Reports

Cell Rigidity: Effect on Concanavalin A—Mediated Agglutinability of Fibroblasts After Fixation

Abstract. *A quantitative hemadsorption assay distinguishes the effects of membrane fixation on concanavalin A–mediated agglutinability of fixed cells with unfixed cells. We observed undiminished adherence of unfixed erythrocytes to glutaraldehyde-fixed normal and virus-transformed hamster fibroblasts coated with concanavalin A. Fixation of the erythrocytes abolished agglutination with fixed fibroblasts. The agglutinability of fixed cells is more likely related to increased cell rigidity than to decreased membrane fluidity.*

Cells transformed by viruses and cells treated with trypsin are agglutinated by lower concentrations of lectins than are normal cells (*1*), but the mechanism of the enhanced agglutination of these cells is not clear. At present, the favored proposal is that the lectin receptor sites of transformed or trypsinized cells are more mobile than those of normal cells, so that lectins can more easily induce clustering of the receptor sites, thus enhancing agglutination. The increased agglutination of transformed and trypsinized cells and clustering of receptor sites are both inhibited after fixation (*2–4*). Marquardt and Gordon (*5*) have reported that fixation of trypsinized human erythrocytes does *not* alter their concanavalin A (Con A)–mediated agglutination when tested with unfixed cells.

Consideration of the data available on the effect of fixation on the agglutinability of cells raises the question whether the contrast in reports of agglutinability of fixed cells reflects either a fundamental difference in membranes or a difference that arises from the methods used to evaluate agglutination. We have, therefore, studied agglutination of glutaraldehyde-fixed Syrian hamster cells with an assay method (*6*) that allows us to distinguish between the effect of fixation on one or both of the agglutinating surfaces.

The extent of agglutination is determined by the number of erythrocytes (indicator cells) that adhere to the exposed surface area of cells in a monolayer (test cells). Hence, the data are expressed as the mean number of erythrocytes adhering per square centimeter of flask surface covered by cells. The agglutination assay offers advantages over the usual techniques involving visual scoring of suspended cells that are often collected from monolayer cultures after the use of trypsin or other means. The cells require little manipulation since the assay is carried out in the flask containing the undisturbed monolayer of cells; the method allows us to distinguish the effect of fixation on either the test cells or indicator cells, and the evaluation of agglutination is easily quantified under conditions relatively free from shear forces (Table 1).

Syrian hamster embryonic cells (SHE) that had been passaged in culture three to five times served as normal controls for the polyomavirus-infected Syrian hamster cells (Py-SHE). The infected cells were tumorigenic and have the morphology, growth patterns, and antigenicity characteristic of transformed cells. The estimates of the flask surface covered with the normal cells were 80 to 90 percent and for the

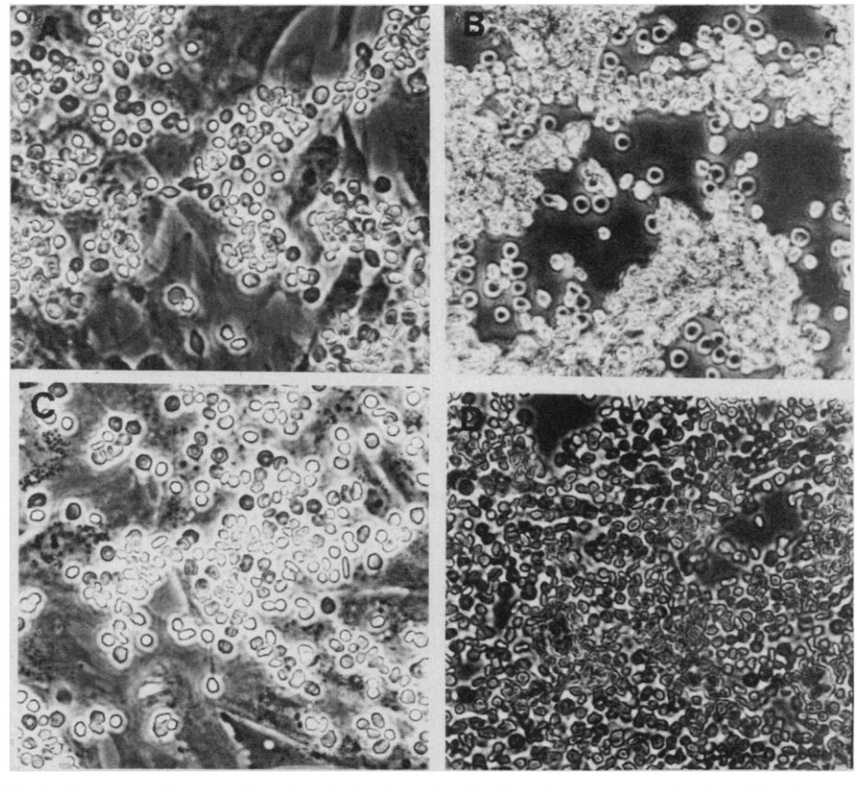

Fig. 1. Concanavalin A–mediated agglutination of unfixed erythrocytes to unfixed and fixed monolayers. (A) Unfixed normal Syrian hamster monolayer. (B) Unfixed polyoma-transformed Syrian hamster monolayer. (C) Fixed normal Syrian hamster monolayer. (D) Fixed polyoma-transformed Syrian hamster monolayer.

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Table 1. Concanavalin A–mediated agglutination of unfixed erythrocytes to unfixed and fixed monolayers. The monolayers were washed with phosphate-buffered saline (PBS), incubated 10 minutes with Con A (50 μg/ml) in PBS at 35°C, washed three times with PBS, incubated 20 minutes with a 1 percent suspension of human erythrocytes (O positive, outdated) in PBS at 35°C, and the nonadherent erythrocytes removed with three washings with PBS. Visual scoring of hemadsorption was by phase microscopy, 0 to 4+. For hemolysis the adherent erythrocytes were lysed with water, and the hemoglobin absorbance was determined (Cary spectrophotometer) at 415 nm. Hemoglobin absorbancy values were subsequently converted to the number of erythrocytes that had adhered to the monolayers, and the total cell surface area available for hemagglutination was calculated from the estimated area covered by cells in the T-30 Falcon flasks (25 cm^2). Hemadsorption was inhibited with 50 m*M* α-methyl-D-mannopyranoside (α-MM) in PBS. Data include results from three experiments; four to five flasks were averaged for each experimental value; the maximum standard error of the mean was 10 percent. RBC, red blood cells.

Monolayers	Hemadsorption assay					
	Visual			Hemolysis (10^{-5} RBC/cm^2)		
	Con A	Con A + α-MM	No Con A	Con A	Con A + α-MM	No Con A
SHE	++	0	0	1.83	0.25	0.23
SHE (fixed)*	+++	0	0	4.41†	0.34	0.17
Py-SHE	++++	+	+	8.92†	1.14	1.12
Py-SHE (fixed)*	++++	±	±	9.16	0.77	0.96

*Monolayers were fixed for 4 hours with 2.5 percent glutaraldehyde followed by a 10-minute incubation with 0.2*M* glycine in PBS at 4°C and washed three times with PBS before initiation of assay. †$P < .001$, by Student's *t*-test, indicates significant differences from SHE unfixed monolayers.

transformed cells about 65 to 75 percent. A simple correction for cell surface area available for agglutination was made on this basis.

We found no inhibition of Con A–mediated agglutination after glutaraldehyde fixation of either the normal or transformed monolayers (Table 1). Fixation of monolayers leads to enhancement of lectin-mediated hemadsorption to SHE monolayers. Photographs showing the characteristic patterns of hemadsorption to unfixed and fixed, normal and transformed monolayers are shown in Fig. 1, A–D. The addition of α-methyl-D-mannopyranoside reduced the number of adherent erythrocytes to nearly the level obtained when Con A was omitted (Table 1).

The number of fixed erythrocytes adhering to the unfixed normal or transformed monolayers was reduced 50 to 60 percent compared to the adsorption of unfixed erythrocytes to the same monolayers (Table 2). It is unlikely that the inhibition of hemadsorption of fixed erythrocytes can be related to inhibition of Con A–induced aggregation of receptor sites, because the Con A was adsorbed to the unfixed Syrian hamster monolayers; the unfixed erythrocytes showed a greater lectin-mediated affinity for fixed monolayers compared to unfixed monolayers (Table 1). A more dramatic reduction (80 to 96 percent) of adherent erythrocytes was observed when fixed erythrocytes interacted with fixed monolayers (Table 2), suggesting a still greater instability of the interaction when both the test cells and indicator cells were fixed.

It has been proposed that the major difference between the sensitivity of normal and transformed or trypsinized cells to agglutination by Con A is due to the difference in the susceptibility of the receptor sites to Con A–*induced* clustering (*2–4, 7*). Several morphological techniques have shown that Con A receptor sites are randomly dispersed after fixation of the cells or incubation at low temperatures, conditions that also presumably inhibit the enhanced agglutination of transformed cells (*4, 8*). However, it is not clear whether lower temperatures inhibit cell agglutination by immobilizing receptors per se because the Con A dissociates to relatively inactive dimers at low temperature (*9*).

Our results with fixed SHE and Py-SHE monolayers indicate that relative agglutinating ability of these cells with Con A is not altered when they contact unfixed cells. This finding is similar to that reported for mixtures of unfixed and fixed erythrocytes under shear conditions (*5*). Erythrocytes are known to be less deformable after glutaraldehyde fixation (*10*). Cell rigidity and not altered clustering of receptors was a better explanation for the effects of fixation on erythrocyte-to-erythrocyte agglutination (*5*). We suggested that large cell-to-cell contacts are still possible if a portion of the cells were unfixed. The unfixed cell can, then, accommodate the rigid surface of the fixed cell while two fixed cells contact each other over a smaller area. This interpretation adequately explains the similar findings of fixation on the fibroblast cells reported here, although we are unable to visualize the contact between cells as in the previous study (*5*). In any event, the immobility of receptor sites after fixation (*2*) cannot explain the observation that agglutinability of fixed cells is undiminished when tested with unfixed cells. The contrast in our results with other reports can be attributed to the use of a method that experimentally separates the effects of fixation when only one cell surface is fixed from the effect of two opposing fixed surfaces in the agglutination reaction.

Table 2. Concanavalin A–mediated agglutination of fixed erythrocytes to unfixed and fixed monolayers. Erythrocytes were fixed in 2.5 percent glutaraldehyde in phosphate-buffered saline for 4 hours at room temperature and washed four times in this buffer. Other conditions of assay were the same as those described in the legend for Table 1, except that lysis of fixed erythrocytes was not possible, and the adherent fixed and unfixed erythrocytes were eluted with 100 m*M* α-methyl-D-mannopyranoside in the same buffer and counted in a Bio/Physics Systems model 6301 cytograf.

Monolayers	Ratio of fixed erythrocytes to unfixed erythrocytes
SHE	0.40
SHE (fixed)	0.04
Py-SHE	0.51
Py-SHE (fixed)	0.19

D. Ann Gibson
M. D. Marquardt
Julius A. Gordon
Department of Pathology, University of Colorado Medical Center, Denver 80220

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