Journal of Cellular Biochemistry 18:469–478 (1982)
Cellular Recognition 749–758

Calcium-Dependent and Calcium-Independent Adhesive Mechanisms Are Present During Initial Binding Events of Neural Retina Cells

David R. McClay and Richard B. Marchase

Department of Zoology, Duke University (D.R.M.) and Department of Anatomy, Duke University Medical Center (R.B.M.), Durham, North Carolina 27710

The hypothesis that intercellular adhesion can be subdivided into two separable phenomena, an initial recognition event and a subsequent stabilization, is supported by the use of a new cell binding assay that provides a quantitative measure of intercellular binding strengths. Radioactive single cells are brought into contact with cell monolayers at 4°C in sealed compartments. The compartments are inverted and a centrifugal force is then applied tending to dislodge the probe cells from the monolayers. By varying the speed of centrifugation, the force maintaining associations between embryonic chick neural retina cells was determined to be on the order of 10^{-5} dynes after incubation at 4°C. Brief incubations at 37°C resulted in significant strengthening of the intercellular bond. Using this cell binding assay, neural retina cells were shown to exhibit both a Ca^{++}-independent and a Ca^{++}-dependent mechanism in their initial binding to one another.

Key words: neural retina cells, adhesion, adhesion calcium effects, cell binding assay

Adhesive specificities have been detected by a variety of experimental approaches and proposed to be responsible for diverse morphogenetic phenomena [1]. Investigations of the biochemical mechanisms underlying adhesive specificity, however, have produced only isolated fragments of information. No coherent understanding has emerged. Several laboratories, for example, have reported factors that selectively promote cell associations [2–4]. Others have separated adhesion into an initial recognition process and a subsequent stabilization [5–7], or into Ca^{++}-independent and Ca^{++}-dependent phenomena [8–13]. To some extent, the unrelated nature of these experimental findings is attributable to the variety of adhesion assays that have been utilized and to difficulty in interpreting the information produced. Many of the assays require hours of incubation and conditions under which recognition is

Received May 1, 1981; accepted November 30, 1981.

0730-2312/82/1804-0469$03.00

confounded with membrane diffusion, cell motility, and countless other cellular processes.

A gap exists between experimental findings and ideas about adhesive recognition as well. The most widely held theory for the mechanism of cell adhesion is that lock-and-key interactions between cell-surface molecules provide the basis for intercellular bonds [14,15]. Yet while lock-and-key interactions such as antigen-antibody or lectin-carbohydrate binding proceed at 4°C, adhesive interactions have usually been undetected at this temperature [16,17].

The approach to these problems discussed here centers on a new cell binding assay that is capable of dissecting adhesive phenomena into individual events accessible to separate study [18]. Our evidence suggests that recognition phenomena do take place at 4°C and in metabolically poisoned cells; however, initial interactions resist shear forces of only 10^{-5} dynes per cell. The cell binding assay allows precise measurements of cellular interactions in these low ranges of forces. When apposed cells are allowed to metabolize, a rapid strengthening of cellular associations occurs. This event is also approachable in the cell binding assay.

Four laboratories have distinguished recently between Ca^{++}-dependent and Ca^{++}-independent mechanisms for the adhesion of neural retina cells [8–13]. These mechanisms can be selectively investigated by varying the dissociation procedures used in the preparation of the retinal cell suspensions. The cell binding assay has been used here to investigate the cation dependence of the initial binding and subsequent stabilization of adhesion among neural retina cells prepared by various dissociation procedures.

MATERIALS AND METHODS

Neural retina tissue was dissected from 7- and 10-day chicken embryos (Pittsboro Hatchery, Pittsboro, NC). Several methods of tissue dissociation were used: 1) Trypsin plus calcium (TC). Tissues were incubated 20 min at 37°C in saline containing 10 mM Ca^{++} and 0.25% trypsin (Gibco, 1:250). The tissues were washed three times with culture medium (CM) consisting of Eagle's MEM, HEPES (10 mM, pH 7.2), penicillin (50 u/ml), streptomycin (50 μg/ml), and DNAase (15 μg/ml, Sigma). The tissues were gently dissociated by pipetting and the cell suspensions were washed twice in CM. 2) Trypsin minus Ca^{++} (TE). Tissues were incubated 20 min at 37°C in Ca^{++}–Mg^{++}-free saline (CMF) containing 0.5 mM EDTA and 0.25% Trypsin (Gibco 1:250). The tissues were then washed and dissociated in the same way as given for the TC procedure. 3) Low trypsin plus calcium (LTC). Tissues were incubated 20 min at 37°C in saline containing 10 mM Ca^{++} and 0.001% trypsin (2× crystalline, Sigma). CM was added and the tissues were gently pipetted. The cells and chunks of tissue were centrifuged then resuspended in the 0.001% trypsin and incubated for a second 20 min. The cells were diluted in CM, gently pipetted, centrifuged at 200g, and resuspended in CM. The washing procedure was repeated 3 times. 4) Low trypsin minus calcium (LTE). Tissues were suspended in CMF containing 0.5 mM EDTA and 0.001% Trypsin (2× crystalline, Sigma). They were then dissociated by the same protocol as given for the LTC cells. 5) EDTA-dissociated cells (E). Tissues were incubated 20 min at 37°C in CMF containing 0.5 mM EDTA. They were dissociated and washed in CM as given for the TC protocol. "Recovered" cells were TE cells that were preincubated in sparse culture for 2–4 hr before being tested in the binding assay [19].

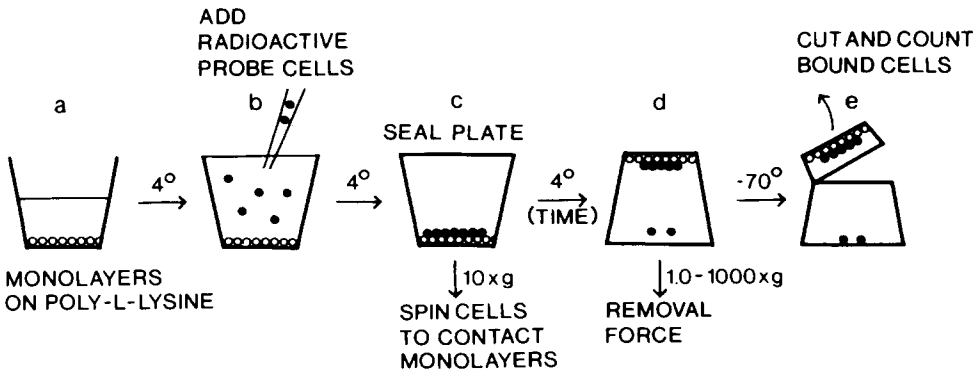

Fig. 1. Cell binding assay. a) Monolayer cells are spread onto PLL-treated microtiter wells. b) Radioactive probe cells are added and each well is filled to the brim. c) The wells are sealed with plate-sealing tape and the probe cells are centrifuged to contact the monolayers. d) The plates are inverted (immediately or after an incubation period) and centrifuged (1-2000g) to provide a dislodgment force tending to remove probe cells from the monolayers. e) After centrifuging, the plates are quick frozen in the inverted position in ethanol-dry ice, and the bottom 3 mm of each well is clipped away to be counted by scintillation methods [18].

Figure 1 illustrates the cell binding assay. Cell monolayers were constructed on flat-bottom polyvinyl microtiter plates (Dynatech, tissue culture treated). The monolayers were established by first treating the microtiter plates with poly-L-lysine in distilled water (PLL: 50 μg/ml; 60,000 MW; Sigma) for 1 hour at 24°C. The plates were washed with distilled water and cell suspensions in CM were added either in 0.1 or 0.2 ml. The cells were centrifuged onto the PLL-coated bottoms (50g, 3 min). The plates were incubated for 30 min to 1 hr either at room temperature, or at 37°C. The monolayers were washed first with CM containing poly-L-glutamic acid (PGA; 10 μg/ml; 30,000 MW, Sigma), which was found to neutralize the binding of probe cells to cell-free PLL-treated wells, and subsequently with CM. When cells were centrifuged onto PLL-treated plates containing no monolayers (10g, 10 min, 4°C), better than 95% of the population resisted a dislodgment force of greater than 300g. However, if the PLL layers were treated with PGA before adding probe cells, less than 3% of the probe cell population remained bound to the wells after a 50g spin. The PGA treatment did not affect probe cell-monolayer binding nor did the PGA remove bound monolayer cells; PGA was therefore used to minimize background binding of probe cells to any PLL-exposed areas within the monolayer (exposed areas never exceeded 10% of the total area).

Probe cells were labeled with ^{3}H-leucine (10 μCi/ml in leucine-free Eagle's medium; 16 hrs; New England Nuclear). Probe cell suspensions in CM were added to the microtiter wells at 4°C to give a final concentration of 1×10^5 cells/well. The final volume in each well was brought to 0.3 ml. This volume resulted in a slightly positive meniscus, a necessary step in the assay to prevent air bubbles when the wells were subsequently sealed. If a 37°C step was included in an experiment the wells were not sealed until after the return to 4°C. The wells were sealed by rolling on an adhesive microtiter plate sealer (Dynatech). Every other row on a microtiter

plate was used. The empty rows received approximately 10 μl of spillover as the wells were sealed (this was found to be a necessary contribution to the 5% experimental error of the assay). Probe cells were then centrifuged into contact with the monolayers (10g, 10 min, 4°C). This force was sufficient to bring better than 90% of the neural retina cell population into contact with the monolayers in control experiments when no dislodgment force was applied.

The plates were inverted and again centrifuged so that the centrifugal force now tended to pull the probe cells away from the monolayers. In control experiments, monolayers were not dislodged by forces greater by threefold than the maximum used in most experiments. The centrifugation time was 10 min. At the completion of a centrifugal spin the plate was placed, still sealed in the inverted position, into a solution of ethanol and dry ice. The wells were frozen and the bottoms of the wells containing the monolayers were clipped off using a pet toenail clipper modified to cut exactly at 3 mm. The well bottoms, containing the monolayers and any attached probe cells plus ~ 40μl frozen CM, were transferred while still frozen to scintillation vials and counted either in Aquasal (New England Nuclear) or in a cocktail of toluene, Triton X100, water, PPO, and POPOP. Each experimental point was performed at least in quadruplicate with standard errors of the means generally less than 5%. Identical experiments performed on separate days were generally within 5% of one another after normalizing probe cell populations for CPM/cell.

At a contact force of 1g it took 90–120 min to bring 90% of the neural retina probe cells into contact with the monolayers, whereas 10 min was more than sufficient when a contact force of 10g was applied. The dislodgment force did not differ significantly when 1g and 10g contact forces were compared. Higher contact forces (50g) did lead to a significant increase in the dislodgment force. Therefore, the 10g contact force was used routinely.

RESULTS

Dislodgment Force

The relative centrifugal force (RCF) required to dislodge populations of single, radioactive neural retina cells from neural retina monolayers was measured after incubation at 4°C. Figure 2 illustrates the percentage of radioactive probe cells in identical aliquots that remained bound to monolayers following exposure to increasing RCFs. At forces below 50g nearly all the cells resisted dislodgment. With increasing centrifugal speeds, progressively more cells were removed during the 10-minute centrifugation time period. In Figure 2, 85% of the probe cells were removed at RCFs between 50 and 400 g.

The force per cell required for dislodgment was calculated using the formula:

$$F_D = (\varrho_{cell} - \varrho_{medium}) \cdot V_{cell} \cdot RCF$$

where F_D = dislodgment force per cell; ϱ_{cell} = specific density of the cell (1.07g/cm³); ϱ_{medium} = specific density of the medium (1.00gm/cm³); V_{cell} = cell volume (a diameter of 10 μ was used for retina cells). With the RCF range of 50–400g the retina probe cell population was removed from monolayers by forces of between 1.8×10^{-6} and 1.4×10^{-5} dynes/cell; the median force for removal was 7×10^{-6} dynes/cell.

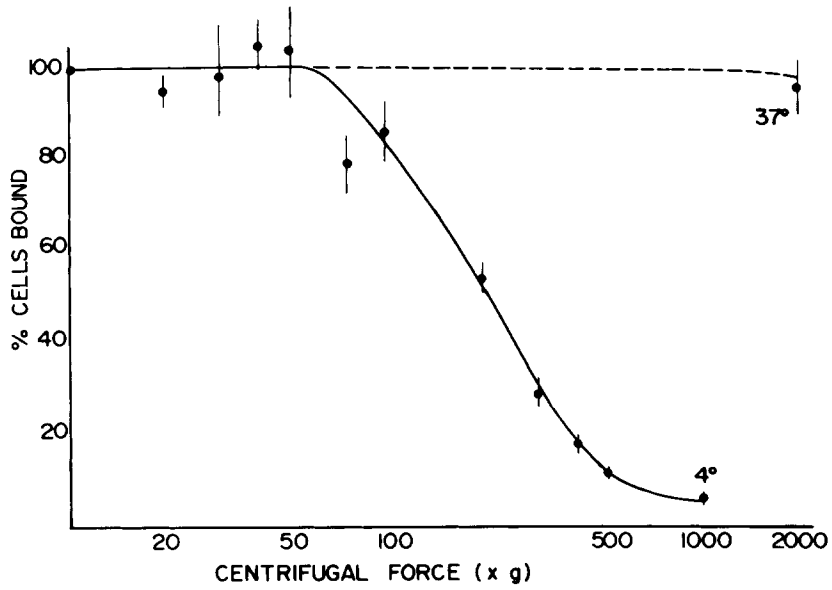

Fig. 2. Removal of neural retina probe cells from neural retina monolayers at different RCFs. Monolayers were established on PLL. Probe cells (10 day embryonic chick neural retinal cells; ^{3}H-leucine labeled; 0.05 cpm/cell; 100,000 cells/well) were added to wells in CM at 4°C. The probe cells were centriguged to contact the monolayers (10g, 10 min, 4°C). The plates were then inverted and centrifuged for 10 min at 12 different g forces. Each point represents the mean of cells remaining bound to 8 wells ± SEM. The dashed line represents one point in which the wells were warmed to 37°C for 8 min prior to inversion and centrifugation at 2000g. After this brief warmup period the entire population of cells resisted a 2000g dislodgment force [18].

When the plates were warmed at 37°C while the probe cells were in contact with the monolayers, a large increase in binding strength occurred. After only 6 min at the higher temperature, most of the probe cells resisted a dislodgment force of 400g and by 8–10 min, almost no probe cells were dislodged even at 2000g (Fig. 2). From these experiments we concluded that there are two phases to the cellular interactions: The first, an initial binding, occurs at 4°C and is reversible in moderate shear forces. The second, a stabilization, requires an incubation period at 37°C and results in intercellular bonds that are relatively irreversible. These data thus support the findings of Umbreit and Roseman [5] that two separable phenomena contribute to adhesive interactions.

Divalent Cation Effects

Four laboratories recently have distinguished between a calcium-dependent and a separate calcium-independent mechanism of cell aggregation [8–13]. Since the cell binding assay allows the separation of initial binding from subsequent stabilization steps, the cation requirements for initial binding could be examined in isolation.

Cells will exhibit predominantly either the Ca^{++}-dependent mechanism for adhesion or the Ca^{++}-independent mechanism, depending on the protocol utilized during dissociation [8–13]. Five different protocols were tested and are reported

here. First, monolayers and probe cells that had been trypsinized and allowed to recover at 37°C in nutrient medium for 2 hr were employed in the standard binding assay using a 200g removal force.

In Table I it can be seen that more cells remain bound in the presence of Ca^{++} than in its absence. Treatment of the recovered cells with high trypsin concentrations (0.25%, TC and TE) removed most of the binding capacity, though some Ca^{++}-dependent binding remained following trypsinization in Ca^{++}. Low concentrations of trypsin (0.001%) in the presence of Ca^{++} (LTC) resulted in the retention of the Ca^{++}-dependent binding, while the LTE treatment actually enhanced the Ca^{++}-independent binding over that in the recovered cells.

Next, the effect of cations on cell binding during the stabilization phase was examined. Table II shows that both the Ca^{++}-dependent and the Ca^{++}-independent binding were greatly strengthened in 10 min at 37°C. All freshly dissociated probes resisted a removal force of 300g after 10-min incubations at 37°C independent of the presence of Ca^{++}. However, differences could be detected if the removal force was increased to 600g. LTC probes confronted with LTC monolayers showed stabilization only in the presence of Ca^{++}, while LTE cells on LTE monolayers stabilized equally well in the presence or absence of the cation. This may point to independent stabilization mechanisms, or alternatively, could reflect a universal stabilization mechanism with the differences in cation dependency observed being a result of differences in initial binding.

When LTE probe cells were added to LTC monolayers, or when LTC probes were added to LTE monolayers, incubation in the presence of Ca^{++} stimulated binding following an incubation at 37°C to a level equivalent to that seen with the LTC-LTC Ca^{++}-dependent adhesion. In the absence of Ca^{++}, values remained significantly lower than seen with Ca^{++}-independent LTE-LTE interactions. This may imply that a Ca^{++}-dependent stabilization mechanism is present in some manner on LTE cells or that its presence on only one member of an apposed pair is sufficient for its operation. On the other hand, the Ca^{++}-independent mechanism seems to be missing from LTC cells and its presence on both cells of an apposed pair seems necessary for stabilization to occur.

Cation Specificity

Earlier we demonstrated two apparent mechanisms for strengthening or stabilizing cell interactions. One mechanism had a requirement for ATP while the second resulted in a modest strengthening that occurred in the presence of uncouplers of ATP production [18,20]. The ATP-independent mechanism was correlated with membrane diffusional properties (18,20). When ATP was present the ATP-dependent mechanism was completely dominant. Mild formaldehyde fixation inhibited the ATP-dependent mechanism without affecting initial binding or the modest strengthening [21]. Table III shows that initial binding with the Ca^{++}-dependent mechanism is cation specific. Recovered cells were used both as probes and as monolayers for this experiment. As can be seen in Table III, the Ca^{++}-dependent effect did not change dramatically when the ATP-dependent process was abolished with formaldehyde. The addition of magnesium did not produce a variation significantly different from the CMF control. Manganese supported binding to some extent at 4°C and also had an increased effect on binding at 37°C. It will be shown elsewhere that the Mn^{++} effect may be related to enzymatic processes (in

TABLE I. Initial Binding in the Presence and Absence of Ca^{++}

Probe treatment	% Binding[a] in 3 mM Ca^{++}	% Binding in CMF
Recovered cells[b]	28	14
TC[c]	10	5
TE[c]	5	3
LTC[c]	25	14
LTE[c]	24	24
EDTA[c]	23	19

[a]Probe cells remaining bound after a 200g removal force. Experiments were at 4°C.
[b]Obtained by incubation of TE cells in CM + 2% fetal calf serum in sparse culture for 2 hr.
[c]Obtained by retreatment of recovered probes.

TABLE II. Calcium-Dependent and Calcium-Independent Binding at 4°C and 37°C

Probe[a] cells	Monolayers	% Binding at 4°[b]		% Binding at 37°[c]	
		$+Ca^{++}$	$-Ca^{++}$	$+Ca^{++}$	$-Ca^{++}$
LTC	LTC	29	8	66	16
LTE	LTE	30	26	63	54
LTE	LTC	23	14	66	32
LTC	LTE	16	13	53	31

[a]Probe cells were freshly dissociated. Monolayers were allowed to recover from their initial LTE dissociation for 2–4 hr. They were then treated in the specified manner.
[b]A 300g removal force was used.
[c]Probes were incubated 10 min at 37°C while in contact with monolayers. All freshly trypsinized probes then resisted a removal force of 300g. The removal force for the data above was 600g. If probes and monolayers were permitted to recover from trypsin treatments prior to the experiment, all probes resisted a 600g removal force (see Figure 2).

TABLE III. Divalent Cation Requirements for Initial Binding

Cation conc (mM)		% Binding at 4°C	% Binding at 37°C
CMF		3 ± 5	8 ± 2
Ca^{++}	1	5 ± 4	9 ± 6
	10	56 ± 3	48 ± 7
Mg^{++}	1	3 ± 5	11 ± 2
	10	5 ± 1	11 ± 3
Mn^{++}	1	9 ± 2	19 ± 3
	10	20 ± 4	43 ± 7

Recovered probes and monolayers were fixed 10 min in 1% formaldehyde to inhibit the ATP-dependent stabilization step. After 15 min in contact, a removal force of 200g was applied. The % cells remaining bound to monolayers is given.

preparation). From the data presented here we conclude that Ca^{++} appears to be specifically required for the cation-dependent initial binding step.

DISCUSSION

The cell binding assay described here differs from traditional cell aggregation assays in that the aggregation assays generally include liquid shear forces of greater

TABLE IV. Calcium-Dependent and Calcium-Independent Binding of Neural Retina Cells: A Comparison of Data From Independent Sources

Group[a]	Cell name	Age	Dissociation treatment: Trypsin	Dissociation treatment: Other	Relative binding 4°C: +Ca	Relative binding 4°C: −Ca	Relative binding 37°C: +Ca	Relative binding 37°C: −Ca	CI or CD[b]	Notes
T	E	7D		1 mM EDTA			9[c]	8	CI + CD	α E Fab's inhibit CI
S	E	7D		1.3 mM EGTA	5		10	10	CI + CD	α E Fab's inhibit CI
M	E	7D		0.5 mM EGTA	3	3			CI + (CD)	
T	TC	7D	0.01%	10 mM Ca^{++}			5	1	CD	α E Fab's do not affect CD
S	TC	7D	0.044%	2.5 mM Ca^{++}	0		8	1	CD	α E Fab's inhibit CD
E	T/Ca	7D	0.04%	10 mM Ca^{++}			7	1	CD	α T/Ca inhibits CD, CD lost with embryonic age
L	CaT	10D	0.05%	1 mM Ca^{++}			10	0	CD	
M	TC	7D	0.25%	10 mM Ca^{++}	2	1	4	1	CD	
T	TE	7D	0.01%	1 mM EDTA			1	1	none	
S	TE	7D	0.044%	1.3 mM EDTA	0		0(3)	0(1)	none	(aggregates after lag)
L	T	10D	0.05%	CMF			1	0	none	
M	TE	7D	0.25%	0.5 mM EDTA	1	1	(2)	(2)	none	(CI returns with monolayer incubation)
T	LTE	7D	0.0005%	1 mM EDTA			5	5	CI	
S	LTE	7D	0.001%	1.3 mM EGTA			8	8	CI	
E	T/E	7D	0.002%	1 mM EDTA			8	8	CI	α NCAM inhibits
M	LTE	7D	0.001%	0.5 mM EDTA	3	3	6	5	CI	
S	LTC	7D	0.001%	2.5 mM Ca^{++}			9	6	CI + CD	
M	LTC	7D	0.001%	10 mM Ca^{++}	3.5	1.5	9	2	CD	
E	T/SC	10D	0.5%	20 hr recovery			5	5	CI	α NCAM inhibits
M	RECOVERED	7D	0.25%	2 hr recovery	3.5	1.5	10	8	CD + CI	

[a]T = Takeichi [8]; S = Steinberg [11-13]; M = McClay and Marchase, this paper; E = Edelman [10]; L = Lilien [9].

[b]CI = calcium independent; CD = calcium dependent

[c]Numbers are an arbitrary scale of from 1-10 to compare data from the five laboratories gathered using a variety of assays. The table is grouped by similar treatments.

than 10^{-4} dynes [22]. Thus initial binding phenomena without subsequent stabilizations or adhesive events with binding strengths of less than 10^{-4} dynes are not detectable with aggregation assays [16,17]. With the cell binding assay, a dislodgment force as low as 10^{-9} dynes/cell can be detected. The results with this assay show that specific interactions do occur at 4°C [18], and that the interactions result in binding strengths on the order of 10^{-5} dynes. Following these interactions, stabilization processes that themselves need possess no mechanism for specificity produce an enhancement of the strength of an interaction that allows the cell to withstand more severe disruptive forces.

The upper limit of force measurement in the cell binding assay was set by the microtiter centrifuge carriers at RCFs of 2000g or 5×10^{-4} dynes/cell. When a 37°C incubation period was added to the basic assay, this magnitude of force did not release probe cells from monolayers. In other studies of adhesive forces, about 1 dyne/cell was required for separation of fibroblasts from a substrate [23] or for the separation of cancer cells from one another [24]. The separation force measurements of such "irreversibly" bound cells may be underestimates, however, because as noted by Weiss [25], at high separation forces membranes might tear before cells separate. In any event the binding strength of the stabilized bonds are beyond the limits of practical measurability using this assay. We can only conclude that the strengthening that occurs at 37°C is at least 13-fold over initial binding.

A transition from a weak initial adhesive interaction to a stronger interaction was previously observed by Umbreit and Roseman [5] using a Coulter counter assay. Their assay distinguished between cell interactions that were resistant to a gentle inversion of a Coulter counting vial from those resistant to 10 rapid inversions of the vial. Although the strengths of the adhesive interactions could not be quantitated, this assay demonstrated a transition from "reversible" to stronger "irreversible" interactions. Gerisch and co-workers, using a rotating drum apparatus to study Dictyostelium cells [6], were able to detect an EDTA-sensitive and reversible bond that formed prior to permanent bonds that were EDTA-resistant. Moscona [7] has described a "primary" and a "secondary" phase of aggregation. These phases however refer to the sorting out of cells in aggregates and thus to later steps in the in vitro histogenetic process.

Table IV compares our results with the four laboratories that have previously described calcium-dependent and calcium-independent aggregation. There is a basic agreement that trysinization in the presence of Ca^{++} ions preserves a Ca^{++}-dependent mechanism. Very low amounts of trypsin in the presence of EDTA preserves a Ca^{++}-independent mechanism. Two approaches show the two mechanisms to be separate: Distinct antibody specificities exist [10,12] and sequential treatments abolish the mechanisms in a predictable manner [8,11]. Our data show both a Ca^{++}-dependent and Ca^{++}-independent mechanism to be present during the initial binding phase of cell interactions (Tables I and II). Both mechanisms are rapidly amplified when the assay is warmed to 37°C. Because of this correspondence, it is likely the Ca^{++}-dependent and Ca^{++}-independent mechanisms observed with aggregating systems [8–13] are associated with the initial binding of cells. These results suggest that ligand-related associations are involved in both mechanisms but do not rule out Ca^{++} effects on membrane physical properties, membrane transport, or the involvement of the cytoskeleton.

ACKNOWLEDGMENTS

We thank Ms Susan Gurganus for excellent secretarial support. This work was supported by grants from the National Institutes of Health GM-22477 and EY-02845. DRM is the recipient of a Research Career Development Award, HD-00259.

REFERENCES

1. Marchase RM, Vosbeck K, Roth S: Biochim Biophys Acta 457:385, 1976.
2. McClay DR, Moscona AA: Exp Cell Res 87:438, 1974.
3. Balsamo J, Lilien J: Biochem. 14:167, 1975.
4. Hausman RE, Moscona AA: Proc Natl Acad Sci USA 72:916, 1975.
5. Umbreit J, Roseman S: J Biol Chem 250:9360-9368, 1975.
6. Beug H, Gerisch G, Kempff S, Riedel V, Cremer G: Exp Cell Res 63:147, 1970.
7. Moscona AA: In Willmer EN (ed): "Cells and Tissues in Culture." New York: Academic Press, 1965, vol 1, pp 489-529.
8. Urushihara H, Takeichi M, Hakura A, Okada TS: J Cell Sci 22:685, 1976.
9. Grunwald GC, Geller RL, Lilien J: J Cell Biol 85:766, 1980.
10. Brackenbury R, Rutishauser U, Edelman GM: Proc Natl Acad Sci USA 78:387, 1981.
11. Magnani JL, Thomas WA, Steinberg MS: Dev Biol 81:96, 1981.
12. Thomas WA, Steinberg MS: Dev Biol 81:106, 1981.
13. Thomas WA, Thomson J, Magnani JL, Steinberg MS: Dev Biol 81:379, 1981.
14. Tyler A: Growth (Symposium 6) 10:7, 1946.
15. Weiss P: Yale J Biol Med 19:235, 1947.
16. Roth S: Dev Biol 18:602, 1968.
17. Walther BT, Öhman R, Roseman S: Proc Nat Acad Sci USA 70:1569, 1973.
18. McClay DR, Wessel GM, Marchase RB: Proc Natl Acad Sci USA 78:4975, 1981.
19. McClay DR, Gooding LR, Fransen ME: J Cell Biol 75:56, 1977.
20. Chambers AF, McClay DR: (submitted for publication).
21. Luster A, Marchase RB, McClay DR: (submitted for publication).
22. Bell GI: Science 200:618, 1978.
23. Rich AM: Thesis, University of North Carolina, Chapel Hill, 1978.
24. Coman DR: Cancer Res 4:625, 1944.
25. Weiss L: Exp Cell Res Suppl 8:141, 1961.