incubation in 1 ml of 5% Na_2CO_3 –0.1 N NaOH for 1 hr. The samples were divided into equal portions for measurements of tritium content and protein as previously described (17, 18). All points in Figs. 1–6 are the average of six determinations. A, cells enriched with nonadecanoic acid ($C_{19:0}$). B, control cells grown in MEM + P. C, cells enriched with linolenic acid ($C_{18:3}$). From Rittenhouse et al. (18) with permission of the publisher.

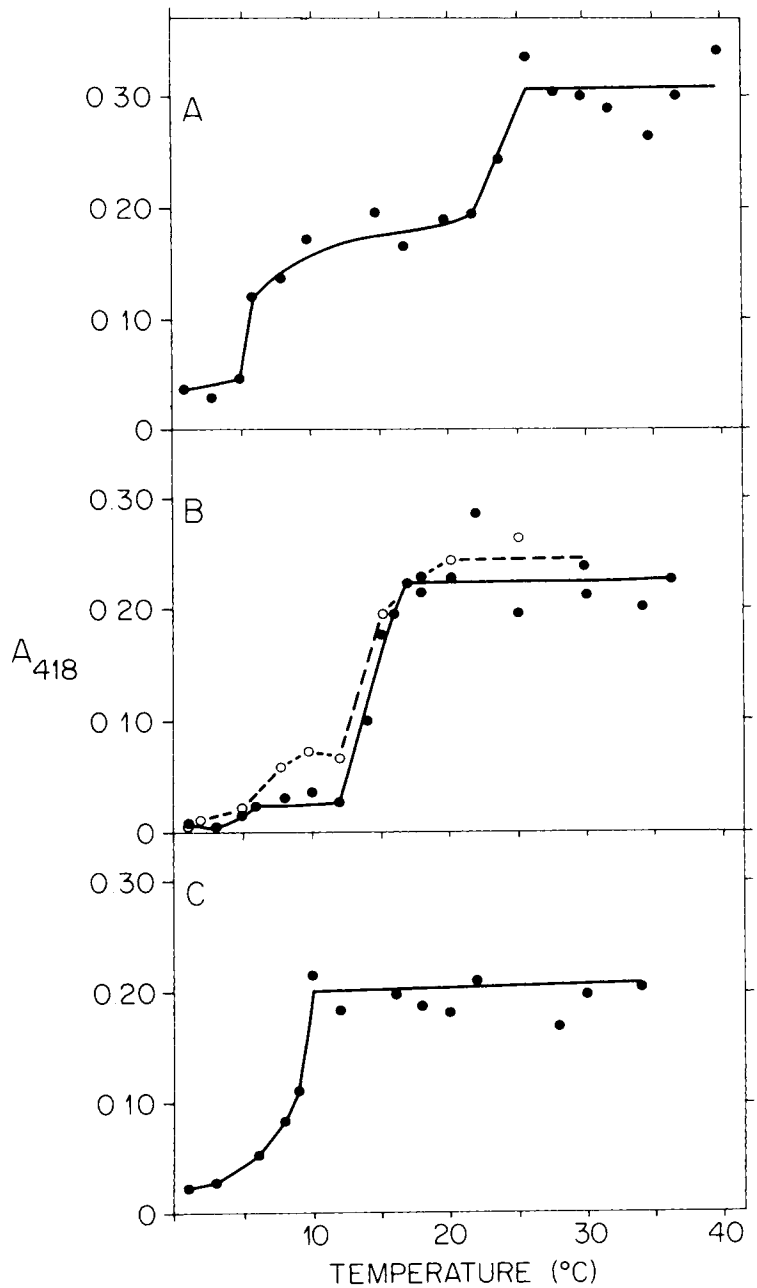

Fig. 2. Effect of fatty acid composition and temperature on the Con A-mediated hemadsorption to LM cells. Cells grown to 80–100% confluency in Linbro multidish wells were incubated for 10 min at the test temperature (all washings and incubations were at the test temperature), washed twice with saline, and incubated for 5 min with Con A in PBS, pH 7.2. The cells were then washed five times with saline and incubated for 10 min with a rabbit erythrocyte suspension (2% v/v) in PBS (17). Finally, the cells were washed five times with PBS, solubilized in 5% sodium dodecyl sulfate (w/v), and analyzed spectrophotometrically for hemoglobin content at 418 nm. A, cells enriched with $C_{19:0}$, 200 μg/ml Con A. B, control cells grown in MEM + P – ○----○, 200 μg/ml Con A; ●—●, 100 μg/ml Con A. C, cells enriched for $C_{18:3}$, 100 μg/ml Con A. From Rittenhouse et al. (18) with permission of the publisher.

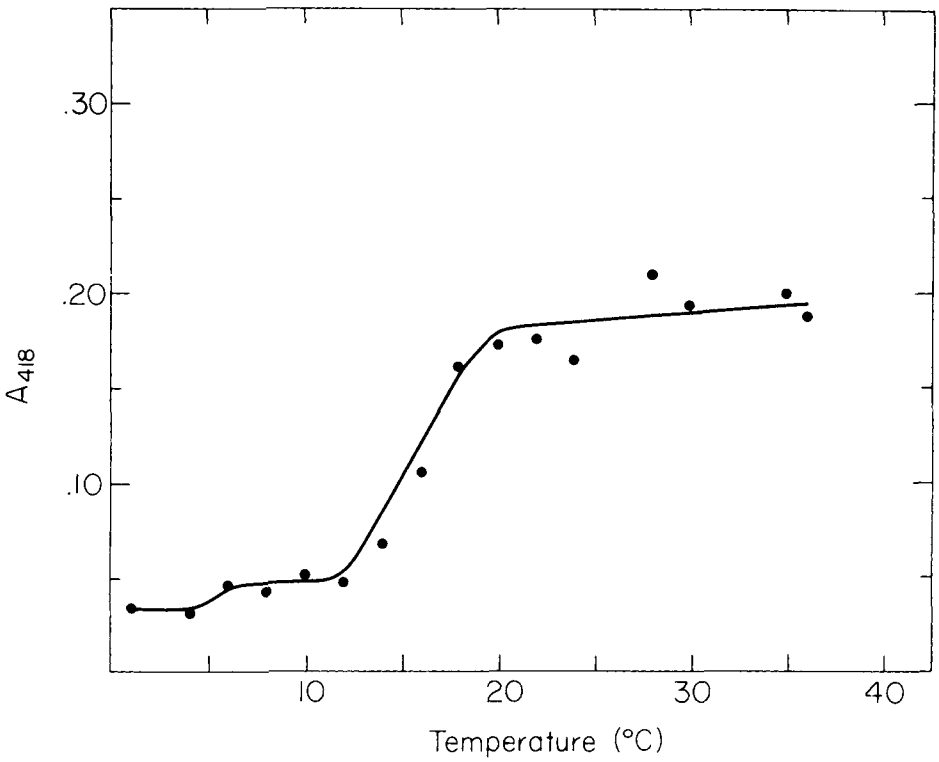

Fig. 3. Effect of temperature on Con A-mediated hemadsorption to LM cells where Con A was bound to all cells at 22°C. Cells were grown to 80–100% confluency in Linbro multidish wells (35 mm) and were incubated for 5 min at 22°C with Con A (100 μg/ml) in PBS, pH 7.2. The cells were washed five times with saline at 22°C and incubated in PBS for 10 min at the test temperature before addition of rabbit erythrocytes (2% v/v) in PBS for a 10 min incubation. Spectrophotometric analysis of hemoglobin content was as described for Fig. 2.

hemadsorption assay at different temperatures. Fig. 3 demonstrates that essentially the same temperature-dependent pattern of the Con A-mediated hemadsorption was observed with control cells as found in Fig. 2. Table I shows that no significant loss of bound Con A occurred upon shifting to higher or lower temperatures after first binding Con A at 22°C. The critical temperatures for Con A-mediated hemadsorption (Fig. 2) cannot therefore be accounted for by differential binding of Con A at different temperatures.

The number of Con A molecules bound does have some effect on Con A-mediated hemadsorption (Fig. 4). Cells incubated at 22°C with Con A require less lectin (150 μg/ml) for saturation of Con A-mediated hemadsorption at 22°C than cells treated with Con A at 10°C and incubated subsequently for hemadsorption assay at 22°C (300 μg/ml).

Microtubule-Disrupting Drugs and Lectin Interactions of LM Cells

Colchicine treatment at concentrations as low as 10^{-6} M significantly increases the Con A-mediated agglutinability of LM cells (Fig. 5A). The increase in Con A-mediated

TABLE I. Effect of Temperature Shifts on Con A Binding to LM cells[a]

Incubation 1		Incubation 2			
Temperature (initial) (°C)	Time (min)	Temperature (final) (°C)	Time (min)	CPM/mg protein[b]	Temperature shift
0	5	–	–	24,200	none
0	5	22	10	29,100	0 → 22
22	5	–	–	61,500	none
22	5	0	10	58,800	22 → 0

[a]Con A binding was performed for 5 min (Incubation 1) as described in the legend to Fig. 1, and unbound Con A was removed prior to Incubation 2. The cells were then incubated in PBS of pH 7.2 for 10 min at the indicated temperatures to test for loss of bound Con A after the indicated shifts in temperature.

[b]Values are the average of six determinations.

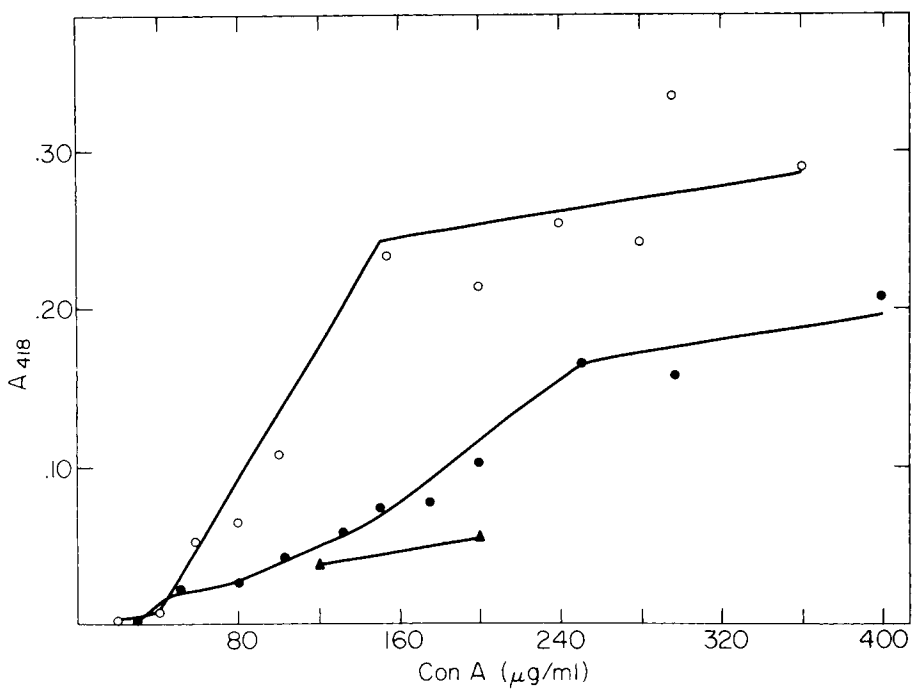

Fig. 4. Effect of temperature for binding of Con A to cells on the concentration dependence of Con A for Con A-mediated hemadsorption. LM cells grown to 80–100% confluency in MEM + P in Linbro multidish wells (35 mm) were incubated for 5 min at 22°C (○—○), 10°C (●—●), or 0°C (▲—▲), with the indicated amounts of Con A. The amount of Con A which binds to cells at 10°C and 0°C is approximately 50% and 30%, respectively, of that which binds to cells at 22°C (17). After the incubations for Con A binding, the cells were washed five times with saline and incubated for 10 min at 22°C with rabbit erythrocytes (2% v/v) in PBS. Spectrophotometric analysis for hemoglobin was performed as described for Fig. 2.

hemadsorption after colchicine treatment was associated with a visually observed change in cell morphology indicative of microtubule disruption. Incubation of cells with lumicolchicine, an inactive photoderivative of colchicine, resulted in no observed morphological change and perhaps a slight inhibitory effect on Con A-mediated hemadsorption (Fig. 5A). Colcemid (2×10^{-7} M) and vinblastine (10^{-6} M) treatments enhance Con A-mediated hemadsorption, which correlated with a visually observed alteration in cellular morphology (Fig. 5B, C).

A study of the concentration effect of colchicine action revealed that even 1,000-fold higher concentrations of colchicine (10^{-3} M) did not significantly increase the enhanced hemadsorption observed with 10^{-6} M colchicine (Fig. 6). In this study the Con A-mediated hemadsorption of LM cells was increased approximately fourfold at 50 μg/ml of Con A (Fig. 6). Colchicine-treated cells were as agglutinable at 50 μg/ml of Con A as control cells at 150 μg/ml of Con A.

Con A binding studies of cells treated with colchicine, lumicolchicine, vinblastine, or colcemid at a 10^{-6} M drug concentration are shown in Table II. The amount of Con A bound to cells is unaltered by alkaloid treatment.

Con A-mediated hemadsorption is dramatically inhibited when LM cells are first incubated at low temperature (7°C or less) (Table III). Colchicine (10^{-6} M) treatment of cells partially reverses this cold inhibition effect (Table III). The amount of Con A bound to cells at 0°C is not affected by colchicine (not shown).

DISCUSSION

The upwards or downwards shift of the upper critical temperature for Con A binding and Con A-mediated hemadsorption of LM cells enriched for saturated or polyunsaturated fatty acids, respectively, demonstrates that these two Con A-related phenomena require a fluid membrane lipid phase. The physical state of membrane lipids in prokaryotes has been shown to affect a number of membrane-associated processes (23–25). Two characteristic temperatures have been demonstrated by physiological and physical techniques (23–25). The higher of these characteristic temperatures is that at which the formation of solid patches of membrane lipids is first detected. The lower is the end-point of the course of lateral phase separations, at which all the membrane lipids are in a solid phase. Recent studies with the plasma membrane fraction of LM cells using electron spin resonance methodology indicate that the higher critical temperature for both lectin binding and lectin-mediated hemadsorption phenomena corresponds to the temperature at which all the cell surface membrane lipids become frozen (26).

The lower critical temperature for Con A binding and Con A-mediated hemadsorption (5–7°C) is unchanged for cells enriched for saturated or polyunsaturated fatty acids (Figs. 1 and 2), indicating that the state of membrane lipids is not the causal factor involved here. The time-dependent reversibility of inhibition of Con A-mediated hemadsorption at 22°C after incubation of LM cells at 7°C or less also indicates that factors other than membrane lipid fluidity are determinants of the lower critical temperature for hemadsorption (Table III). If a microtubule/microfilament cytoskeleton system is an important determinant of the restriction of membrane protein mobility (4, 27), disruption of microtubule structure, which is known to occur at low temperature (4–10°C) (28, 29),

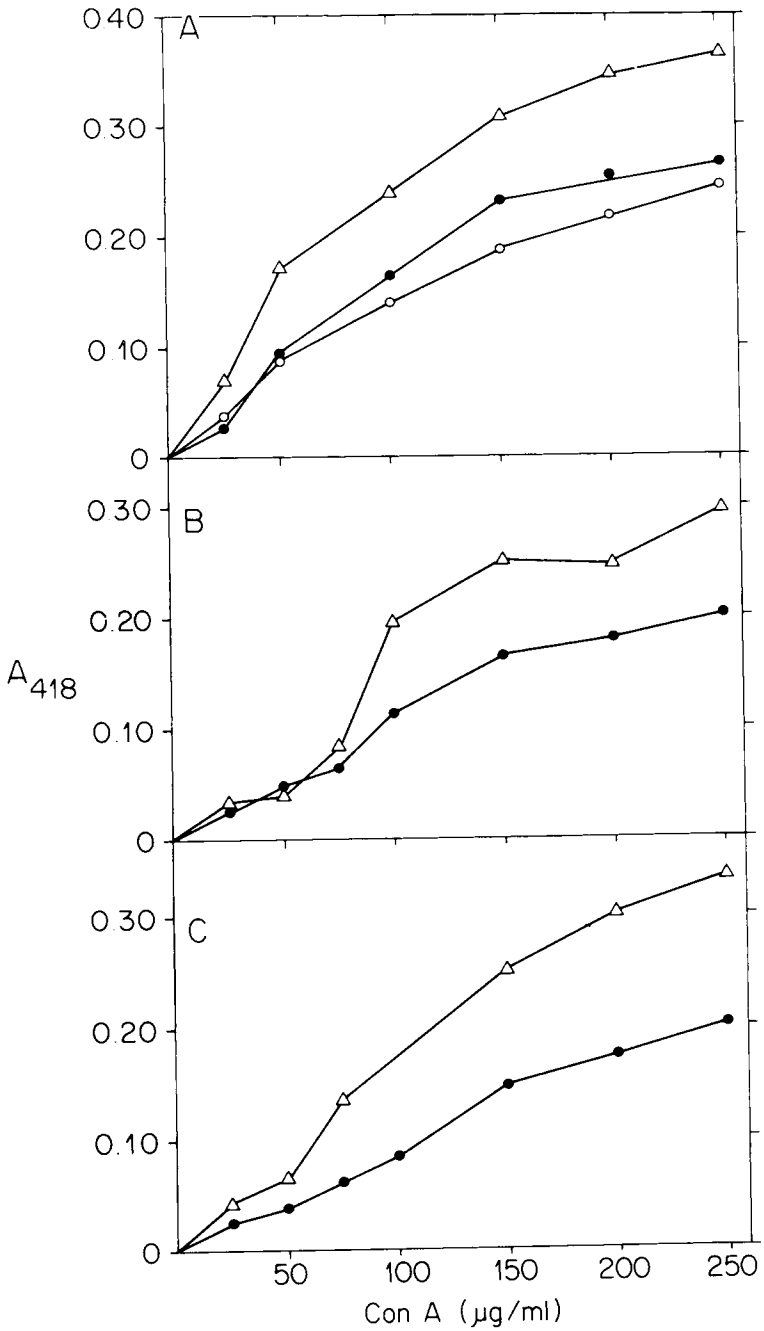

Fig. 5. Effect of tubulin-binding drugs on the concentration dependence of Con A for Con A-mediated hemadsorption ot LM cells. A, cells were incubated for 3 hr at 37°C with 10^{-6} M colchicine in MEM (△—△), 10^{-6} M lumicolchicine in MEM (○—○), or MEM alone (●—●). B, cells were incubated for 3 hr at 37°C with 2×10^{-7} M colcemid in MEM (△—△) or in MEM alone (●—●). C, cells were incubated for 3 hr at 37°C with 10^{-6} M vinblastine sulfate (△—△) or in MEM alone (●—●). The Con A-mediated hemadsorption assay was performed on all samples at 22°C as described in the legend to Fig. 2.

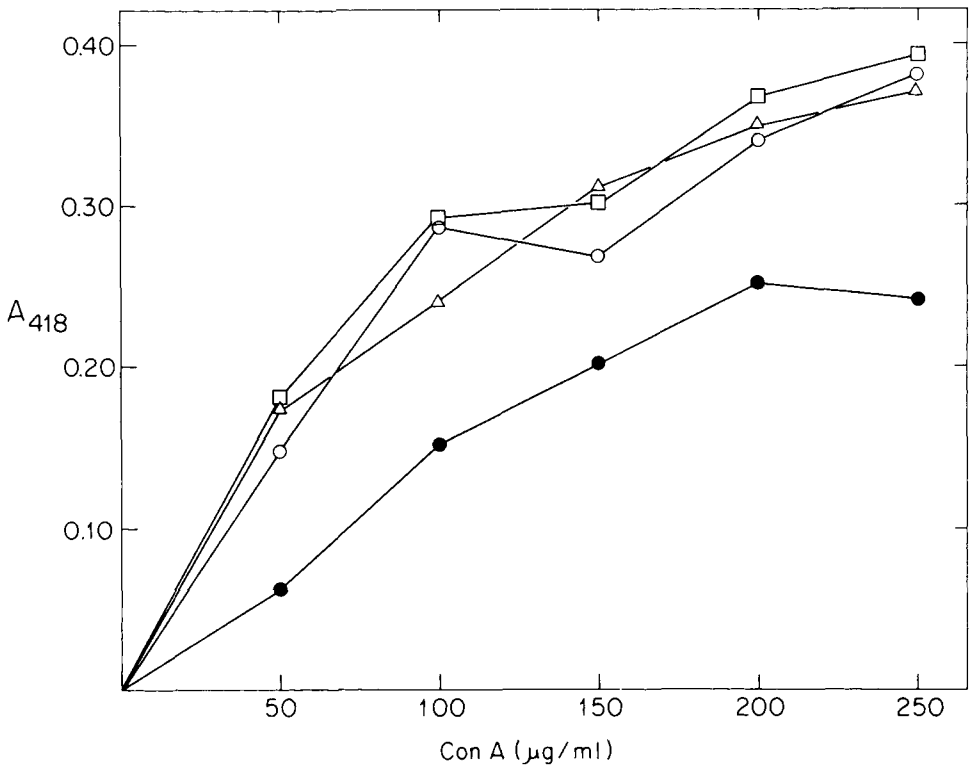

Fig. 6. Effect of colchicine concentration on Con A-mediated agglutinability of LM cells. Cells were incubated for 3 hr at 37°C with 10^{-3} M colchicine in MEM (□—□), 10^{-5} M colchicine in MEM (△—△), 10^{-6} M colchicine in MEM (○—○), or in MEM alone (●—●). The Con A-mediated hemadsorption assay was performed on all samples at 22°C as described in the legend to Fig. 2.

might account for the inhibition of Con A-mediated hemadsorption caused by incubation below 7°C. However, colchicine (10^{-6} M) treatment of LM cells incubated below 7°C has a two- to threefold stimulatory effect on hemadsorption (Table III), as it also has at 22°C. As found previously (17), a recovery incubation period of greater than 10 min is required to substantially reverse the inhibition of Con A-mediated hemadsorption from prior cold incubation (0°C). Cells treated with colchicine responded in a similar fashion (Table III). Cytoskeletal elements in addition to microtubule structures may therefore be involved in the low-temperature inhbition of hemadsorption. Kaneko et al. (30) have shown that cytochalasin B inhibits the Con A-mediated agglutination of rat ascites tumor cells, possibly by acting on a microfilament system. Microfilaments might act as structural bridges between microtubules and cell surface receptors (4).

Drugs which bind tubulin and prohibit microtubule assembly (31) significantly increase the susceptibility of LM cells to Con A-mediated agglutination (Figs. 5 and 6). The same drugs (colchicine, colcemid, and vinblastine) do not alter the quantitative binding of Con A to cells (Table II). These results indicate that a change in cell surface topography and/or Con A receptor mobility induced by disruption of microtubule structure

TABLE II. Effect of Tubulin-Binding Drugs on Con A Binding to LM Cells

Drug[a] (10^{-6} M)	^{3}H-Con A[b] (cpm/mg)
None	47,400
Colchicine	46,600
Vinblastine	47,100
Colcemid	48,200
Lumicolchicine	47,100

[a]Cells were grown to 80–100% confluency in 35 mm diameter wells. The cells were then incubated for 3 hr in the presence of each drug at 37°C, washed twice with saline, and the Con A binding assay performed as described in the legend to Fig. 1.
[b]Values are the average of six determinations.

potentiates lectin-mediated agglutinability. Nicolson (4) and Berlin et al. (27) have suggested that submembranous structures (microtubule/microfilament system) may influence the mobility of membrane proteins. Berlin's group has shown that microtubule-disrupting agents decrease the agglutinability of Simian virus 40 transformed 3T3 cells (32). The effects of tubulin-binding drugs on Con A-mediated agglutination of LM cells and SV3T3 cells appear to be in different directions. The probable reason for the apparent difference between their results and ours are the different experimental conditions used in these two studies. Con A binding (10 min) and hemadsorption (20 min) incubation times used in the SV3T3 cell study (32) were twice the length of the times used in the experiments with LM cells. The microtubule-disrupting drugs apparently increase the formation of caps or clusters of Con A receptors at the center of SV3T3 cells, and this may account for the decreased agglutinability of these cells (33). The capping of Con A receptors may not allow large amounts of red blood cell adsorption (hemadsorption) to occur because of steric inaccessibility. We have recently found that *prolonged* incubation of colchicine-treated LM cells with Con A results in a dramatic inhibition of Con A-mediated hemadsorption in accord with possible cap formation of Con A receptors (Rittenhouse et al., in preparation). Thus in both cases (LM and SV3T3 cells), colchicine, vinblastine, and colcemid could indirectly promote an increased mobility of Con A receptor proteins, presumably by altering the cytoskeleton. Inhibition or enhancement of Con A-mediated agglutinability by tubulin-binding drugs would depend upon the extent of cap formation. Exposure of new Con A receptor sites by the drugs used in this study resulting in more bound Con A is not likely since the quantitative binding of radioactively labeled lectin is unaltered by these drugs (Table II). This is in agreement with the finding by Edelman's group that various microtubule-disrupting drugs do not alter the amount of Con A which binds to lymphocytes (34).

The apparent alteration of the cell surface by microtubule-disrupting drugs may simulate cell surface changes which occur during the cell cycle. Numerous studies have shown significant changes in the agglutination and binding properties of Con A as a function of cell cycle with various cell lines including 3T3 cells (5, 35), Epstein-Barr virus trans-

TABLE III. Effect of Colchicine and Cold Treatment on Con A-Mediated Hemadsorption to LM Cells

Incubation 1[a] (Con A binding) temperature (°C)	Incubation 2[b] recovery		Incubation 3[c] (hemagglutination) temperature (°C)	+Colchicine[d] A_{418}[e]	−Colchicine A_{418}[e]
	Temperature (°C)	Time (min)			
0	–	–	0	.042	.016
22	–	–	22	.221	.095
0	–	–	22	.114	.038
0	22	5	22	.094	.029
0	22	10	22	.111	.056
0	22	20	22	.174	.078

[a]Con A binding was performed for 5 min as described in the legend to Fig. 1, and unbound Con A was removed before Incubation 2.

[b]Incubation in PBS, pH 7.2, for the indicated time intervals at 22°C after the Con A binding step to allow for recovery from the effect of cold temperature incubation.

[c]Hemadsorption with rabbit erythrocytes (2% v/v) was performed as described in the legend to Fig. 2 for 10 min.

[d]Cells were incubated for 3 hr at 37°C with 10^{-6} M colchicine in MEM prior to Incubation 1. The cells were exposed to no additional colchicine during this or subsequent incubations.

[e]Values are the average of six determinations.

TABLE IV. Observations of Cell–Cell and Cell–Lectin Interactions Consistent with the Proposed Model of Con A Receptor Proteins

Experiment	Observation	References
Con A-mediated agglutinability	In general transformed cells are agglutinated at lectin concentrations which do not induce the agglutination of the normal cell counterparts.	1–4
Cell surface topography of Con A receptors	Con A induces the clustering of Con A receptors of transformed cells at 22°C, whereas no clustering of Con A receptors is revealed for the normal cell counterparts after treatment with Con A. A random arrangement of Con A receptors is found for both transformed and normal cells in the presence of Con A at 4°C.	11–13
Effect of cAMP on lectin-mediated agglutinability	Lectin-mediated agglutinability of transformed cells decreased.	46
Production of cAMP in transformed cells	Transformed cells contain less cAMP than their normal cell counterparts.	48, 49
Effect of cAMP on regulation of cell growth	Treatment of transformed cells with dibutyryl adenosine cyclic 3′-5′-monophosphate restores the cells to density-dependent inhibition of growth.	50, 51
Effect of microtubule-disrupting alkaloids on lectin-mediated agglutinability	Agglutinability of transformed cells dramatically decreased (32) or increased (this study) depending upon the experimental conditions.	32
Distribution of "cytoskeletal" elements – i.e., microfilaments and microtubules	Microfilaments associated with the plasma membrane are diminished in transformed cells compared to the normal cell counterparts. Colcemid relieves contact inhibition of growth in mouse fibroblasts, and this is associated with a disappearance of microtubules from the cytoplasm.	41–45
Effect of microtubule-disrupting alkaloids on cell surface topography	Vinblastine and colchicine promote the redistribution of certain transport receptors on the cell surface of macrophages and polymorphonuclear leucocytes.	52
Effect of Con A and colchicine on the cap formation of Con A receptors	At 4°C without colchicine or at 37°C with colchicine treatment, extensive cap formation of Con A receptors occurs in lymphocytes.	53
Protease treatment of cells, microtubule structures, and lectin-mediated agglutinability	Trypsin treatment of normal cells promotes the lectin-mediated agglutinability of these cells. Trypsin treatment of cells is associated with a loss of microtubule structures.	2–4

formed human lymphocytes (36), and hamster kidney fibroblasts (37). Numerous other studies also implicate the Con A receptor protein(s) directly or indirectly in the regulation of cell proliferation. In Table IV we summarize some of these studies. A scheme which would bring together the large number of experimental observations on phenomena involving Con A receptor protein(s) would obviously be useful. We have therefore developed a working hypothesis (Fig. 7) for cell–cell interactions which assigns a central role to the Con A receptor protein(s).

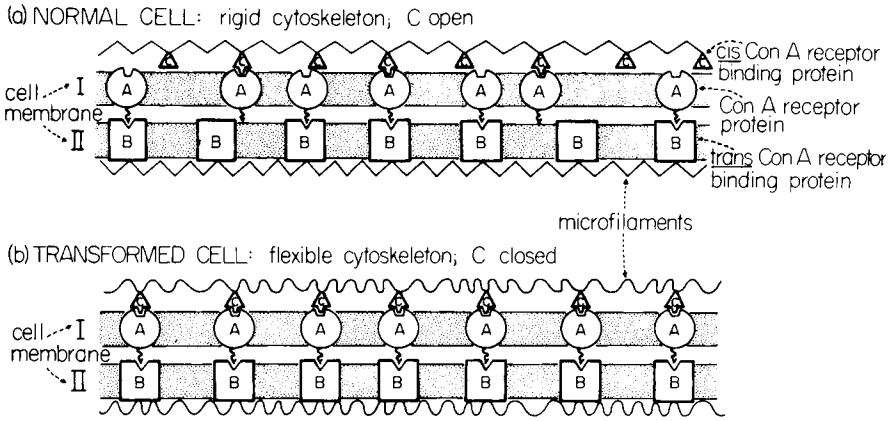

Fig. 7. Model of cell–cell interactions involving Con A receptor protein(s). Four major elements are depicted in the model: (1) the Con A receptor protein(s) (A); (2) the trans Con A receptor binding protein (B) – that is, the protein on the opposing cell surface that interacts with the carbohydrate moiety on A; (3) the cis Con A receptor binding protein (C) – that is, the receptor binding protein on the inner surface of the membrane within which the Con A receptor (A) resides; and (4) the cytoskeleton (microtubule/microfilament system) underlying the plasma membrane as it affects directly or indirectly the distribution or mobility of the Con A receptor protein(s) (A).

The working hypothesis (Fig. 7) depicts the interactions of the Con A receptor protein with neighboring cells. In this scheme, we identify four major elements: A, the Con A receptor protein; B, the trans Con A receptor binding protein -- that is, the protein in the opposing cell surface that interacts with the carbohydrate moiety on A; C, the cis Con A receptor binding protein – that is, the receptor binding protein on or in the inner surface of the membrane within which the Con A receptor A resides; and the cytoskeleton underlying the plasma membrane as it affects directly or indirectly the distribution or mobility of the Con A receptor protein A. In the schemes shown, the components A, B, and C are shown to be present in only one membrane. This is done for simplicity of presentation, for all these would be components of both cell membranes.

The first case shows the interactions of the Con A receptor protein at regions of cell–cell contact in normal cells (Fig. 7a). Both C and B are firmly bound to the cytoskeleton (microtubule/microfilament system) possibly to an actin-like protein, and A has affinity for both B and C. It is possible, of course, that multiple species of Con A receptor proteins exist, but for the purpose of simplicity only one receptor species is depicted in this model. We propose that the cytoskeleton is rigid in the normal cell for most of the cell cycle. A flexible cytoskeleton may occur transiently during a portion of the cell cycle – for example, during the M phase, which would affect accordingly the relative apparent affinities of A for B and C. When the cytoskeleton is rigid, B and C must compete for binding A, leaving much of C in the "open" state. This competition could arise as a consequence of the attachment of B and C to cytoskeletal structures exhibiting different periodicity (as shown), as a consequence of random disposition of B and C on a nonordered (but rigid) cytoskeleton, or as a consequence of random disposi-

tion on a cytoskeleton having regular repeating units where B and C do not fill every point on the lattice. The relative positions of B and C are fixed in their respective membranes, and the rigidity of the cytoskeleton prevents their lateral adjustment and alignment into positions opposite each other. We postulate that under the condition where the cytoskeleton is rigid, and binding of A to C is therefore prevented by competition with binding to B, C either produces a substance which inhibits cell proliferation or cannot produce a substance which potentiates it. Thus the net result of a rigid cytoskeleton at a critical cell density is the prevention of cell proliferation.

The transformed cell differs from its normal counterpart by having a more flexible cytoskeleton. This greater flexibility permits lateral adjustment in the disposition of components B and C – that is, it permits A to bind to both (Fig. 7b). The net result of this is an increase in the amount of C in the "closed" state, in which C either synthesizes an effector which potentiates cell proliferation or is inhibited from synthesizing an effector which prevents cell proliferation. The model does not require that all C be in the "open" or "closed" state, as the response to the effector produced by C could display sigmoid kinetics. Finally, this hypothesis is not limited to any single species of receptor – that is, either to a single Con A receptor or to Con A receptors in general. Any other surface receptor which has mobility restricted by a submembranous structural network might also interact with component C or a component with similar properties.

Table IV illustrates a number of observations on cell–cell or cell–lectin interactions upon which this model was predicated. A critical feature of the model is the role of the cytoskeleton as a determinant of cell surface receptor topography. Numerous electron microscopic studies have shown that microfilaments are associated with the plasma membrane (38–40). Recent findings which indicate a marked difference in the distribution of microfilaments in transformed cells from that of untransformed or revertant cell counterparts are likewise consistent with this model (41–44). In particular, McNutt et al. (44) have found that a close relationship exists between decreased microfilament density and loss of density-dependent inhibition of growth in transformed 3T3 cells (SV3T3). The link between microtubules and microfilaments is less clear. However, colcemid has been shown to relieve contact inhibition in mouse fibroblasts in culture and this phenomenon was associated with a disappearance of microtubules from the cytoplasm (45).

A large body of data suggests that transformed cells are more agglutinable with Con A than is the normal cell counterpart (1–4). The normal cell can in general be agglutinated only by high concentrations of Con A. Experiments using ferritin, fluorescent, and hemocyanin labeling techniques indicate that Con A receptors (A) are more easily clustered by Con A than are their counterparts in normal cells (11–13).

The model predicts that agents which decrease the rigidity of the cytoskeleton will increase Con A receptor protein mobility and that drugs which stabilize the microtubule/microfilament system will restrict the mobility of these surface proteins. Inhibition or enhancement of Con A-mediated agglutinability will depend upon the extent of capping in the case of microtubule-disrupting agents. Con A-mediated agglutinability should be inhibited by drugs which stabilize the cytoskeleton. In the present report we have described how drugs which may act to disrupt microtubules dramatically increase the Con A-mediated agglutinability of LM cells. Recently, Puck's group has shown that dibutyryl adenosine cyclic 3′-5′-monophosphate (cAMP) significantly inhibits the WGA-mediated agglutinability of Chinese hamster ovary cells (46). Cyclic AMP may promote

microtubular aggregation (47, 48). We have found that 10^{-3} M dibutyryl adenosine cyclic 3′-5′-monophosphate strongly inhibits Con A-mediated hemadsorption to LM cells (Rittenhouse et al., in preparation).

Utilizing this basic working hypothesis, an attempt has been made to analyze some of the extensive literature regarding the interactions of Con A with transformed and normal cells (Fig. 7). The model is capable of accounting for much of the existing data on the interactions of lectins with cells. An important prediction made by this model is that a protein (C) exists at the inner plasma membrane and can bind both the Con A receptor protein A and actin (microfilaments). A second important prediction is the existence in membranes of protein B – that is, a component which is bound to actin and has affinity for the concanavalin A receptor. Finally, this model predicts that cyclic AMP acts to inhibit cell proliferation indirectly via its effect on the rigidity of the cytoskeleton. The identification of components with properties of A, B, or C would greatly aid our understanding of the mechanism of density-dependent inhibition of growth and lectin interactions with the cell surface.

ACKNOWLEDGMENTS

This work was supported in part by USPHS grants AI-10733 and GM-18233. Harry G. Rittenhouse is an American Cancer Society Postdoctoral Fellow. C. Fred Fox is the recipient of USPHS Research Career Development Award GM-42,359.

REFERENCES

1. Aub, J. C., Tieslau, C., and Lankester, A., Proc. Nat. Acad. Sci. U.S. 50:613 (1963).
2. Sharon, N., and Lis, H., Science 177:949 (1972).
3. Lis, H., and Sharon, N., Ann. Rev. Biochem. 43:541 (1973).
4. Nicolson, G. L., Int. Rev. Cytol. 39:89 (1974).
5. Ozanne, B., J. Virol. 12:79 (1973).
6. Wright, J. A., J. Cell Biol. 56:666 (1973).
7. Arndt-Jovin, D. J., and Berg, P., J. Virol. 8:716 (1971).
8. Cline, M. J., and Livingston, D. C., Nature New Biol. 232:155 (1971).
9. Ozanne, B., and Sambrook, J., Nature New Biol. 232:156 (1971).
10. Nicolson, G. L., Nature New Biol. 239:193 (1972).
11. Singer, S. J., and Nicolson, G. L., Science 175:720 (1972).
12. Inbar, M., and Sachs, L., FEBS Lett. 32:124 (1973).
13. Rosenblith, J., Ukena, T. E., Yin, H. H., Berlin, R. O., and Karnovsky, M. J., Proc. Nat. Acad. Sci. U.S. 70:1625 (1973).
14. Nicolson, G. L., Nature New Biol. 243:218 (1973).
15. Noonan, K. D., and Burger, M. M., J. Biol. Chem. 248:4286 (1973).
16. Noonan, K. D., and Burger, M. M., J. Cell Biol. 59:132 (1973).
17. Rittenhouse, H. G., and Fox, C. F., Biochem. Biophys. Res. Commun. 57:323 (1974).
18. Rittenhouse, H. G., Williams, R. E., Wisnieski, B. J. and Fox, C. F., Biochem. Biophys. Res. Commun. 58:222 (1974).
19. Wisnieski, B. J., Williams, R. E., and Fox, C. F., Proc. Nat. Acad. Sci. U.S. 70:3669 (1973).
20. Williams, R. E., Wisnieski, B. J., Rittenhouse, H. G., and Fox, C. F., Biochemistry 13:1969 (1974).
21. Agrawal, B., Goldstein, I., Hassing, G., and So, L., Biochemistry 7:4211 (1968).
22. Mizel, J., and Wilson, L., Biochemistry 11:2573 (1972).

23. Fox, C. F., in "Membrane Molecular Biology," C. F. Fox and A. D. Keith (Eds.). Stamford, Conn., Sinauer Associates, p. 345 (1972).
23a. Machtiger, N. A. and Fox, C. F., Ann. Rev. Biochem. 42:575 (1973).
24. Linden, C. D., Wright, K. L., McConnell, H. M., and Fox, C. F., Proc. Nat. Acad. Sci. U.S. 70:2271 (1973).
25. Linden, C. D., Keith, A. D., and Fox, C. F., J. Supramol. Struc. 1:523 (1973).
26. Parkes, J. G., Rittenhouse, H. G., Wisnieski, B. J., and Fox, C. F., Proc. Can. Fed. Biol. Soc. 17:148 (1974).
26a. Wisneiski, B. J., Huang, Y. O. and Fox, C. F., J. Supramol. Struc. 2:593 (1974).
26b. Fox, C. F., in "Biochemistry of Cell Walls and Membranes," C. F. Fox (Ed.). MTP International Reviews of Science: Biochemistry, Vol. 2, p. 279.
27. Berlin, R. D., Oliver, J. M., Ukena, T. E., and Yin, H. H., Nature New Biol. 247:45 (1974).
28. Marsland, D., and Asterita, H., Exp. Cell Res. 42:316 (1966).
29. Roth, L. E., J. Cell Biol. 34:47 (1967).
30. Kaneko, I., Satoh, H., and Ukita, T., Biochem. Biophys. Res. Commun. 50:1087 (1973).
31. Olmstead, J. B., and Borisy, G. G., Ann. Rev. Biochem. 43:507 (1973).
32. Yin, H., Ukena, T., and Berlin, R., Science 178:867 (1972).
33. Ukena, T. E., Borysenko, J. Z., Karnovsky, M. J. and Berlin, R. D., J. Cell Biol. 61:70 (1974).
33a. Berlin, R. D., personal communication.
34. Edelman, G. M., Cunningham, B. A., Reeke, G. N., Jr., Becker, J. W., Waxdal, M. J., and Wang, J. L., Proc. Nat. Acad. Sci. U.S. 69:2580 (1972).
35. Fox, T. O., Sheppard, J. R., and Burger, M. M., Proc. Nat. Acad. Sci. U.S. 68:244 (1971).
36. Smets, L. A., Nature New Biol. 245:113 (1973).
37. Glick, M. C., and Buck, C., Biochemistry 12:185 (1973).
38. McNutt, N. S., Culp, L. A., and Black, P. H., J. Cell Biol. 56:412 (1973).
39. Yamada, K. M., Spooner, B. S., and Wessels, N. K., J. Cell Biol. 49:614 (1971).
40. Stachelin, L. A., Chaplowski, F. J., and Bonnaville, M. A., J. Cell Biol. 53:73 (1972).
41. Revel, J.-P., and Wolken, Exp. Cell Res. 78:1 (1973).
42. Heaysman, J. E. and Pegrum, S. M., Exp. Cell Res. 78:71 (1973).
43. Heaysman, J. E. and Pegrum, S. M., Exp. Cell Res. 78:479 (1973).
44. McNutt, N. S., Culp, L. A., and Black, P. H., J. Cell Biol. 56:412 (1973).
45. Vasiliev, I. M., Gelfand, L. V., Domnina, L., Ivanova, O., Komm, S. and Olshevskaja, L. V., J. Embryol. Exp. Morphol. 24:625 (1970).
46. Hsie, A. W., Jones, C., and Puck, T. T., Proc. Nat. Acad. Sci. U.S. 68:1648 (1971).
47. Kram, R., and Tompkins, G. M., Proc. Nat. Acad. Sci. U.S. 70:1659 (1973).
48. Olsen, R. W., J. Theoret. Biol. (in press).
49. Bürk, R. R., Nature 219:1272 (1968).
50. Hsie, A. W. and Puck, T. T., Proc. Nat. Acad. Sci. U.S. 68:358 (1971).
51. Sheppard, J. R., Proc. Nat. Acad. Sci. U.S. 68:1316 (1971).
52. Ukena, T. E. and Berlin, R. D., J. Exp. Med. 136:1 (1972).
53. Yahara, I., and Edelman, G., Exp. Cell Res. 81:142 (1973).
54. Revel, J.-P., Hoch, P., and Ho, D., Exp. Cell Res. 84:207 (1974).