

IMMUNE T LYMPHOCYTE TO TUMOR CELL ADHESION Magnesium Sufficient, Calcium Insufficient

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ABSTRACT

The prelytic adhesion of immune cytolytic thymus-derived lymphocytes to specific antigen-bearing ascites tumor target cells has been studied. A new assay was used in which adhesions are permitted to form for 2.5 min; the cells are then dispersed to prevent further adhesion, and the predispersion adhesions are quantitated by subsequent ^{51}Cr release from the tumor cells as a result of the cytolytic activity of the adhering lymphocytes. There were the following new findings: (a) magnesium is sufficient to support optimal adhesion formation even when EGTA is added to remove contaminating traces of calcium; (b) calcium supports no adhesion formation when traces of contaminating magnesium are removed by pretreating the medium with a chelating ion exchange resin; (c) calcium synergizes with suboptimal magnesium, increasing the apparent adhesion-supporting potency of magnesium 20-fold in the presence of $50\ \mu\text{M}$ calcium; (d) in the presence of optimal magnesium (2–4 mM), calcium has no effect on the properties of the adhesion by any of six criteria; and (e) manganese supports adhesion better than magnesium, and strontium is ineffective. A survey of previous literature indicates that these results are remarkably similar to the predominant pattern for nonimmunologic cell adhesion (e.g., fibroblasts) involving cells from a variety of tissues in late embryonic and adult avians and mammals. This suggests that a “magnesium sufficient, calcium insufficient” mechanism may be found among the latter types of cell adhesions when appropriately examined. Moreover, it seems likely that the present lymphocyte–tumor cell adhesion, although evoked by specific receptor–antigen recognition, relies predominantly on mechanisms common to nonimmunologic intercellular adhesion processes.

KEY WORDS calcium · magnesium · manganese · intercellular adhesion · new adhesion assay · immune lymphocyte adhesion

Thymus-derived (T) lymphocytes are recognized as playing a central role in all forms of immune responses, including resistance to infectious agents and certain neoplasms, graft rejection, and various immunopathologies including allergies, other hypersensitivities, and autoimmune disease (81). One

lineage of T lymphocytes differentiates, in response to antigen, into cytolytic effector cells, that is, cells capable of killing specific antigen-bearing target cells. Cytolytic T lymphocytes (CTL) can kill nucleated eukaryotic cells such as lymphocytes, fibroblasts, macrophages, or tumor cells, and are believed to provide the major effector mechanism in graft rejection (18).

Following intercellular contact, the interaction between CTL and target cells has been resolved

into three stages: (a) the formation of a strong adhesion between the two cell membranes within about 2 min, (b) within about 10 additional min, the administration of a lethal hit which programs the target cell for subsequent lysis, and (c) the process of lysis itself, which usually requires an hour or more, occurs independently of continued contact with the CTL, and terminates in the disintegration of the target cell membrane and release of soluble cytoplasmic constituents including the conventional radioisotopic marker for lysis, ^{51}Cr (see 6 and Chapters 7–9 in 81). The chemical basis for the lethal hit remains enigmatic, but it is noteworthy that the CTL remains unscathed and that each can administer many lethal hits in sequence (55, 97).

It is remarkable that CTL form such strong adhesions (withstanding vigorous vortex shearing) so quickly with ascites tumor target cells, although the adhesion formation is triggered by a specific receptor-antigen interaction. Neither the CTL nor the ascites tumor cells adhere spontaneously to glass or to other cells in the absence of immune recognition.

Specific adhesion of CTL to target cells was first studied by incubating the lymphocytes on monolayers of macrophages or fibroblasts and then separating the fractions of the lymphocyte population which are nonadherent or adherent to the monolayer (9, 14, 35, reviewed in 54). The CTL activity of these two fractions gives a depletion/enrichment ratio which has been shown to reflect specific adhesion. Results with this technique have indicated that magnesium is more effective than calcium in supporting specific CTL-target adhesion (33, 65, 79), and that cytochalasin B (34, 37, 80) and inhibitors of energy metabolism (7, 34, 85) reversibly block adhesion formation.

We recently introduced a new assay for quantitating the percentage of target cells that have adhered to CTL (51, 54, 63). CTL and target cells are intermixed and centrifuged into contact in the cold (0° – 4°C), which prevents any detectable interaction. The cell pellet is then warmed to 37°C for precisely timed intervals during which adhesions may form, producing stable cellular conjugates. These conjugates may be counted microscopically (8, 51), and although this provides the most direct quantitation of adhesion, it is a tedious assay that severely limits the number of samples that can be evaluated. Alternatively, the adhesion process may be interrupted by the addition of a large volume of viscous (dextran-containing) me-

dium into which the cells are dispersed by vortex mixing. This medium maintains the cells dispersed in suspension during a subsequent 5-h incubation, preventing any new intercellular contacts. Thus, the only opportunity for target cells to adhere to CTL is in the brief, predispersion incubation at 37°C ; those that do are lysed and release ^{51}Cr during the 5-hr postdispersion incubation period. In short, the percentage of ^{51}Cr release reflects the percentage of target cells that adhered to CTL.

This isotope release-based postdispersion lysis assay for CTL-target cell adhesion has previously been used to study the kinetics, temperature dependence, drug sensitivity, and other properties of specific as well as nonspecific lectin-induced adhesion formation and detachment (31, 32, 51, 52, 54, 63; see also footnote 1). The present report contains a detailed examination of the role of divalent cations in CTL-target cell adhesion (see also 32). Preliminary mention of some of the present findings has been made elsewhere (32, 53, 54).

MATERIALS AND METHODS

Media

Unless otherwise specified, L15 medium (Grand Island Biological Co., Grand Island, N. Y.) was used because it lacks bicarbonate buffer and thus has a stable pH in air. To this was added penicillin (100 U/ml), streptomycin (100 $\mu\text{g}/\text{ml}$), 10% fetal calf serum (Grand Island Biological Co., previously heated to 56°C for 90 min), and, in some cases, 10 mM HEPES buffer. This medium is designated L15S. Eagle's minimum essential medium (MEM) was used in an atmosphere of 5% CO_2 in air where specified, including antibiotics and serum as above (MEMS).

In one experiment (Table V), modified RPMI 1640 (RHS) was used: sodium chloride was substituted for sodium bicarbonate; the divalent cation concentrations were increased to equal those in L15 medium (1.3 mM calcium; 1.8 mM magnesium); and 10 mM HEPES buffer, antibiotics, and 10% fetal calf serum were added.

Calcium- and magnesium-free medium (CMF) was made with analyzed reagent-grade salts to contain per liter: 80 g of sodium chloride, 4 g of potassium chloride, 1.9 g of Na_2HPO_4 , and 0.6 g of KH_2PO_4 . To this were added 10 g/liter of glucose, 0.1 g/liter of phenol red, and amino acids, vitamins, and glutamine at the concentrations in Eagle's MEM (concentrated solutions from Grand Island Biological Co.), antibiotics as described above, and 1% fetal calf serum that had been dialyzed three times (6 h each) against 10 vol of the above-described salt solution. The pH of CMF was adjusted to 7.3 ± 0.1 . The known contamination of CMF with divalent cations, based on the manufac-

¹ Gately, M. K., W. J. Wechter, and E. Martz. 1979. Early steps in specific tumor lysis by sensitized mouse T lymphocytes. IV. Inhibition of programming for lysis by pharmacologic agents. *J. Immunol.* In press.

turer's analysis of the sodium chloride and the calcium pantothenate included, is at least 0.011 mM. Golstein and Smith (34) reported 0.046 mM calcium in similar medium by absorption spectrometry.

Where specified, EDTA (S-311, Fisher Scientific Co., Pittsburgh, Pa.) and EGTA (E-3251, Sigma Chemical Company, St. Louis, Mo.) were added from stock solutions whose pH had been adjusted so that when they were mixed with medium, the final pH remained at 7.3 ± 0.1 . All metal ions were added as the chloride salts.

Resin-treated CMF

Chelex 100 analytical grade chelating resin (Bio-Rad Laboratories, Richmond, Calif.), 100–200-mesh, sodium form, was suspended in 0.1 M phosphate buffer (pH 7) containing phenol red and neutralized with concentrated hydrochloric acid until the pH was stable for at least 1 h. The resin was then rinsed five times in distilled water (standing 10 min each during the last three) and two times in CMF, allowing the second rinse to equilibrate for 30 min or more. This was stored at 4°C until use (not >1 mo). For resin-treatment, the resin was rinsed one time in CMF and resuspended in an equal volume of CMF. Then, 10 ml of this resin suspension was added to 100 ml of CMF, and the mixture was stirred overnight at 6°C. The supernatant medium (resin-treated CMF) was allowed to contact only plastic containers. During all experimental manipulations with this medium, the experimenter wore plastic gloves, and all experimental tubes were kept capped whenever possible, especially during centrifu-

gation, to minimize the possibility of contamination with divalent cations.

Cells

CTL were produced by allografting C57BL/6J mice (obtained from The Jackson Laboratory, Bar Harbor, Maine) with intraperitoneal injection of 10^7 P815 mastocytoma cells (of DBA/2 origin). After 9–11 d, the cells in the peritoneal cavity were washed out with L15S. The adherent cells were depleted by one or more 30-min incubations at 37°C in 10-cm diameter plastic tissue culture dishes (Falcon Labware, Div. Becton, Dickinson Co., Oxnard, Calif.), the cells from each mouse being incubated in one dish in 10 ml of MEMS. This usually reduced the obvious (larger, debris-laden) macrophages from ~40 to <10% of the cell population.

Target cells were P815 mastocytoma cells grown intraperitoneally in DBA/2J or (BALB/c \times DBA/2) F_1 mice (The Jackson Laboratory or Cumberland View Farms, Clinton, Tenn.) and harvested at densities yielding 10^7 – 10^8 cells per mouse. For chromium labeling, about 1×10^7 cells in 0.5 ml of MEMS were mixed with 0.15 ml of $\text{Na}_2^{51}\text{CrO}_4$ in isotonic saline (1 mCi/ml, NEZ 030, 200–500 Ci/g chromium, New England Nuclear, Boston, Mass.), and incubated at 37°C for 90 min with gentle resuspension ~ every 10 min. The cell suspension was then layered onto 3 ml of fetal calf serum and centrifuged for 3 min at 1,000 g. The supernate was discarded, and the cell pellet was resuspended in 10 ml of MEMS and incubated at 37°C in a plastic dish until needed (but at least 15 min), to release any

TABLE I
Summary of Technical Variables

Experiment	[EDTA] during depletion <i>mM</i>	Test in resin-treated CMF?	Spontaneous release %	Mg/Ca/EGTA concentration giving maximum adhesion <i>mM</i>	Specific release values used for normalization	
					Max	Min‡
4	1	No	18–30	4/0/0.1	46	4
5	1	No	15–19	4/0/0.1	60	6
6	1	No	16–21	10/0/0	61	11
7	1*	No	18–29	1/1/0	63	4
9	0.1	No	17–25	10/0/0	52	2
10	0.1	No	15–23	4/1/0	54	6
		Yes	15–22	4/1/0	59	7
11	0.1	Yes	15–22	4/1/0	60	10
12	0.1	Yes	20–30	4/1/0	58	11
13	0.1	Yes	21–40	4/1/0	53	17
14	0.1	No	14–16	3/0/0.2	61	19
		Yes	14–16	3/0/0.2	49§	5§
15	0.1	Yes	21–25	3/1/0	57	4
16	0.1	Yes	22–29	3/0/0.2	52	4

Data in the present report were obtained primarily from 11 experiments for which pertinent details are given above. These experiments were part of a series of 16 experiments. Experiments 1–3 are not reported because the methods were substantially different and had not been standardized. Experiment 8 is excluded because the CTL activity was unusually low (maximum specific release 26% at 2.5 min).

* Plus 1 mM EGTA for part of this experiment.

‡ Tested in CMF/resin-treated CMF with no additions except in experiment 4, 1 mM EDTA.

§ For 1 min at 37°C before dispersion. All other values in this table are for 2.5 min.

loosely bound isotope. Target cells were then rinsed once and used.

Depletion of Divalent Cations and Equilibration with Test Media

Lymphocytes and chromium-labeled P815 cells were depleted separately. About 2×10^7 cells/ml (less for P815) were rinsed twice into CMF containing EDTA (concentrations are given in Table I), and incubated at 37°C for 30 min. The depleted cells were then divided into aliquots for each medium to be tested. Each aliquot was rinsed twice into the test medium, which reduced the concentration of EDTA during testing to <0.001 mM (<0.01 mM in experiments 5-7, <0.1 mM in experiment 4). Lymphocytes and target cells, still separate, were then allowed to equilibrate with the test media containing various concentrations of divalent cations for 20 min at 37°C (except 10 min in experiments 4-6).

Adhesion Quantitation Procedure

We placed 2×10^4 radiochromium-labeled P815 cells and 3×10^5 immune lymphocytes in 12×75 -mm round-bottomed plastic test tubes (Falcon Labware) standing in crushed ice. (Previously, both cell types had been separately depleted of divalent ions and equilibrated as detailed above in media containing various concentrations of divalent cations to be tested). The total volume of medium per tube for adhesion testing was 0.2 ml. The tubes were placed in a 37°C water bath for the times indicated (usually 1.0, 2.5, and 7.0 min). At the end of each incubation, 2 ml of L15S containing 10% Dextran T500 (Pharmacia Fine Chemicals, Div. Pharmacia Inc., Piscataway, N. J.) was added and the cells were dispersed by vortex mixing for 6 s, Setting 3 on a Vortex Genie Mixer (Scientific Products Co., Div. American Hospital Supply Corp., McGaw Park, Ill.). Tubes were then incubated at 37°C for $5 \text{ h} \pm 15 \text{ min}$. To sample radiochromium release at the end of this time, we added 2 ml of ice-cold isotonic saline, vortex-mixed the contents of each tube, and centrifuged the tubes at 1,500 *g* for 10 min at 4°C. The upper 2 ml of supernate was then removed with a 5-ml capacity Cornwall syringe (Scientific Products Co.) fitted with a 4-in. 14 cannula. The supernates were counted in a gamma scintillation spectrometer (Packard Instruments Co., Inc., Downers Grove, Ill.).

Duplicate spontaneous release (see values in Table I) control tubes were made for each medium to be tested by omitting the lymphocytes. The spontaneous release (*c*) and experimental (*e*) counts were averaged for duplicate pairs, corrected for counter background, and expressed as a decimal fraction of the counts released from target cells by one cycle of freezing in liquid nitrogen and thawing. (About 75% of total isotope uptake is released by this procedure.) Since small but variable losses of target cells occurred during rinsing before testing, freeze-thaw controls were made for each medium to be tested. Specific radiochromium release was calculated as $(e-c)/(1-c)$. Where indicated, the observed specific release values (obs) were normalized relative to the maximum (max) and minimum (min) values in each experiment (listed in Table I): percentage of normalized adhesion = $100(\text{obs}-\text{min})/(\text{max}-\text{min})$.

RESULTS

The rationale behind the use of postdispersion lysis (see beginning of this article) to study the role of divalent cations in the formation of CTL-target

cell adhesions is to vary the divalent ion concentrations only during the adhesion process, and to return to standard physiological concentrations during the programming for lysis and lysis itself. Thus, the assay consists of two stages. In the first stage (adhesion formation), CTL and target cells are allowed to interact for brief intervals in a total volume of 0.2 ml under conditions in which varying concentrations of divalent cations are rigorously controlled. In the second stage (dispersion and lysis), the addition of 2 ml of dextran-containing L15S medium (containing 1.4 mM Ca^{++} and 1.8 mM Mg^{++}) dilutes the original medium 11-fold, restoring divalent ion concentrations to physiological levels. This is especially important because it has been shown that programming for lysis, unlike adhesion, is strongly calcium dependent (31, 32, 33, 54, 65). This two-stage procedure is used for all experiments reported here.

Magnesium Sufficient for Adhesion

The results of one experiment using resin-treated EGTA-containing CMF medium are given in Fig. 1 and show that magnesium in the presence of EGTA (to remove trace Ca^{++}) appears sufficient to support adhesion formation (cf. reference 31).

The observation that, in the presence of magnesium, the chelating agent EGTA does not block adhesion provides the most compelling evidence that magnesium, in the near-absence of free calcium, supports adhesion formation between CTL and target cells. This is because magnesium does not compete effectively with calcium for the binding site of EGTA, unlike EDTA (17). Thus, unlike EDTA, EGTA can remove traces of calcium in the presence of free magnesium concentrations equal to or exceeding the EGTA concentration.

The cells used in these experiments were depleted of divalent cations before use by incubating in EDTA (30 min, 37°C) and then rinsed into resin-treated CMF. Such treatment may deplete extracellular membrane-bound cations but leaves intracellular divalent ion stores relatively intact (13). Thus, the participation of traces of calcium supplied from intracellular stores cannot be excluded from the present experiments.

Normalized data from 10 independent experiments for various magnesium concentrations in the presence of EGTA are summarized in Fig. 2. Data for both CMF and resin-treated CMF are included and are not significantly different. The

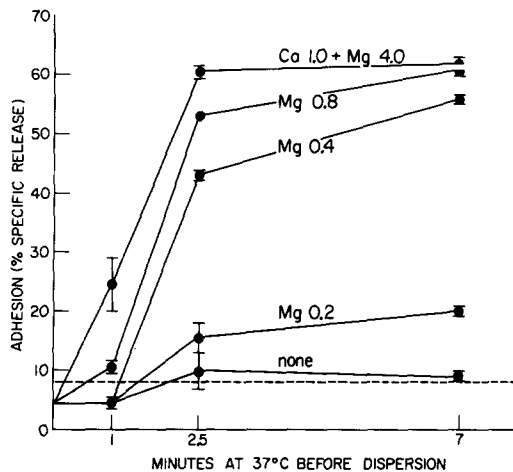


FIGURE 1 Magnesium with EGTA supports adhesion formation in resin-treated CMF. Lymphocytes and target cells were separately depleted of divalent ions and equilibrated in test media containing the indicated mM concentrations of magnesium plus EGTA (0.05 mM) in resin-treated CMF medium. Control media contained 1 mM calcium plus 4 mM magnesium or neither (*none*). Cells were mixed (15 lymphocytes per target cell) and centrifuged at 0°–4°C, and the tubes were placed in a 37°C water bath for the times indicated. Cells were then dispersed in dextran-containing medium (thereby restoring ion concentrations to physiological levels), incubated for 5 h at 37°C, and sampled for radiochromium release. Data points are from experiment 11 (Table I) and show mean \pm SD for duplicate observations. Values below the dotted line represent insignificant adhesion: this line shows the mean plus SD for quadruplicate cultures not incubated at 37°C before dispersion. This value indicates the maximum amount of “background” lysis resulting from interactions occurring at 0°–4°C before dispersion or occurring at 37°C after dispersion.

data show that optimal adhesion occurs at physiological concentrations of magnesium (10) around 3 mM, both 10 mM and 1 mM being less effective. The estimated concentration of magnesium giving half-maximal adhesion formation falls between 0.2 and 0.6 mM in eight of these 10 experiments. Omission of EGTA has a large effect, which is dealt with in a later section.

Calcium Insufficient for Adhesion

When calcium was added to CMF, it appeared to support adhesion formation (broken lines, Fig. 3). However, the curves were markedly different from the magnesium curves (dotted lines, Fig. 3). The optimum for calcium (about 0.3 mM) was 10-fold lower than that for magnesium. Moreover,

the average adhesion with optimum calcium was <70% of the maximum observed with magnesium.

Dramatically different results were obtained when calcium was tested in resin-treated CMF (solid lines, Fig. 3). In this medium, calcium alone was unable to support significant adhesion formation. This difference raised the possibility that traces of magnesium contaminating the CMF were synergizing with calcium to support adhesion formation.

Calcium Synergy with Suboptimal Magnesium

The concentrations of magnesium giving half-maximal adhesion in CMF without EGTA were 0.03–0.05 mM (experiments 5, 6, and 9 in Table I), 10-fold lower than the concentrations required in the presence of EGTA (0.2–0.6 mM). Resin

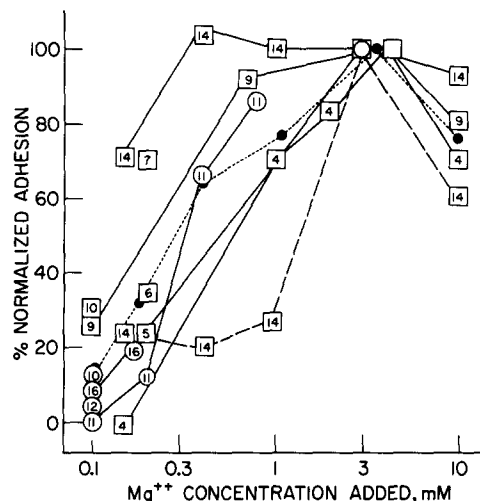


FIGURE 2 Magnesium concentration vs. adhesion formation in EGTA. This figure includes data from 10 experiments, each point representing the mean of duplicate tests. Numbers within open symbols designate the experiment numbers in Table I. EGTA concentrations were 0.03–0.5 mM (usually 0.05–0.1 mM) and were always less than the total magnesium concentration. The percentage of specific release values, representing adhesions, has been normalized relative to the maximum and minimum values listed in Table I. For all experiments, cells were depleted of divalent ions before testing. Medium was CMF (squares) or resin-treated CMF (circles). All data are for 2.5 min at 37°C before dispersion (solid lines) except one curve for experiment 14, 1 min (broken line). Solid circles with dotted line represent averages of all observations divided into six arbitrary concentration ranges: 0.1, 0.15–0.2, 0.4, 0.7–2, 3–4, and 10 mM.

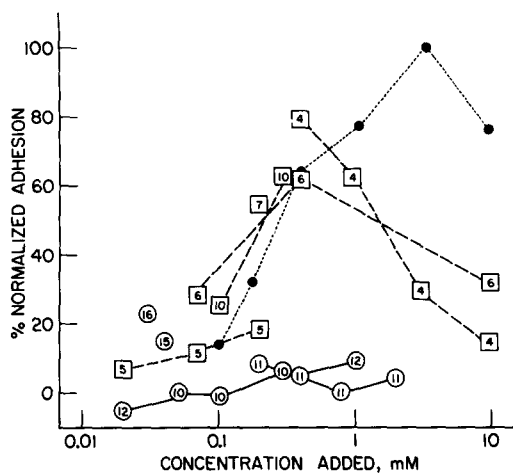


FIGURE 3 Calcium is insufficient to support adhesion formation. This figure includes data from nine experiments, each point representing the mean of duplicate tests dispersed after 2.5 min at 37°C. Numbers within open symbols designate the experiment numbers in Table I, where pertinent details may be found. Experimental procedures were the same as in Figs. 1 and 2. Calcium was tested in CMF medium (squares, broken lines) or resin-treated CMF medium (circles, solid lines). The averaged points for magnesium (solid circles, dotted lines) are copied from Fig. 2 for comparison.

treatment of the CMF had little effect on the half-maximal value for magnesium without EGTA, the range for four experiments (experiments 10–12 and 16) being 0.04–0.06 mM (difference from values in CMF not significant).

In 11 experiments, the adhesion supporting-ability of magnesium (0.1–0.2 mM) with and without EGTA (0.05–0.1 mM) was directly compared. The average difference in normalized adhesion was 59%, range 19–87%, the presence of EGTA giving the lower value in each case. Again, differences in resin-treated CMF were not less than those in CMF; indeed, the former averaged slightly larger than the latter. Thus, EGTA dramatically reduces the adhesion-supporting ability of magnesium in both CMF and resin-treated CMF.

The nature of the EGTA effect on adhesion formation in magnesium was explored in the experiments summarized in Table II. Theory predicts that EGTA should not be able to chelate sufficient magnesium to cause the reduction in adhesion, and this was experimentally verified. (cf. line 7 and 15 with 9 and 16, respectively, in Table II. Bear in mind that although EGTA and EDTA each have four potentially ionizable carboxyl

groups, only one divalent cation is chelated per EGTA or EDTA molecule [17, 27].) Thus, EGTA does not act by reducing the free magnesium concentration. Presumably, it acts by chelating traces of calcium. Based on this assumption, four predictions can be made, all of which were verified. First, EDTA in concentrations less than that of magnesium should fail to act like EGTA (cf. lines 1–3 in Table II). Second, the effect of EGTA should be neutralized by an equivalent amount of calcium (lines 7, 8, and 10 in Table II). Third, EGTA neutralized calcium should not inhibit adhesion even in very large amounts (lines 1 and 4 in Table II). Fourth, depletion of divalent ions by EGTA before testing should not alter the results (cf. lines 5 and 6 with 1 and 2 in Table II).

We conclude that, in the absence of EGTA, traces of calcium act synergistically with magnesium to support adhesion formation. The combination is superadditive: a substantial increment in magnesium concentration has much less effect than does the trace of calcium (lines 7, 9, 15, and 16 in Table II). The apparent concentration of synergizing calcium in resin-treated CMF was estimated to be ~0.02 mM by varying the concentration of EGTA (Lines 11–14 in Table II). It seems probable that this calcium originates from intracellular stores rather than bulk contamination of the medium. In this case, the active calcium may be concentrated in the region of the cell membranes, and the overall concentration of calcium released from the cells may be much <0.02 mM. The possibility that the synergizing element is a multivalent cation other than calcium seems unlikely but is not excluded.

In view of the above conclusion, direct tests for synergy between calcium and magnesium were carried out in resin-treated media. Synergy was observed in some experiments but not all. The conditions for synergy appeared to be quite narrow but on three occasions, clear-cut synergy occurred (Table III). On two of these occasions, the level of adhesions seen with calcium plus magnesium could not be achieved with either ion alone at a concentration equal to the total of the divalent ions in the combined test (Table III). This indicates that calcium could not be acting indirectly by displacing magnesium which might otherwise be bound, for example, to serum proteins.

In summary, three types of evidence provide strong support for the conclusion that micromolar concentrations of calcium synergize with subopti-

TABLE II
Effect of EGTA on Adhesion-supporting Ability of Magnesium

Experiment*	Line	[Mg ⁺⁺]	[EGTA]	[EDTA]	[Ca ⁺⁺]	Normalized adhesion (± SEM)‡
		mM	mM	mM	mM	%
7	1	0.2				97 ± 2§
	2	0.2	0.05			78 ± 0.5§
	3	0.2		0.05		97 ± 3
	4	1.0	5.0		6.0	102 ± 3
	5	0.2				98 ± 2
	6	0.2	0.05			67 ± 2
11	7	0.1				73 ± 9
	8	0.1	0.05			-2 ± 2
	9	0.2	0.05			17 ± 4
	10	0.1	0.05		0.05	65 ± 6
12	11	0.1				91 ± 5
	12	0.1	0.003			74 ± 6
	13	0.1	0.01			40 ± 1
	14	0.1	0.05			4 ± 0.3
16	15	0.12				102 ± 8
	16	0.17	0.05			19 ± 2

* Experiment numbers correspond to those in Table I.

‡ Adhesion (chromium-specific release) values at 2.5 min were normalized relative to the minima and maxima listed in Table I. In experiment 11, values at 2.5 min and 7.0 min were averaged.

§ Difference statistically significant (d.f. = 2, $t = 9.1$, $P < 0.02$).

|| Divalent cations were depleted from the cells with 1 mM EGTA + 1 mM EDTA before testing. EDTA alone was used in all other experiments (see Table I for concentrations). Difference statistically significant (d.f. = 2, $t = 12.4$, $P < 0.01$).

mal concentrations of magnesium in supporting adhesion formation: (a) calcium appears to support adhesion formation when added to CMF, but fails to do so when added to resin-treated CMF; (b) the apparent adhesion-supporting ability of magnesium is drastically reduced in EGTA; and (c) synergy can be demonstrated in direct tests.

Calcium Not Necessary for Optimal Adhesion

I concluded above that magnesium can support adhesion formation without calcium. However, the above demonstration that calcium synergizes with suboptimal concentrations of magnesium raised the possibility that calcium might augment adhesion formation even in optimal concentrations of magnesium. A more detailed examination of kinetics was made. In experiment 14, samples were taken at 0.33, 0.67, 1.0, 1.5, and 2.5 min at 37°C before dispersion. Various near-optimal concentrations of magnesium were tested, and the maximal discrimination occurred at the 1.0-min time points (experiment 14, Fig. 2). These confirmed my previous conclusion that 3 mM magnesium gives optimal adhesion in the presence of

EGTA. Addition of calcium failed to augment the initial rate of adhesion formation at any time point (data not shown). This was confirmed in a second similar experiment.

Indistinguishability of Adhesions Formed in Magnesium with and without Calcium

Adhesions formed in optimal concentrations of magnesium (1–4 mM) with calcium (1–1.4 mM) or without calcium (with EGTA) were found to be indistinguishable by any of six criteria. The similarity of the rates of adhesion have been noted in the previous section.

The strength of adhesions was tested by vigorous vortex shearing. This was previously noted to have little or no effect on adhesions formed in ordinary media (51); nor did it affect adhesions formed without calcium (Table IV).

EDTA detaches adhesions formed in the presence (51) or absence (31) of calcium. As calcium apparently plays some role in adhesion formation, at least at suboptimal magnesium concentrations, I wondered if adhesions formed in calcium plus magnesium would be transiently weakened or de-

tached upon addition of EGTA. Adhesions formed at 37° (2 min) or at 15°C (30 min) were incubated in EGTA (10 mM), EDTA (10 mM), or both for 3 and 10 min at 37°C, then vigorously vortexed and dispersed. EGTA had no effect (data not shown); EDTA detached all adhesions in the presence or absence of EGTA (thus showing that EGTA did not prevent EDTA-induced detachment). Similar results were seen in three experiments. Thus, EGTA does not detach adhesions formed in the presence or absence of calcium provided adequate magnesium is present. (My previous statement to the contrary [53] was based on a misinterpretation of certain controls. However, EGTA can induce detachment when magnesium is inadequate².)

The temperature dependence of adhesion formation in ordinary medium is well characterized (31, 51, 54) and has a Q_{10} of 2–3. Removal of calcium did not alter this temperature dependence (Table IV). Finally, cytochalasin B has been shown to block adhesion formation in ordinary medium (34, 37, 54, 80). Moreover, we have recently found that this drug weakens previously formed adhesions sufficiently to permit detachment by vortex shearing (74; see also footnote 1). Both effects have been confirmed for adhesions formed in the absence of calcium as well (data not shown).

Inhibition at Superoptimal Concentrations and Its Neutralization

It may be seen in Fig. 2 that 10 mM magnesium (with EGTA) supported only 75% as much adhesion (on the average) as did 3–4 mM. In experiment 9, omission of EGTA prevented this inhibition by 10 mM magnesium.

Results with calcium were similar but more dramatic (Table V). Traces of magnesium contaminating the CMF together with moderate amounts of calcium (0.4 mM, optimal) support 60–80% normalized adhesion (see Fig. 3). Increasing the calcium to 10 mM reduces adhesion drastically (Fig. 3, Table V). This inhibition by high calcium is prevented by 0.02 mM magnesium, an amount which alone supports no adhesion (experiment 6, Table V). Nor does 10 mM calcium inhibit adhesion in 1 mM magnesium (experiment 4, Table V). Thus, either calcium or magnesium at a high concentration tends to inhibit adhesion formation

² W. L. Parker and E. Martz. Unpublished observations.

TABLE III
Calcium Synergizes with Magnesium in Supporting Adhesion Formation

Experiment*	Added [Mg ⁺⁺]	Added [Ca ⁺⁺]	Normalized adhesion
	mM		(± SEM)‡
		mM	%
10	0.02		8 ± 3
		0.05	0 ± 1
	0.02	0.05	60 ± 5
12		0.1	-1 ± 3
	0.02		6 ± 5
		0.02	-4 ± 1
	0.02	0.02	42 ± 5§
16		1.0	14 ± 0.1
	0.04		22 ± 1§
	0.02		23 ± <0.1
		0.03	23 ± 1
	0.01	0.03	88 ± 4
	0.02	0.02	77 ± <0.1
	0.03	0.01	63 ± 5
	0.04	40 ± 0.4	

* Experiment numbers correspond to those in Table I.

‡ Adhesion (chromium-specific release) values at 2.5 min (except experiment 12, 7 min) were normalized relative to the minima and maxima listed in Table I.

§ Difference statistically marginal (d.f. = 2, $t = 4.1$, $0.1 > P > 0.05$).

|| Difference statistically significant (d.f. = 2, $t = 12.6$, $P < 0.01$).

if the other ion is at very low levels; this inhibition is antagonized by higher levels of the other ion.

Manganese, Strontium, Cobalt, and Nickel

Divalent manganese ions were found to support adhesion with about twice the potency of magnesium, even in resin-treated CMF. In three experiments (experiments 9, 11, and 12), the concentrations supporting half-maximal adhesion were in the range of 0.021–0.028 mM. For comparison, half-maximal adhesion required 0.032–0.057 mM magnesium in seven experiments (including the above-mentioned three). The possibility that manganese requires synergistic traces of calcium or magnesium to support adhesion formation cannot be excluded. (Manganese has a higher affinity for EDTA than do either calcium or magnesium [12]. We have not found a binding constant for manganese and EGTA.) Manganese plus magnesium appeared to support adhesion in an approximately additive fashion (data not shown).

Strontium appeared unable to support adhesion formation in four experiments covering the range 0.02–0.5 mM. In a single test, the latter concentra-

TABLE IV
Calcium Affects Neither the Strength Nor the Temperature Dependence of Adhesion

[Ca ⁺⁺]	[Mg ⁺⁺]	[EGTA]	Temperature and time for adhesion	Vigorous shearing after adhesion*	Adhesion, specific release‡
mM	mM	mM			%
1.0	2.0		15°C, 65 min	-	47 ± 6
1.0	2.0		"	+	48 ± 2
	4.0	0.1	"	-	47 ± 6
	4.0	0.1	"	+	43 ± 5
1.4	1.8		37°C, 1 min		36 ± 3
1.4	1.8	5.0	"		34 ± 4
1.4	1.8		15°C, 5 min		19 ± 3
1.4	1.8	5.0	"		18 ± 3

Data on shearing are from experiment 10. Data on temperature dependence are from an experiment (not listed in Table I) which was conducted in RHS medium (see Materials and Methods). Adhesions in 5 mM EGTA were conducted after depletion of calcium from the cells by a 15-min 37°C preincubation in 5 mM EGTA-containing RHS medium.

* Cells in 0.2 ml in a 12 × 75-mm test tube were given 10 s at setting 5 on a Vortex Genie Mixer (Scientific Products).

‡ Values for adhesion at 2.5 min are mean ± SE.

TABLE V
Inhibition by Suboptimal Calcium and Its Antagonism by Magnesium

Experiment*	Ca ⁺⁺	[Mg ⁺⁺]	Normalized adhesion‡
	mM	mM	%
4	0.4	Trace§	79 ± 1
	10.0	Trace§	14 ± 3
	10.0	1.0	98 ± 2
		1.0	71 ± 2
6	0.4	Trace§	62 ± 7
	10.0	Trace§	32 ± 9
	10.0	0.02	62 ± 0.4
		0.02	10 ± 1

* Corresponds to those in Table I.

‡ Mean ± SE for duplicate observations of specific release were normalized using the maxima and minima listed in Table I.

§ It was concluded in an earlier section (see Fig. 3) that calcium supports no adhesion formation unless a contaminating trace of magnesium is present (concentration unknown).

|| Plus 0.1 mM EGTA.

tion had no effect on the level of adhesion in a suboptimal concentration of magnesium. These results were confirmed and extended elsewhere (32).

In two experiments, divalent cobalt and nickel each appeared to support adhesion at 0.5 mM. In a single test, divalent barium, zinc, and copper appeared unable to support adhesion formation at 0.05 or 0.5 mM.

DISCUSSION

The Cytolytic Interaction

Using depletion of CTL on target cell monolayers to detect adhesion (see the beginning of this article), three previous reports (33, 65, 79) concluded that magnesium is more potent than calcium at supporting adhesion formation. The present results confirm and extend this conclusion.

The previous conclusion that magnesium alone is sufficient to support adhesion formation (33, 65, 79) was based on tests without EGTA. Our previous (31) and present finding that magnesium remains sufficient to support adhesion formation in the presence of EGTA and near-absence of calcium confirms and greatly strengthens this conclusion, since the EGTA should have reduced the level of free contaminating calcium in our studies by three orders of magnitude relative to that in studies not using EGTA.

On the other hand, the previous results in which calcium alone appeared to support adhesion formation (33, 65, 79) were probably due to the synergy of calcium with traces of contaminating magnesium. In this study, calcium was unable to support adhesion formation in medium from which contaminating divalent cations had been depleted by treatment with a chelating ion exchange resin. The previous results were confirmed, however, because medium not so treated, plus calcium, did support limited adhesion formation.

A new and unexpected finding in the present

study is a striking synergy between calcium and suboptimal concentrations of magnesium in supporting adhesion formation. This is a true synergy in the sense that magnesium (or calcium) alone supports little adhesion formation at a concentration equal to the sum of calcium plus magnesium concentrations able to support extensive adhesion formation. Thus, calcium can play a direct role in adhesion and probably does not act indirectly, for example, by freeing some magnesium which would otherwise be bound to serum components in the bulk medium. As a result of this synergy, the apparent potency of magnesium in supporting adhesion formation increases >20-fold as the concentration of calcium increases over the range from trace levels to about 0.05 mM (summarized in Table VI).

Given that calcium is crucial for the lethal hit (31, 32, 33, 65), the previously reported (65) calcium-magnesium synergy supporting the cytolytic process (studied as a whole) is probably accounted for simply by the fact that calcium alone does not support adhesion well. The previous failure to find synergy in the adhesion process (65) can be explained by the limited concentration range studied.

The previous evidence that calcium is required for the CTL lethal hit but not for the adhesion process (31, 33, 65, 79) suggests that calcium may play some direct role in the lytic mechanism, in which magnesium cannot substitute, similar to the calcium influxes that trigger secretory processes (5). Recent results (32) have shown that the roles of calcium in adhesion and in the lethal hit are distinguishable by the fact that strontium will substitute for the latter but not the former. Thus, calcium acts upon at least two sites during T-lymphocyte-mediated cytotoxicity. Only its action in the lethal hit is necessary for killing, however, since magnesium plus strontium supports killing (32).

Comparison with Lectin-induced Adhesions

It is noteworthy that several of the properties of the specific immune recognition-triggered adhesion between CTL and tumor cells have been found also in concanavalin A (Con A)-induced nonspecific adhesion between CTL and syngeneic (antigenically unrecognizable) tumor cells. Such adhesions will form at an undiminished rate in magnesium plus EGTA (relative to magnesium plus calcium) and are detached in EDTA (31).

TABLE VI
Summary of Divalent Cation Concentrations
Required for Adhesion Formation between CTL and
Tumor Cells

Contents of test medium	Variable cation added	Concentration for half-maximal adhesion	Concentration for optimal adhesion
mM		mM	mM
EGTA 0.05-0.1	Mg	0.2-0.6	3-4
Trace of Ca*	Mg	0.03-0.06	~0.5-10
Ca 0.03-0.07	Mg	≤0.01	~0.1->2
Trace of Mg‡	Ca	~0.2	0.4§
Mg 3.0	Ca	<10 ⁻⁴	<10 ⁻⁴ ->10
Resin-treated CMF	Mn	0.02-0.03	≥0.1

All values given in this table were arrived at in the text.
* In either CMF or resin-treated CMF (no significant difference).

‡ In CMF. Calcium alone was unable to support adhesion formation in resin-treated CMF.

§ Suboptimal peak (Fig. 3).

(The efficacy of calcium alone and the possibility of synergy have not been tested.) Cytochalasin B blocks both specific and Con A-dependent adhesion formation and weakens established adhesions of both types (unpublished data; see also footnote 1). The rates and temperature sensitivities of the two types of adhesions are closely similar (31). These observations add weight to the concept (87, 95) that some lectins agglutinate by causing expression of a natural cell adhesion mechanism, rather than solely by bridging surface glycosidic moieties between cells. The latter mechanism appears to occur more rapidly when the target cells are precoated with Con A, since the adhesions cannot then be detached with EDTA¹.

Comparison with Spontaneous Adhesions of Other Cell Types

It is clear that the adhesion process studied here is initiated by the specific binding of immunological T-cell surface receptor molecules to surface antigen on the target cell. The T-cell receptor molecule has not been well characterized, as its detection and isolation in cell-free form have only recently begun to be possible (11, 58, 82). Thus, the divalent cation requirements, if any, for T-cell receptor-antigen interaction are not known. The binding of B-lymphocyte-synthesized immunoglobulin receptors to specific antigen, however, is divalent cation independent. Ultrastructurally, no specialized structures or junctions have been seen

in the adhesions of CTL to target cells (40, 46, 97; see also footnote 3). We doubt that the adhesions studied here are held together primarily by the receptor-antigen bond. Rather, we suspect that the immune recognition triggers another more general, nonimmunologic cell-adhesion process, which has features in common with adhesions between other types of tissue cell. Recent evidence suggests that the recognition event is divalent cation independent (49) and can lead to killing in the absence of strong adhesion (74), reinforcing the idea that the adhesion event is separate from immune recognition for the CTL. Similarly, although the initial attachment of immunoglobulin Fc receptor-bearing lymphocytes to immobilized antibody-antigen complexes is divalent cation independent (98), divalent cations appear to promote a stronger adhesion associated with flattening (1).

Calcium vs. Magnesium in Intercellular Adhesion

It has often been stated or implied that calcium plays a much more important or direct role in intercellular adhesion than does magnesium (22, 50, 77, 78, 92). This view seems to have originated with early work on invertebrates and amphibians (68, 92). Indeed, the literature concerning amphibian embryos and adult epithelia is unanimous in finding calcium to be much more potent than magnesium in supporting intercellular adhesion formation (4, 29, 48, 76, 77). In the case of homeothermic vertebrates (avians and mammals), however, results have been more confusing. Some authors have concluded that calcium is more potent than magnesium (16, 24, 26, 36, 47, 56, 84, 86, 89, 94), others have concluded the reverse (3, 33, 38, 39, 65, 79, 86), and a few found no substantial difference (70, 91).

Magnesium appears to be better than calcium (each at 2–6 mM) in supporting cell aggregation for 4–5-d embryonic chick limb bud cells (3, 39) and in supporting polymorphonuclear neutrophil (PMN)-endothelial cell adhesion (38), although none of these studies provides dose-response data. Ueda and Takeichi (86) studied the aggregation of embryonic chicken dermal fibroblasts and obtained dose-response curves strikingly similar to those obtained in the present study (Fig. 4).

Although the mechanism of cell-to-substratum

³ E. Raviola, M. J. Karnovsky, E. Martz, and B. Benacerraf. Unpublished observations.

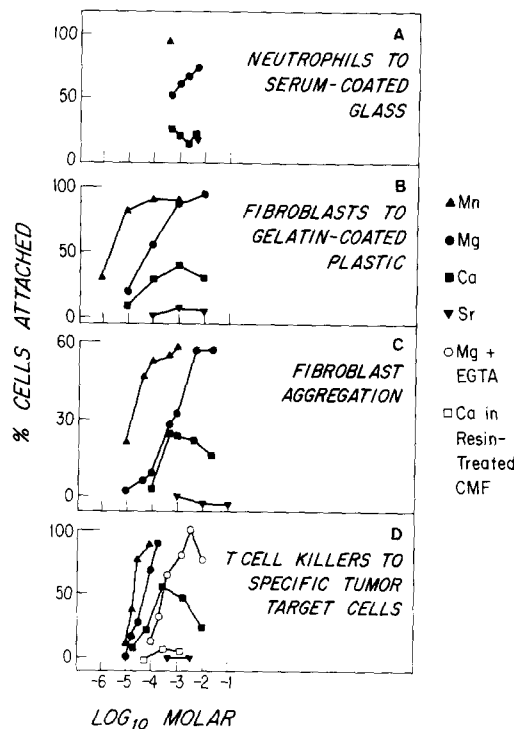


FIGURE 4 Comparison of divalent cation dose responses for immunologic and nonimmunologic cell adhesion. Data from this report and two previous reports were replotted in a comparable manner. In all systems, the indicated divalent ions were added singly to divalent cation-free (sic) media, and adhesion was studied at 37°C. (A) Purified rat peritoneal neutrophils were passed through a column of serum-coated glass beads. The data shown are from Fig. 1 and Table 5 in reference 30. (From Garvin [30]). (B) and (C) 11–12-d chick embryo dermal fibroblasts were cultured 4–5 d in monolayer and dissociated with trypsin in CMF. Data for adhesion of cells to gelatin-coated plastic during 60 min (B) were taken from Fig. 8 (in reference 86). Data for cell aggregation in gyrated cultures (C) (Fig. 2 in 86) were obtained by measuring the reduction in total particle number in a Coulter counter, plotted here as $100(1 - [\text{number at } 60 \text{ min}/\text{number at } 0 \text{ min}])$. (From Ueda and Takeichi [86]). (D) Immunologic adhesion of CTL to specific tumor target cells quantitated by postdispersion lysis (^{51}Cr release). Average $\text{Mg}^{++} + \text{EGTA}$ points for 10 experiments were taken from dotted curve (Fig. 2); average points for Mg^{++} without EGTA represent six experiments. Average Ca^{++} points were taken from five experiments (broken lines, Fig. 3); average Ca^{++} points for medium pretreated with a chelating ion exchange resin (to remove traces of Mg^{++}) were taken from three experiments (solid lines, Fig. 3). Average Mn^{++} points were taken from six experiments; Sr^{++} observations were made only once. (From this report).

adhesion may differ from that of cell-to-cell, it is noteworthy that in all studies in which magnesium and calcium have been compared for ability to support adhesion of cells to protein-coated glass or plastic or to collagen, magnesium was found to be more potent than (30, 41, 42, 45, 57, 62, 71, 72, 84, 86, 89) or equal to calcium (2, 43). Similar results have been seen in studies of cell spreading on culture substrata (66, 96). The adhesion studies included baby hamster kidney cells, mouse SV-3T3, rat hepatocytes, Chinese hamster ovary cells, rat neutrophils, and chick embryonic fibroblasts. In the latter three systems (30, 41, 86), dose responses again were strikingly similar to those for cell-to-cell adhesion (86 and this study, see Fig. 4).

Calcium has appeared to support cell-to-cell adhesion better than magnesium in 10 reports cited above. One report concerning the eight-cell mouse embryo is quite convincing, but in view of the above-mentioned results with ontogenetically more advanced cells, it seems possible that the adhesive mechanisms in the very early embryo may differ from that in the later embryo or adult. We shall tentatively set aside the very early embryo for the remainder of this discussion. Upon critical examination, six of the remaining nine reports are questionable with regard to the relative effects of calcium vs. magnesium (16, 24, 36, 47, 84, 94). One presents no data for magnesium (84). One uses an assay of enzyme-induced dissociation where factors other than cell adhesion, such as effects on enzyme activity, may play a predominant role (16). One is questionable because the combinations tested were few and the calcium-EDTA control produced unexplained inhibition (47). In one, the adhesion involves a glutaraldehyde-fixed tissue section (94). Another is questionable because the assay is not a very direct reflection of cell aggregation, and the effect of calcium appears very slight (24).

The three remaining studies provide more convincing data concerning embryonic neural retina cells (86), liver cells (56) and baby hamster kidney cells (89). Since all three studies used trypsin-treated cells, it should be noted that that Steinberg and co-workers, as well as other groups, have shown that calcium protects cells from trypsin-induced damage to their adhesiveness (78, 83, 90, 93). Moreover, Urushihara and co-workers found that trypsin treatment greatly slowed the aggregation of BHK cells and that the recovery of BHK cells from trypsin-induced damage occurred in calcium but not magnesium (89, 90). Therefore, it is

possible that the greater aggregation-promoting ability of calcium observed in some studies reflects a calcium requirement for the repair of trypsin-induced damage rather than a direct participation of calcium in the adhesion process. (Vosbeck and Roth, using trypsinized 3T3 cells allowed to recover for 2 h, found calcium no better than magnesium in supporting cell-to-cell adhesion [91].) On the other hand, for certain cell types, it appears that trypsin, in the presence of calcium, induces a state of calcium-dependent adhesiveness which does not require completion of any repair process, and for which magnesium will not suffice (78, 83, 88; see also footnote 4).

In summary, I know of no report that provides compelling evidence that calcium supports cell-to-cell adhesion better than magnesium in any system involving late embryonic or adult avian or mammalian cells not in a trypsin-damaged state. (Early embryo blastomeres appear to be an exception; 26). On the other hand, I think that compelling evidence has been provided that magnesium supports more adhesion than calcium in three systems: nontrypsinized embryonic chick limb bud (3), trypsinized embryonic chick dermal fibroblasts (86), and immune lymphocytes plus nontrypsinized tumor target cells (this report). In the latter two cases, detailed dose responses were obtained which show remarkable agreement (Fig. 4). In conclusion, it seems quite plausible that the properties I have defined here for lymphocyte-tumor cell adhesions (magnesium necessary, calcium insufficient) will be found to apply to several cell types when appropriately examined.

In addition, there is clearly a second category of cell adhesions for which calcium is more effective than magnesium, characterizing all amphibian cells presently tested plus very early mammalian embryo cells. Whether any avian or mammalian late embryo or adult cell types fall into this second category is not clear on the basis of present evidence.

Previous Use of EGTA

Adhesion of various cell types to protein-coated substrata has been observed in magnesium +

⁴ Magnani, J. L., W. A. Thomas, and M. S. Steinberg. 1980. Two distinct adhesion mechanisms in embryonic neural retinal cells. I. A kinetic analysis. Thomas, W. A., and M. S. Steinberg. 1980. Two distinct adhesion mechanisms in embryonic neural retinal cells. II. An immunologic analysis. Manuscripts submitted for publication.

EGTA (15, 71, 72). The present study (see also 31) appears to be the first in which Mg + EGTA has been shown to support cell-to-cell adhesion. The frequent observation that EGTA will detach cells from culture substrate (20, 59, 60, 73) probably does not reflect a direct participation of calcium in adhesion. Rosen and Culp (69) have shown that EGTA does not cause detachment per se but rather rounding up of the cell, perhaps due to effects on calcium-dependent cytoskeletal function. The rounding up causes the formation of fragile cytoplasmic threads connected to the original attachment points of the formerly flattened cell. Gentle agitation breaks these threads, releasing the cell but leaving the adherent portions of the cell surface attached to the substratum as small fragments.

Manganese, Strontium, Barium, Cobalt, and Nickel

The finding that manganese is a more potent supporter of adhesion than magnesium is consistent with previous reports on cell-to-cell (28, 38, 86, 91) or cell-to-substratum (2, 30, 42, 62, 67, 86) adhesion. Our data, which suggest that cobalt and nickel may support adhesion formation, are consistent with several previous reports in both homeotherms and poikilotherms (28, 30, 38, 42, 48, 75, 76, 86). That strontium supported no adhesion is consistent with other studies of homeothermic cell systems, which were similar in the respect that magnesium supported more adhesion than calcium (3, 30, 41, 42, 86). Interestingly, several studies of amphibian systems, in which calcium supports more adhesion than magnesium, found that strontium supported adhesion formation weakly but better than magnesium (4, 48). We did not study barium, but this ion has supported no adhesion formation (3, 4, 29, 48, 75, 86) except very weakly in two cases (30, 38).

Synergy and Two Binding Sites

The quantitative demonstration of a synergy between calcium and magnesium in supporting cell-to-cell adhesion formation in the present study is not unprecedented, to our knowledge. Such synergy was documented, however, in one study of the adhesion of neutrophils to protein-coated glass (30). It seems likely that such synergy will be found in other systems when they are examined appropriately. When added to nominally divalent cation-free media, chelating agents have been seen

to affect cell adhesion in other systems (28, 70, 71, 91).

Synergy suggests that the two cations are acting at two different sites. This idea has already been proposed by Garvin, who first observed synergy (30; see also 57), and by Ueda and Takeichi (86), based on comparative dose-response studies for two cell types. The proposal that the divalent cations operate primarily through surface charge reduction (31, 64, 76) has been excluded by elegant electrophoretic studies (3, 19, 23, 38; see also footnote 5) that show selectivity of ions in promoting adhesion, which cannot be explained by their charge neutralization properties.

A study by Deman and co-workers (24) raised the possibility that the role of calcium in adhesion may be mediated through sialic acid residues, a point that deserves further exploration. Another possible site for calcium action at the cell surface is phospholipids. Onishi and Ito (61) showed that calcium is more effective than magnesium in producing fluid to solid phase transitions by binding to phosphatidyl serine in mixtures with phosphatidyl choline. Enzymes using a calcium or magnesium cofactor are a possibility (44). Takeichi (83) found a trypsin-sensitive cell surface protein, the radioiodination or trypsin degradation of which was inhibited by calcium. The loss of this protein was correlated with loss of adhesion. Rutz and Lilien (70) have identified an agglutinin believed to be involved in natural cell-cell adhesion in the chick embryo neural retina. Its activity is divalent cation dependent.

Little can be said about the nature of the magnesium-binding site involved in cell adhesion. Present theory suggests that the preference of this site for magnesium over calcium must reside either in noncoulombic factors (e.g., steric restriction) or in a very high coulombic affinity, due to a high anionic field strength at the site (25). Though this places some physicochemical restrictions on the nature of the magnesium-binding site, elucidation of the molecules involved awaits further work.

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⁵ Hoover, R. L., R. Folger, W. Haering, B. Ware, and M. J. Karnovsky. 1980. Adhesion of leucocytes to endothelium: roles of divalent cations, surface charge, chemotactic agents, and substrate. Manuscript submitted for publication.

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