erythrocytes. This is consistent with the report (*6*) that antibody-forming cells do not bear C3 receptors.

Rat serum activated by CoF diminished by threefold the ability of normal rat spleen cells to differentiate into hemolytic plaque-forming cells in syngeneic irradiated hosts challenged with sheep red blood cells (Table 1). The same treated cells were unimpaired in their ability to induce a graft-versus-host reaction in semiallogeneic LBN rats (Table 1), suggesting that CoF-activated serum had no effect on T lymphocytes.

Other procedures have been used to eliminate B cells (*6, 12*). Differential centrifugation and treatment on columns containing immunoadsorbents—such as immune complexes or antibody to immunoglobulins—may be effective methods, but are quite laborious. The specific lysis of B cells by antibody to immunoglobulin and complement may be obscured by the presence of some immunoglobulin determinants on T cells (*13*). Our procedure is simple and rapid; it can, in theory, be used on the lymphoid cells from any species. Indeed, our recent experiments have shown it effective with both mouse and human cells (*14*).

While it is interesting to postulate that CRL were destroyed by passive cytolysis in vitro, two other possibilities must be considered. Cells coated with C3 may be more susceptible to phagocytosis in vivo, or these cells may be sterically hindered from performing their function by cell-bound complement components. Whether all B cells are affected by our procedure and whether the effects totally spare T cells remain to be determined.

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7. Pooled rat serum [44 hemolytic complement (CH_{50}) unit/ml] was obtained from Suburban Serum Laboratories, Silver Spring, Maryland. It contained no measurable cytotoxic activity against Lewis rat spleen cells.
8. For separation of cobra factor (CoF), lyophilized *Naja siamensis* venom (Miami Serpentarium) was placed on a DEAE cellulose column (100 by 2.5 cm) equilibrated with 0.005*M* phosphate buffer (*p*H 7.4); the column was eluted with a continuous salt gradient (0.005 to 0.5*M* NaCl) in 0.005*M* phosphate buffer. The CoF activity appeared in the second protein peak and, at a 1 : 10,000 dilution, diminished by greater than 50 percent the CH_{50} activity of whole guinea pig serum (188 CH_{50} unit/ml), but was nontoxic when injected undiluted into 4-day-old mice.
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Effect of Colchicine, Colcemid, and Vinblastine on the Agglutination, by Concanavalin A, of Transformed Cells

Abstract. *The sensitivity of transformed 3T3 fibroblasts to agglutination by concanavalin A is reduced by alkaloids that bind specifically to protein subunits of microtubules.*

Tumor cells that have been transformed by viruses or other agents develop a sensitivity to agglutination by concanavalin A (Con A) and some other lectins (specific proteins that bind carbohydrates) (*1*). The increased agglutinability appears not to reflect an increase in the number of Con A binding sites (*2*), but rather a change in their surface topography: a shift from a normal homogeneous or random pattern to a heterogeneous or patchy distribution (*3*). In an attempt to determine the factors that facilitate agglutination, or effect the segregation of binding sites, or both, we have examined the agglutination, by Con A, of polymorphonuclear leukocytes (PMN) (*4*). These normal cells have Con A binding sites distributed heterogeneously over their surfaces (*5*), and are agglutinable by Con A. Low concentrations of colchicine and vinblastine, alkaloids that bind to protein subunits of microtubules, reduced the sensitivity of PMN to agglutination. We report here the effect of these alkaloids on the agglutination, by Con A, of the mouse fibroblast 3T3, and of 3T3 cells transformed by simian virus 40 (SV3T3). The characteristics of these cells are the prototypes of the above-mentioned differences between normal and transformed cells with respect to agglutinability and surface topography. We will show that colchicine, Colcemid, and vinblastine reduce the sensitivity of SV3T3 cells to agglutination by Con A.

The 3T3 and SV3T3 cells (*6*) were grown to confluence in plastic tissue culture flasks (Falcon) in Dulbecco's modified Eagle's medium (Grand Is-

Table 1. Effect of colchicine on the adherence of RBC's to 3T3 and SV3T3 cells treated with Con A. Nonconfluent monolayers of fibroblasts were incubated for 1 hour at 37°C with or without alkaloids in culture medium, washed three times in phosphate-buffered saline (PBS), incubated for 10 minutes with Con A (Miles-Yeda), and washed three times in PBS. A washed suspension of human type O outdated RBC's from a blood bank, at a hematocrit of 2 percent, was then layered over the monolayers and incubated for 20 minutes. The monolayer was then rinsed thoroughly with PBS, and fixed with 1 percent glutaraldehyde for 2 minutes. The adherent RBC's were enumerated under phase contrast microscopy. Colchicine was added at a concentration of $10^{-6}M$.

Treatment	Mean number of adherent RBC's per fibroblast* at Con A concentrations (μg/ml) of:					
	0	20	40	100	300	500
			3T3 Cells			
None	0.0	—	—	0.42	0.32	—
Colchicine	0.0	—	—	0.24	0.10	—
			SV3T3 Cells			
None	0.0	0.20	1.33	2.43	3.56	3.48
Colchicine	0.0	0.16	0.13	0.31	0.19	0.36

* Numerical index for hemagglutination was obtained by adding the total number of adherent RBC's, and dividing by the numbers of fibroblasts counted in random fields. When the numbers of RBC's adherent per fibroblast exceeded four (often in clusters), precise enumeration was difficult. Consequently, an arbitary value of ten RBC's per fibroblast was assigned for purposes of calculation.

land Biological) supplemented with 10 percent calf serum (Microbiological Associates) inactivated by heat (56°C, 1 hour), without antibiotics in 10 percent CO_2 in air. The cultures were examined periodically for mycoplasma contamination (*7*). The 3T3 and SV3T3 cells were removed from the tissue culture flasks with 0.25 percent (1 : 250) trypsin, were plated into individual wells of multidish trays (Linbro), were incubated for 24 to 48 hours, and were then assayed for agglutination by the microhemadsorption method of Furmanski *et al.* (*8*). This assay is based on the enumeration of red blood cells (RBC) adherent to fibroblast monolayers treated with Con A. The data are given as the mean number of adherent RBC's per fibroblast (a representative of four experiments is shown in Table 1). The hemagglutination assay was more sensitive and reproducible in our laboratory than were assays involving agglutination in suspension (*9*).

In the absence of Con A, virtually no hemagglutination occurred to either 3T3 or SV3T3 cells (Table 1). However, in accord with Furmanski *et al.*, differences were found when fibroblasts were treated with Con A, were washed, and were then exposed to RBC's (Table 1, lines 1 and 2). However, when monolayers were treated first for 1 hour with colchicine, the hemagglutination to SV3T3 cells was reduced (Table 1, line 4) to levels seen with untransformed cells. The dose response of hemagglutination with changing Con A concentration, in the presence or absence of colchicine, shows significant differences at all concentrations (Table 1). One of the effects of treatment was the virtual elimination of a population of fibroblasts with multiple adherent RBC's. Vinblastine and Colcemid, at concentrations of $10^{-6}M$ and $3 \times 10^{-7}M$ (the lowest concentration tested), produced effects of similar magnitude.

Colchicine has been observed to produce morphological changes in cells, including contraction from an elongate to a polygonal form with shortened processes (rounding), ruffling or activity of the entire surface, and directionally random movements (*10*). By 1 hour, most (~ 80 percent) of the cells treated with colchicine appear somewhat rounded. Morphologically similar, but untreated SV3T3 cells, which constitute roughly 5 percent of the population, also are not hemagglutinated. Cells treated with alkaloid are fully viable, as judged by their exclusion of eosin Y.

Table 2. Effect of colchicine on the adherence to SV3T3 cells of RBC's treated with Con A. Fibroblasts were treated with $10^{-6}M$ colchicine as described in Table 1. However, they were not incubated with Con A solutions. Instead, RBC's were incubated with Con A for 10 minutes, and were then washed by centrifugation and resuspension three times in PBS. Such RBC's were then added as 2 percent suspensions to monolayers of SV3T3 cells, and were incubated for 20 minutes. The monolayers were then washed, and the adherent RBC's were enumerated as in Table 1.

Treatment	Mean number of adherent RBC's per fibroblast at Con A concentrations (μg/ml) of:			
	0	20	100	300
None	0.0	2.7	3.1	4.6
Colchicine	0.0	3.0	3.9	4.4

When RBC's are treated with Con A and washed, they adsorb readily to 3T3, and only slightly more avidly to SV3T3 cells (*8*). Similarly, RBC's treated with Con A adsorb equally to SV3T3 cells and to SV3T3 cells treated with colchicine (Table 2). (Red blood cells have no morphologically demonstrable microtubules.) This indicates that Con A binding sites are available on the surface of SV3T3 cells with and without colchicine treatment. If SV3T3 cells treated with Con A are hemagglutinated, and then incubated with colchine ($10^{-6}M$, 1 hour), the RBC's detach.

These results establish that the agglutination of transformed cells [as of PMN (*4*)] is inhibited by colchicine, Colcemid, and vinblastine, which at low concentrations bind specifically to protein subunits of microtubules (*11*). The sensitivity to agglutination is reduced to nearly that of untransformed cells. Receptors for Con A are available on untreated cells and on cells treated with alkaloid, as RBC's treated with Con A were bound equally well to SV3T3 cells whether or not the SV3T3 cells were treated with colchicine. The difference in hemagglutination between 3T3 and SV3T3 cells, when they are treated with Con A, is not present when they are hemagglutinated with RBC's treated with Con A (*8*). Since, as noted, the topographical distribution of Con A binding sites is the feature most singularly associated with the increased agglutinability of transformed cells, our results suggest that protein subunits of microtubules are major determinants of the topographical arrangement of Con A binding sites. Other effects of such microtubular proteins on the surface, such as an increase in the mechanical activity of the surface, which could destabilize Con A bridges linking cells, are alternative possibilities. However, several additional observations favor the former interpretation. For example, colchicine and vinblastine abolish the topographical localization of membrane transport systems, as determined by functional analyses of PMN (*12*), while phagocytosis by PMN, which depends on stable surface-to-particle contact, is only slightly inhibited by colchicine (*12*).

It is becoming clear that the topographical distribution of antigenic determinants and other functional elements in the cell membrane is heterogeneous (*13*). On the other hand, the fluidity of the lipid matrix, which permits the rapid lateral migration of proteins within the surface layer, has been demonstrated (*14*). Our results suggest that protein subunits of microtubules either stabilize arrangements of elements within this fluid matrix that may otherwise provide a medium for their randomization, or are essential for the induction of such arrangements by exogenous multivalent substances like Con A.

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