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MEMBRANE MOBILITY AGENT ALTERS THE CONSEQUENCES OF LECTIN-CELL INTERACTION IN A MALIGNANT CELL MEMBRANE

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SUMMARY

The membrane mobility agent 2-(2-methoxyethoxy)-ethyl 8-(*cis*-2-*n*-octyl-cyclopropyl)-octanoate promotes cap formation from wheat germ agglutinin-receptor combinations at the expense of agglutination in membranes of malignant mastocytoma cells.

INTRODUCTION

Malignant, transformed cells are agglutinated by several plant lectins at lectin concentrations which do not cause agglutination of the corresponding normal cell (for reviews, see refs 1 and 2). Alteration in the membrane by transformation is implied, with the nature of the change and the mechanism of its expression in terms of agglutinability still unknown.

Differences in agglutinability cannot be explained by differences in the quantity of lectin bound, since normal and transformed cells may bind the same amount of lectin [2]. In some studies, clustering of binding sites after lectin attachment has been observed in transformed but not in normal fibroblasts [3–5]. Cluster formation, which is assumed to facilitate efficient cell to cell contact, has been ascribed to a greater freedom of movement of lectin-receptor complexes within the membrane of the transformed cell [2, 3]. Others, however, have failed to demonstrate a correlation between lectin-receptor complex clustering and agglutination [6]. In addition, although lectins induce agglutination of lymphoma cells under conditions for which no agglutination of normal cells occurs, the mobility of some lectin-receptor or antibody-receptor complexes is greater within the normal cell membrane than within the transformed (malignant) cell membrane [7, 8]. The relationship of membrane site mobility to agglutinability is thus unclear.

Abbreviations: A_2C, 2-(2-methoxyethoxy)-ethyl 8-(*cis*-2-*n*-octylcyclopropyl)-octanoate; FITC, fluorescein isothiocyanate.

A number of cyclopropane derivatives which enhance the lateral mobility of antibody-Ig site combinations (promoting cap formation) on lymphocyte membranes have been developed by Kosower et al. [9]. These agents, called membrane mobility agents, provide one means of altering the distribution of membrane sites and examining the consequences of this alteration to the behavior of the cell. In the present study, we have used one of these reagents, A_2C (see formula), to probe the interaction of wheat germ agglutinin with mastocytoma cells.

$$CH_2$$

$$CH_3(CH_2)_7CH\text{-}CH(CH_2)_7COOCH_2CH_2OCH_2CH_2OCH_3$$

We have now found that the membrane mobility agent A_2C promotes a redistribution of lectin-membrane site complexes, a cap-like arrangement forming in place of the diffuse arrangement observed in the absence of the agent. The membrane mobility agent simultaneously diminishes the degree of agglutination by the lectin, and in addition, increases the sensitivity of interphase cells to the cytolytic effect of the lectin used (wheat germ agglutinin). The latter sensitivity is characteristic of mastocytoma cells in mitosis, as shown by Lustig and Pluznik [10].

MATERIALS AND METHODS

Cells. A mastocytoma cell line (P-815-X2) [11] was used. The cells were grown in Dulbecco's modified Eagle's medium (Gibco, USA), supplemented with 10 % heat inactivated horse serum. The cell line was maintained by transferring 10^5 cells to 5 ml fresh medium every 4–5 days. Cell populations with low mitotic indices (less than 1 %) were used in all the experiments. Cells were washed and suspended in buffer containing 140 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.3.

Wheat germ agglutinin. Unlabelled and labelled wheat germ agglutinin with fluorescein isothiocyanate (FITC-agglutinin) were obtained from Miles-Yeda, Rehovot. The lectins were kept frozen until use, then diluted with PBS to the proper concentration.

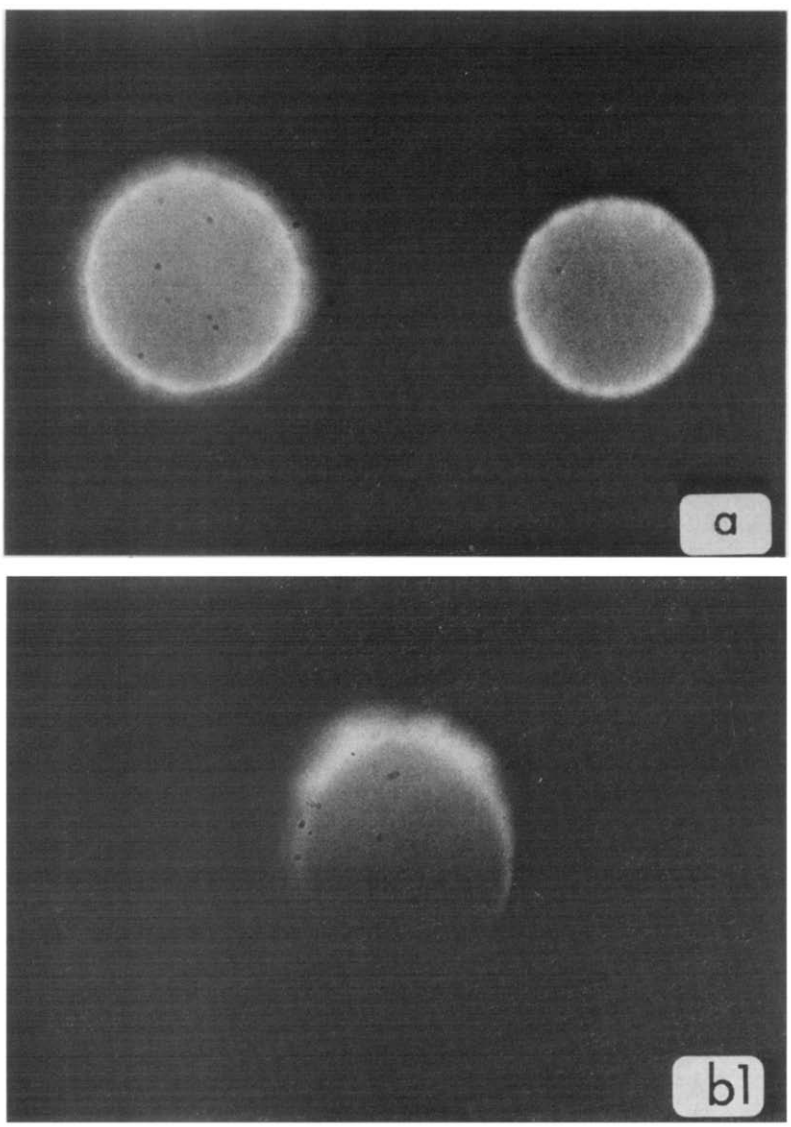
Membrane mobility agent. A_2C, [2-(2-methoxyethoxy)-ethyl 8-(*cis*-2-*n*-octyl-cyclopropyl)-octanoate)] was dispersed in phosphate-buffered saline by sonication for 2 min (MSE ultrasonic disintegrator) just before use.

Incubation and assays. A_2C, dispersed in buffer, was added to the cell suspension to final concentrations of 0.05 μl to 0.5 μl/ml containing $5 \cdot 10^6$ cells. Cell suspensions were incubated in the presence or absence of A_2C at 24 °C for 30 min. Wheat germ agglutinin (10 μg/ml cell suspension) was then added for additional incubation at 4 °C and 37 °C. Aliquots were withdrawn at various time intervals for determination of (a) extent of agglutination, (b) distribution of FITC-agglutinin bound to cell surface and (c) cytotoxicity.

Extent of agglutination of the cells by the lectin was determined according to Eckhart et al. [12] and calculated on the basis of the number of cells which were not agglutinated out of the total number of cells present in a control sample incubated without lectin. The distribution of FITC-agglutinin on the cells was observed with a Zeiss incident light fluorescence microscope.

Cytotoxicity was determined by counting cells in 0.4 % trypan blue solution in

phosphate-buffered saline. In samples treated with wheat germ agglutinin, counting was performed after desaggregation of cell agglutinates to single cells by the addition of 0.3 M *N*-acetyl-D-glucosamine. Percent of lysis was determined by comparing the number of cells found after incubation with wheat germ agglutinin to number of cells found after incubation without the lectin.



RESULTS AND DISCUSSION

Effect of A_2C on the distribution of bound FITC-agglutinin and on agglutination of cells by wheat germ agglutinin. FITC-agglutinin added to mastocytoma cells at 37 °C is distributed in the form of a ring of clusters (Fig. 1a), an appearance which is unchanged for samples taken throughout the incubation period. Mobility agent alters the observed distribution of bound lectin. In the first few minutes, the distribution of lectin sites on the cell surface of A_2C pretreated cells is similar to that of control cells. After 10–15 min, the fluorescent label is found in the form of a cap-like arrangement within a limited area on the cell surface (Fig. 1b).

The addition of 10 μg/ml of wheat germ agglutinin to mastocytoma cells causes their agglutination at 37 °C. Preincubation of the cells in the presence of A_2C diminishes markedly the agglutination, and the agglutinates which do form are composed of only a few cells rather than the large, multicellular aggregates seen with wheat germ agglutinin alone, as shown in Fig. 2. The distribution of FITC-agglutinin on the aggregated cells is shown in Fig. 3. The control cells forming the multicellular aggregates exhibit the fluorescence from the FITC-agglutinin as an uninterrupted ring around the cell periphery (Fig. 3a). In the A_2C pretreated cells, the fluorescent label occupies only particular portions of the cell membrane, those engaged in cell to cell contact. No contact or further aggregation is seen on those portions of the cell membrane free of FITC-agglutinin (Fig. 3b). Decreased aggregation is clearly a consequence of the decreased opportunity for cell to cell interaction presented by the limited area occupied by the lectin-receptor site complexes.

The diminution in the extent of agglutination with increasing concentration

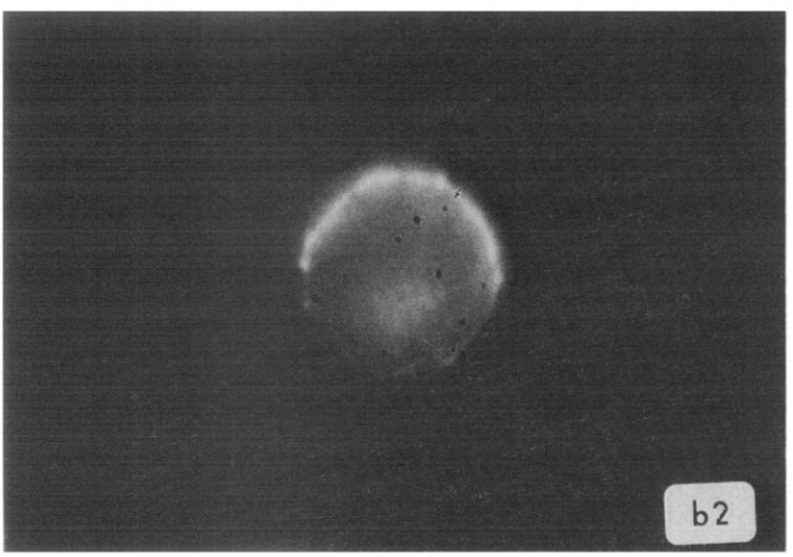


Fig. 1. Distribution of FITC-agglutinin on control and A_2C-treated cells. The A_2C treatment was with 0.15 μl ml cell suspension. For other details, see text. (a) Control cells: 15 min after FITC-agglutinin addition. (No further change is observed on additional incubation.) (b1 and 2). A_2C-treated cells: 15 min after FITC-agglutinin addition.

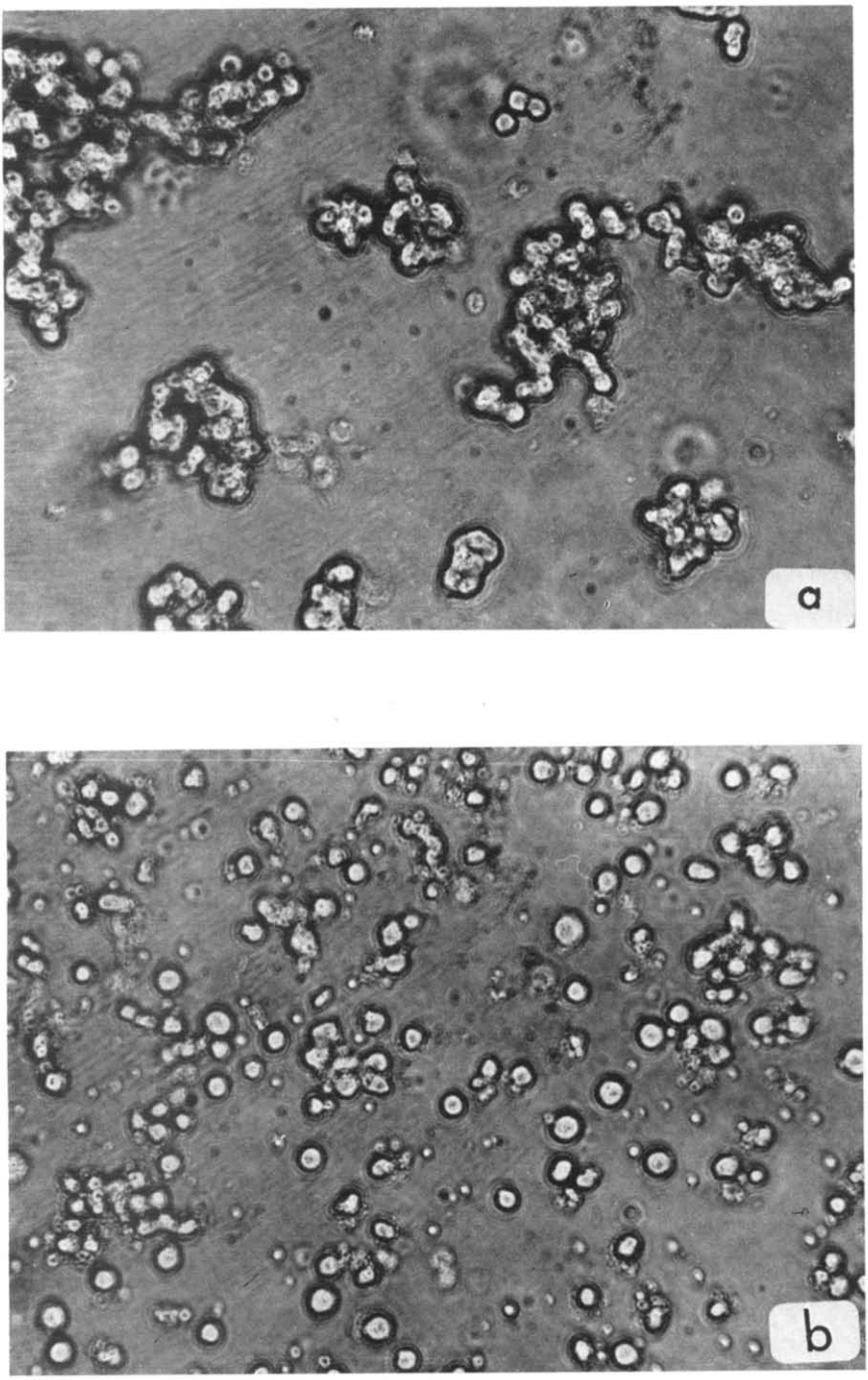


Fig. 2. Effect of A_2C on agglutination of mastocytoma cells by wheat germ agglutinin. A_2C 0.25 μl ml cell suspension. Aliquot removed 15 min after wheat germ agglutinin addition. viewed and photographed at room temperature. (a) Control cells. (b) A_2C treated cells.

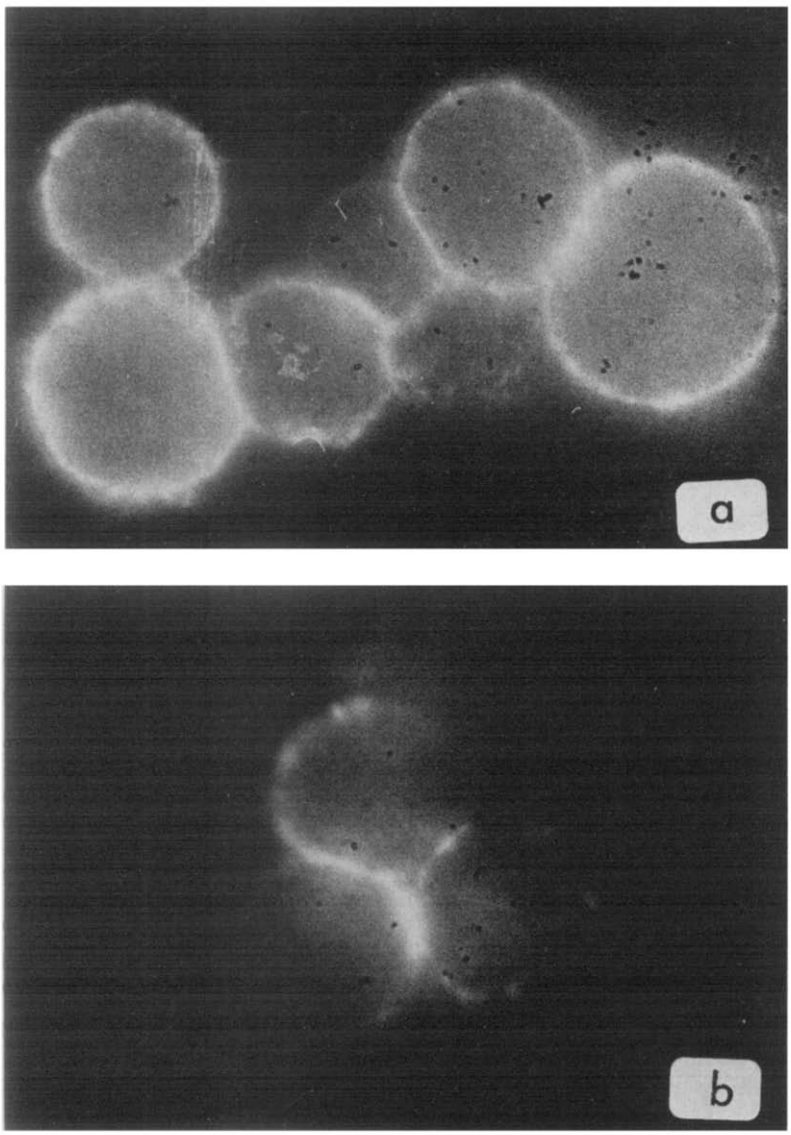


Fig. 3. Distribution of FITC-agglutinin on agglutinated cells in control and A_2C-treated cells. (a) Control sample. (b) A_2C-treated sample.

of A_2C is paralleled by the increase in the number of cells showing caps, as graphically illustrated in Fig. 4.

Effect of A_2C on the viability of interphase mastocytoma cells. The effect of A_2C (with and without added wheat germ agglutinin) on cell viability is shown in Fig. 5. Incubation of cells with A_2C alone for 90 min at 37 °C did not affect cell viability when the reagent was used in amounts up to 0.25 μl/ml. Higher concentrations caused cell death, as manifested by lysis. All intact cells remained viable, as shown by the trypan blue exclusion test. No cytotoxicity was produced by incubation of cells with

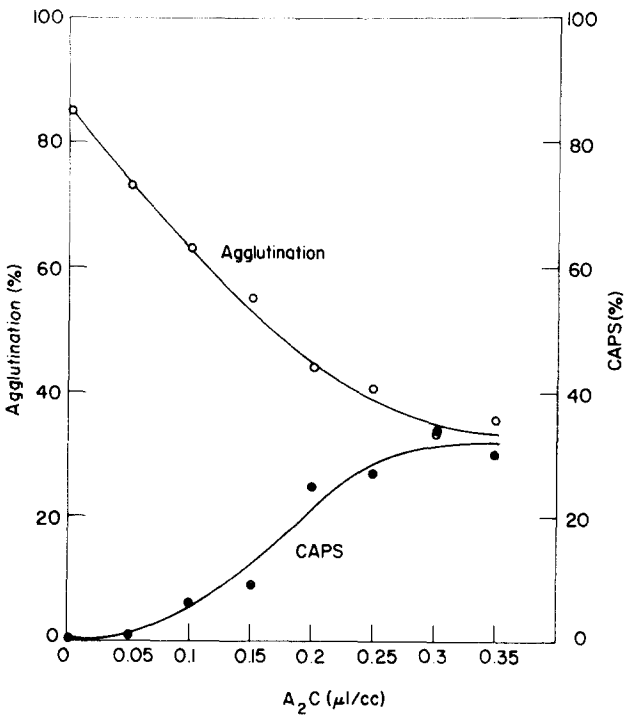


Fig. 4. Relationship of cap formation to agglutination in A_2C-treated cells. For details of preincubation and wheat germ agglutinin addition, see text. Following incubation in the presence of wheat germ agglutinin (15 min), the cells were cooled to 1–2 °C and aliquots taken for determination of agglutination and for evaluation of cap formation. "Caps" were defined as an accumulation of fluorescent label over 1/2 or less of the visible cell surface.

wheat germ agglutinin alone. Preincubation of the cells with A_2C increased the sensitivity of the interphase cells to lysis by wheat germ agglutinin, the degree of lysis being proportional to the amounts of A_2C above 0.05 μl/ml. It had been previously found that wheat germ agglutinin alone had no cytolytic effect on interphase mastocytoma cells whereas mitotic cells were lysed by the lectin [10]. The sensitivity of the interphase cells towards wheat germ agglutinin in the presence of A_2C is thus reminiscent of the sensitivity observed for cells in mitosis.

Effect of low temperature and of sodium azide on agglutination, capping and cytolysis. Incubation of control cells with 10 μg/ml of wheat germ agglutinin at 4 °C did not result in agglutination and FITC-agglutinin was distributed as a "ring" on the cells. Neither "cap" formation nor cytolysis occurred in A_2C-treated cells incubated with wheat germ agglutinin at 4 °C. The addition of 10 mM sodium azide to A_2C-preincubated cells at 37 °C 5 min before the addition of wheat germ agglutinin promoted cell agglutination by the lectin, prevented "cap" formation and inhibited cell lysis. The results of the experiments are presented in Table I.

Significance. The interaction of wheat germ agglutinin with membrane receptor sites is shown in our work to be modulated by the membrane mobility agent A_2C. The agent promotes "cap" formation, diminishes cell agglutination and enhances the

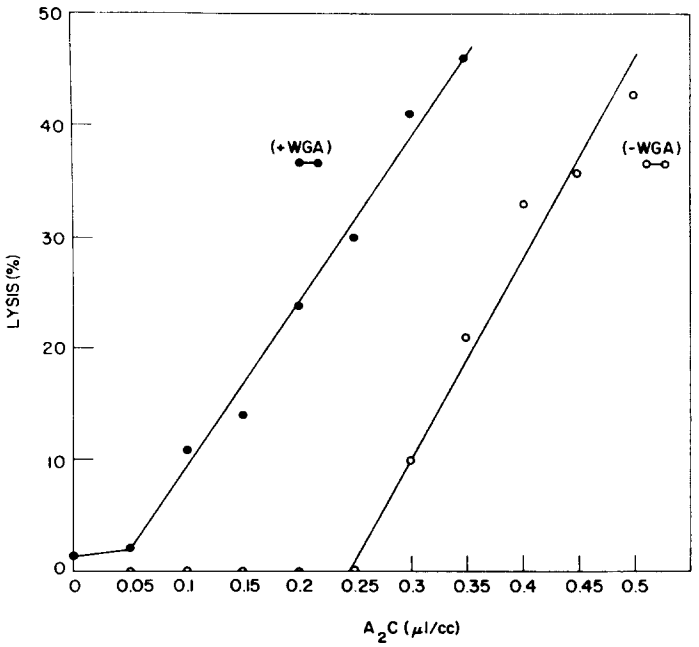


Fig. 5. Cytotoxic effect of wheat germ agglutinin on A_2C treated mastocytoma cells. An effect proportional to the concentration of A_2C is shown by the line marked + WGA. A parallel line is found for the effect of A_2C in the absence of wheat germ agglutinin (− WGA line) at much higher A_2C concentrations.

TABLE I

THE EFFECT OF SODIUM AZIDE AND OF LOW TEMPERATURE ON THE COURSE OF WHEAT GERM AGGLUTININ INTERACTION WITH A_2C-TREATED MASTOCYTOMA CELLS

	Conditions								
	4 °C[a]			37 °C			NaN_3[b]		
	agglut. (%)	caps (%)	lysis[c] (%)	agglut. (%)	caps (%)	lysis[c] (%)	agglut. (%)	caps (%)	lysis[c] (%)
+ A_2C	0	0	2	34	32	42	84	0	2
− A_2C	0	0	0.5	85	0	1.5	84	0	0

[a] Cell samples were preincubated in the absence or presence of 0.3 μl/ml A_2C for 30 min at 24 °C, following which samples were cooled to 1–4 °C, wheat germ agglutinin added and the estimation of agglutination and the extent of cap formation done 15 min later without warming the samples.

[b] Cell samples were preincubated in the absence of presence or 0.3 μl/ml A_2C for 30 min at 24 °C, following which cells were transferred to 37 °C and NaN_3 was added to give a final concentration of 0.01 M. Wheat germ agglutinin was added 5 min later and incubation continued at 37 °C for 15 min, at which time aliquots were utilized for the determination of the extent of cap formation and the degree of agglutination.

[c] Lysis was determined on samples incubated for 60 min at 37 °C after the addition of wheat germ agglutinin.

cytolytic effect of wheat germ agglutinin. It has been suggested that agglutinability of such cells as transformed fibroblasts reflects rapid lateral mobility of lectin sites in those cell membranes, a mobility greater than that in normal cell membranes [3, 13]. A more "fluid" state of the membrane [14] and an altered interaction with submembrane structures [15, 16] have been suggested as possible explanations for the difference in mobility [13]. However, clustering in both normal and transformed cells has been reported [6], a fact which weakens the concept cited above. It is also of interest that the enhanced mobility of clustered lectin sites induced by colchicine is accompanied by a decrease in agglutinability [17].

In studies on cells in suspension, there appears to be an inverse relationship between agglutinability and ease of membrane site motion in lymphocytes. Normal lymphocytes are capable of cap formation but do not agglutinate in the presence of the appropriate lectin, whereas the malignant lymphoma cell agglutinates but does not exhibit facile motion of receptor sites [7]. In addition, the capacity of some myeloblastic cells to differentiate to normal forms is associated with reduced agglutinability and enhanced membrane site mobility [18]. (See also ref. 19.)

The results found in our work are consistent with those found for cells in suspension. The observed redistribution of wheat germ agglutinin-membrane site complexes and their accumulation within a restricted area of the membrane ("cap" formation) reflect an enhanced lateral mobility of some membrane components induced by A_2C. This increased mobility is associated with decreased agglutination. The reciprocal relationship is preserved with the changes brought about by sodium azide (presumably functioning as a metabolic inhibitor) which allows agglutination, prevents "cap" formation and inhibits cytolysis. Further, the enhanced susceptibility of A_2C treated cells to wheat germ agglutinin-induced lysis is similar to that observed for mastocytoma cells in mitosis [10]. Thus, A_2C alters the response of the interphase mastocytoma cell to wheat germ agglutinin into a response resembling that of cells in mitosis.

We may also note that in preliminary experiments we have found that A_2C does not alter ConA-mastocytoma cell interaction, observed as agglutination of cells and evenly distributed clusters. Thus different receptor sites within a particular cell membrane may exhibit different responses to membrane mobility agents, this fact suggesting that there is some specificity in the action of these agents.

The "cap" formation promoted by A_2C and any other mobility process which permits the accumulation of sites in a restricted area of the membrane, would be expected to diminish the probability of multiple bridge formation between cells. For the mastocytoma cells, as well as for other malignant cells in suspension, increased agglutinability is associated with lower mobility for certain sites within the membrane.

In conclusion, it appears that caution is required before retaining the generalization that overall higher membrane mobility and higher "fluidity" within the membrane are characteristic of malignant cells.

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REFERENCES

1 Rapin, A. M. C. and Burger, M. M. (1974) Adv. Cancer Res. 20, 1–91
2 Hynes, R. O. (1974) Cell 1, 147–156
3 Nicolson, G. L. (1973) Nat. New Biol. 243, 218–220
4 Rosenblith, J. Z., Ukena, T. E., Yin, H. H., Berlin, R. D. and Karnovsky, M. J. (1973) Proc. Natl. Acad. Sci. U.S. 70, 1625–1629
5 Shoham, J. and Sachs, L. (1974) Exp. Cell. Res. 85, 8–14
6 Raff, M., dePetris, S. and Mallucci, L. (1974) in Control of Proliferation in Animal Cells, Cold Spring Harbor Conf. on Cell Proliferation, Vol. 1, pp. 271–281
7 Inbar, M. and Sachs, L. (1973) FEBS Lett. 32, 124–128
8 Yefenof, E. and Klein, G. (1974) Exp. Cell. Res. 88, 217–224
9 Kosower, E. M., Kosower, N. S., Faltin, Z., Diver, A., Saltoun, G. and Frensdorf, A. (1974) Biochim. Biophys. Acta 363, 261–265
10 Lustig, S. and Pluznik, D. H., submitted for publication
11 Dunn, T. B. and Dotter, M. J. (1957) J. Natl. Cancer Inst. 18, 587–601
12 Eckhart, W., Dulbecco, R. and Burger, M. M. (1971) Proc. Natl. Acad. Sci. U.S. 68, 283–286
13 Nicolson, G. L. (1974) in Control of Proliferation in Animal Cells, Cold Spring Harbor Conf. on Cell Proliferation, Vol. 1, pp. 251–270
14 Singer, S. J. and Nicolson, G. L. (1972) Science 175, 720–731
15 Yahara, I. and Edelman, G. M. (1973) Nature 236, 152–154
16 Berlin, R. D., Oliver, J. M., Ukena, T. E. and Yin, H. H. (1974) Nature 247, 45–46
17 Ukena, T. E., Borysenko, J. Z., Karnovsky, M. J. and Berlin, R. D. (1974) J. Cell. Biol. 61, 70–82
18 Inbar, M., Ben-Bassat, H., Fibach, E. and Sachs, L. (1973) Proc. Natl. Acad. Sci. U.S. 70, 2577–2581
19 Edidin, M. (1974) Annu. Rev. Biophys. Bioengineer. 3, 179–201