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THE RELATION OF TEMPERATURE AND LIPID COMPOSITION TO CELL ADHESION

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Summary

We have examined as a function of temperature the effect of changes in the composition of the fatty acid chains of membrane phospholipids on the rate of cell to cell adhesion in the neuronal cell line B103. The rate of cell to cell adhesion in this cell line is highly temperature dependent but is not influenced by changes in the fatty acid composition of the plasma membrane generated by growing the cells either in the presence of oleic acid or elaidic acid. In contrast the temperature dependence of the rate of cell to cell adhesion, measured in a monolayer adhesion assay, is highly dependent on the shear force used during the assay. A two-step model of cell to cell adhesion involving multiple adhesion ligands is presented which can be used to explain these observations.

Introduction

A number of biological processes are thought to be mediated by specific cell-cell interaction [1]. Two types of hypothesis have been used to explain cell to cell binding; (a) binding by specific recognition proteins present in the cell surface or secreted by the cell [2—4]; (b) binding as a result of non-specific forces [5]. As has been pointed out [6] these mechanisms are not mutually exclusive. However, biological processes such as synaptogenesis or the highly ordered spatial arrangements of cells in a tissue must primarily involve highly specific cell surface ligands.

The molecular mechanisms involved in cellular adhesion phenomena are poorly understood, because with some exceptions [7—11] the nature of the specific cell surface ligands involved in cellular adhesion are not known. Previous work from this laboratory has shown that cloned neuronal cell lines

Abbreviations: Solution 1, calcium and magnesium free Hanks solution buffered with 0.02 M 4-(2-hydroethyl)-1-piperazinemethanesulfonic acid, pH 7.4.

provide an interesting model system for the study of specific cell adhesion using a homogeneous cell population [12].

Cell to cell adhesion is an energy-dependent process [6] which we found also shows a striking sigmoidal dependence on temperature. One possible interpretation of this phenomenon is that it is related to a transition in the physical properties of the lipid bilayer of the plasma membrane. In this communication we examine the effect of alterations in the acyl chains of membrane phospholipids on cell to cell adhesion as well as the effect of changes in shear force on the temperature dependence of cell to cell adhesion. Cell to cell adhesion proceeds by an initial recognition step followed by the formation of a stable cell-cell attachment [6]. The data to be presented relate to the second step in this process.

Materials and Methods

The cell lines B103 and B65 were grown as described previously [12]. A surface membrane-enriched fraction was prepared from these cells by published procedures [12]. In order to alter the fatty acid composition of the cellular phospholipids the cells were grown for 1 week in Dulbecco minimum essential medium [13] with 10% fetal calf serum (Gibco) in the presence of 10 μg/ml of avidin (Sigma). The cells were replated in the same medium which was replaced after 24 h with medium containing delipidated serum, avidin and where indicated fatty acid supplements. These were added directly to the medium in hexane and the excess solvent was evaporated with a stream of sterile nitrogen. Fatty acids were added at a concentration of 40 μg/ml, either as a single fatty acid or as a mixture of fatty acids as indicated in individual experiments. These methods are modification of those published by other investigators in different systems [14—16].

Delipidated serum was prepared by extraction of fetal calf serum with a mixture of *n*-butanol and di-isopropyl ether [17] followed by dialysis against calcium and magnesium free Hanks solutions buffered with 0.02 M 4-(2-hydroethyl)-1-piperazinemethanesulfonic acid, pH 7.4 (Gibco) (solution 1) to remove residual solvent. Other methods of delipidation were used occasionally with same results [18,19].

Phospholipids were extracted from cells or plasma membrane-enriched fractions by published methods [20]. Fatty acids were analyzed as the methyl esters after transesterification by gas liquid chromatography on 6-ft columns of 10% diethylene glycol succinate on 80/100 chromasorb W AW (Supelco). For the analysis of lipids containing *trans*-unsaturated fatty acids chromatography was carried out on a 6-ft column of 15% SP-2340 on 100/120 chromasorb P AW-DMCS (Supelco). This column allows a separation of oleic acid (18 : 1 *cis*) from elaidic acid (18 : 1 *trans*). The presence of arachidonic acid was confirmed by mass spectrometry by Dr. M. Hoffman of this department.

Cell to cell adhesion assays were carried out using the monolayer adhesion assay of Gottlieb and Glaser [21]. Radioactive probe cells for the assay were prepared by growing the cells in the presence of [^{3}H]leucine [12].

Fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene was measured by the method of Shinitzky and Barenholz [22] as follows. Cells were broken

in a Dounce homogenizer and a crude membrane fraction was collected by centrifugation at 30 000 × *g* for 20 min. To the crude fraction from $3 \cdot 10^8$ cells in 10 ml of solution 1 was added 10 ml of $2 \cdot 10^{-5}$ M 1,6-diphenyl-1,3,5-hexatriene (Aldrich) in solution 1 and the suspension was shaken at 37°C for 30 min. The 1,6-diphenyl-1,3,5-hexatriene solution had been prepared by the addition of 15 µl of $2 \cdot 10^{-2}$ M 1,6-diphenyl-1,3,5-hexatriene in tetrahydrofuran to 15 ml of solution 1 and stirred mechanically for 15 min before use.

The crude membrane fraction was collected by centrifugation at 39 000 × *g* for 20 min in Sorvall SS-34 rotor, and washed once with solution 1. The pellet was then suspended in 3 ml of 60% w/v sucrose in solution 1 and the surface membrane was isolated by isopycnic density centrifugation as described previously [12] except that no serum albumin was used in any of the buffers since it binds 1,6-diphenyl-1,3,5-hexatriene. The final membrane suspension was suspended in 5 ml of solvent 1. Fluorescence was measured in a thermostated cell with a Spex fluorometer using a 357 nm excitation and measuring the emission of 427 nm. Fluorescence was excited for 10-s periods and the samples allowed to recover for at least 30 s in the dark before a new measurement was made. All experiments were carried out at least in duplicate.

For preparation of lipid suspensions 2 ml of extracted phospholipids (0.285 µmol phosphate) were dried under nitrogen, suspended in diethyl ether and dried again. The lipids were suspended in 2.5 ml of solution 1 containing 10^{-6} M 1,6-diphenyl-1,3,5-hexatriene and sonicated under a stream of N_2 at room temperature with the micro probe of a Biosonic oscillator at 20 W output for 10 min, at which time the suspensions had become clear. The lipid dispersion was heated at 55°C for 45 min before measurement of fluorescence as described [23].

Results

Fig. 1 illustrates the temperature dependence of the rate of adhesion of B103 cells to a monolayer of the same cells measured under standard conditions in a rotatory shaker at 64 rev./min. Under these conditions the rate of cell adhesion shows a marked temperature dependence with an inflection point at about 25°C. A possible interpretation of these observations is based on the assumption that a stable linkage between two cells requires the formation of multiple cell to cell contacts. If the formation of these multiple adhesive sites requires movement of the adhesive molecules in the plane of the membrane, then one may anticipate that as the temperature decreases, and the viscosity of the lipid bilayer increases, the rate of movement of molecules in the plane of the membrane will decrease, and therefore that the rate of adhesion will decrease drastically at temperatures below that at which the rate of lateral motion becomes rate limiting for cell adhesion. In order to test this possibility we have attempted to alter the viscosity of the plasma membrane by changes in the fatty acid composition of membrane phospholipids.

Alteration of the fatty acid composition of plasma membrane phospholipids

A number of investigators have demonstrated that it is possible to change the fatty acid composition of cellular lipids by growth of cells in delipidated serum

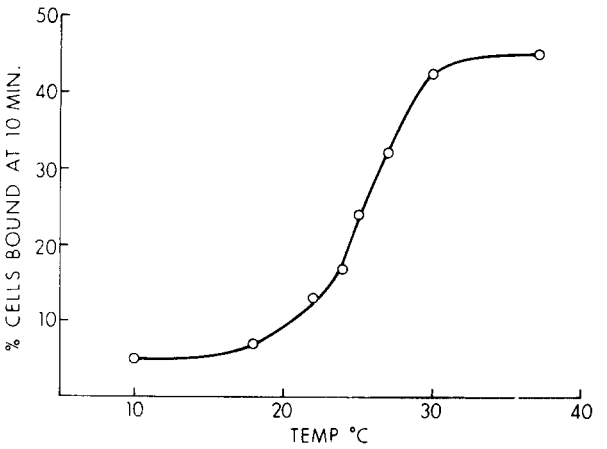

Fig. 1. Rate of adhesion of B103 cells to homologous monolayers as function of temperature. The rate of adhesion of [^{3}H]leucine-labeled B103 cells to monolayers prepared with B103 cells was measured as a function of temperature. Rate is expressed as percentage of cells bound at 10 min. Each point is the mean of 3 experiments (S.D. ± 4.0%).

(or serum-free medium) and a specific fatty acid supplement. These experiments are usually performed in the presence of an inhibitor of endogenous fatty acid synthesis such as avidin or desthiobiotin. B103 cells will not grow in the absence of serum. In the presence of delipidated serum, or delipidated serum with a single fatty acid supplement such as oleic acid or elaidic acid the cells will grow normally for about 40 h, after which growth ceases and extensive morphological changes are noted in the cells *. Normal growth in delipidated serum can be maintained by the addition of a mixture of myristic acid (14 : 0), palmitic acid (16 : 0), stearic acid (18 : 0), oleic acid (18 : 1 *cis*), arachidic acid (20 : 0), arachidonic acid (20 : 4), and cholesterol or by the addition of small supplements of normal fetal calf serum to the delipidated serum (data not shown). It is noteworthy that in the absence of arachidonic acid the maximal rate of growth cannot be maintained in these cells, nor can it be maintained in the absence of cholesterol in the medium.

In order to alter the composition of membrane phospholipids we have grown B103 cells in delipidated serum with a single fatty acid supplement either oleic acid (18 : 1 *cis*) or elaidic acid (18 : 1 *trans*) for 28 h. This period of time is short enough that the cells showed no morphological abnormalities.

The fatty acid composition of the total cellular phospholipids and the phospholipids isolated from a plasma membrane-enriched fraction are shown in Table I. All cells grown in delipidated serum show an increase in the arachidonic acid (20 : 4) content of phospholipid (e.g., compare line 2 vs. 1), which must be derived from neutral lipids or cellular fatty acids. This shift may in part be responsible for the requirement for arachidonic acid for cell growth,

* The morphological changes observed in delipidated serum initiate the formation of long processes, which in other neuronal cells have been interpreted as differentiation [24] but may really represent the effects of nutrient deprivation on cells.

TABLE I

FATTY ACID COMPOSITION OF PHOSPHOLIPIDS FROM B103 GROWN IN DIFFERENT MEDIA

Cells were grown as described in Materials and Methods in the growth media indicated. The phospholipids were isolated either from whole cells or from a plasma membrane-enriched fraction and their fatty acid content determined as described under Materials and Methods. Results are expressed as percent of total fatty acids. Reproducibility of duplicate analyses of the same sample was ±1%. Reproducibility within different cell preparations was ±3%. Representative experiments are shown in this table. The samples used to obtain the data shown in lines 5—7 were the same samples as were used in Figs. 2 and 3, to determine membrane microviscosity.

Medium	14 : 0	16 : 0	18 : 0	12 : 1	14 : 1	16 : 1	18 : 1 (*cis*)	18 : 1 (*trans*)	20 : 4
Total cellular phospholipids									
Fetal calf serum	0.9	19.3	23.3	—	—	8.2	44.0	—	1.0
Delipidated serum + avidin	2.9	23.1	18.0	—	—	6.0	36.0	—	6.5
Delipidated serum + avidin + oleate (18 : 1 *cis*)	1.0	11.2	13.0	—	—	2.6	59.0	—	8.1
Delipidated serum + avidin + elaidate (18 : 1 *trans*)	2.6	13.8	8.9	0.8	1.0	8.8	22.5	29.0	10.5
Plasma membrane phospholipids									
Fetal calf serum	2.2	28.0	18.0			11.5	38.6	—	1.3
Delipidated serum + avidin + oleate (18 : 1 *cis*)	0.7	16.6	15.5	—	—	2.6	62.3	—	6.2
Delipidated serum + avidin + eliadate (18 : 1 *trans*)	3.8	20.7	12.9	2.9	1.7	6.2	22.1	20.4	6.5

as noted above. As has been observed previously by others in different cell types, growth of cells in the presence of oleic acid results in a substantial increase in oleic acid content of membrane phospholipids derived from whole cells (lines 3 vs. 1, 59 vs. 44%) or from the plasma membrane fraction (lines 6 vs. 5, 39 vs. 62%). This increase is accomplished at the expense of a decrease in saturated fatty acid as well as a decrease in palmitoleic acid (16 : 1).

Growth of the cells in delipidated serum + elaidic acid results in the incorporation of substantial quantities of elaidic acid into membrane phospholipid (lines 4 and 7, Table I). This modification is accompanied by a decrease in content of saturated fatty acids, oleic acid and palmitoleic acid for the plasma membrane preparation. There is some selection against the incorporation of elaidic acid-containing phospholipids into the plasma membrane as opposed to non-plasma membranes.

A change in the composition of the phospholipids of the plasma membrane does not necessarily reflect a change in the physical properties of the lipid bilayer. We have therefore examined the microviscosity of the plasma membrane using 1,6-diphenyl-1,3,5-hexatriene as a probe [25]. This method has

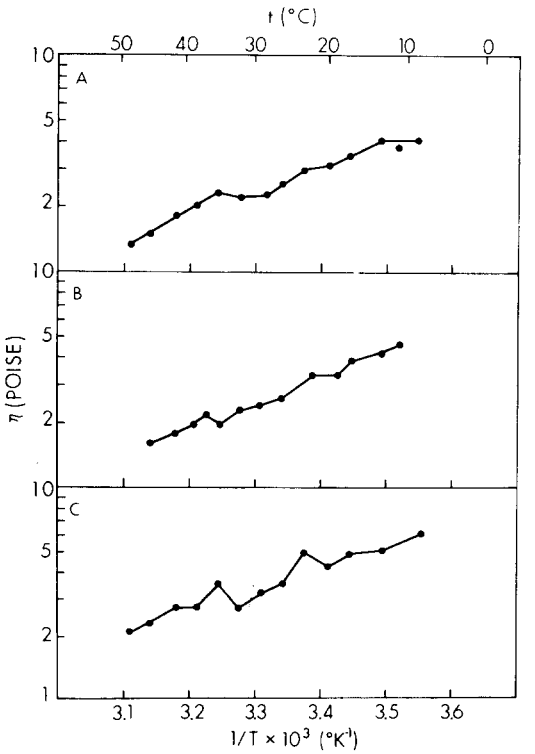

Fig. 2. Microviscosity of plasma membranes. The microviscosity of plasma membranes was calculated from fluorescence polarization data of 1,6-diphenyl-1,3,5-hexatriene [25]. The fluorescence intensity was also determined and used to correct the data for changes in excited lifetime as described [22]. This correction is small (see Fig. 3). (A) Plasma membranes prepared from cells grown in fetal calf serum and labeled with 1,6-diphenyl-1,3,5-hexatriene as described in Materials and Methods. (B) Plasma membranes from cells grown in delipidated serum + oleic acid. (C) Plasma membranes from cells grown in delipidated serum + elaidic acid.

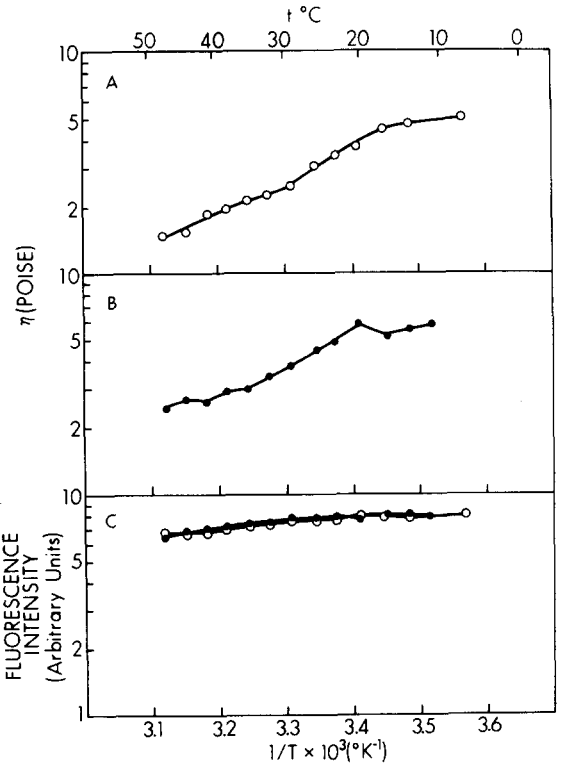

Fig. 3. Microviscosity of lipid dispersion. Panel A shows the microviscosity of a lipid dispersion prepared from plasma membranes of cells grown in 10% fetal calf serum. Panel B shows the microviscosity of a lipid dispersion obtained from plasma membranes of cells grown in delipidated serum + elaidic acid. 1,6-Diphenyl-1,3,5-hexatriene was incorporated into lipid vesicles as described in Materials and Methods. Panel C shows changes in fluorescent intensity with temperature for the samples shown in panels A (○) and B (●), which gave identical values of fluorescent intensity.

been widely used for this purpose [16,22] but the results may not be interpretable in a totally unambiguous manner [26,27]. For the purposes of this investigation changes in microviscosity measured with 1,6-diphenyl-1,3,5-hexatriene can be taken as a rough indication of changes in membrane fluidity.

The data in Fig. 2 show the apparent microviscosity of the plasma membrane obtained from cells grown in normal serum or delipidated serum supplemented with oleate or elaidate. Fig. 3 shows similar data for suspensions of lipids obtained from plasma membranes of cells grown in normal serum and in delipidated serum plus elaidic acid. Addition of elaidic acid to cells resulted in a substantial increase in plasma membrane microviscosities at all temperatures (Fig. 2, curve c vs. a), while little, if any, change in this parameter was observed when membranes enriched with oleic acid were studied (Fig. 2, curve b). We note parenthetically that in studies using *trans*-parinaric acid as a probe Rintoul et al. [37] have observed that an increase in the oleic acid content of membrane phospholipids in Chinese hamster ovary cells decreases the temperature at which a solid phase first appears [28,29]. Unfortunately in that study no measurements were made of the presence of solid phase in membrane phospholipids enriched in elaidic acid.

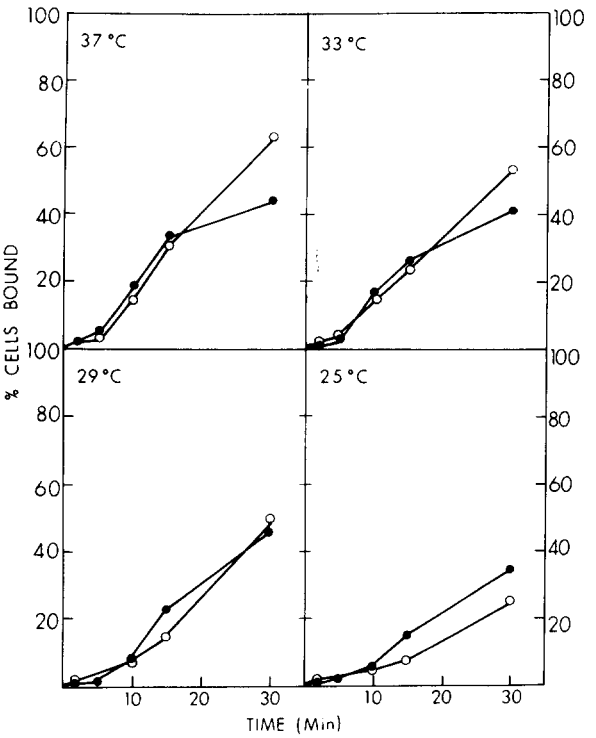

Fig. 4. Rate of adhesion of B103 cells to homologous monolayers. The panels show the rate of adhesion of cells grown in 10% fetal calf serum (○) and cells grown in 10% delipidated serum + elaidic acid (●) to homologous monolayers. The assays were carried out under standard conditions at 100 rev./min at the temperatures indicated. These data represent the results of single experiments carried out in duplicate. The results of 3 similar experiments are summarized in Fig. 7.

Cellular adhesion of normal cells and cells with altered fatty acid composition

The data in Fig. 4 show the rate of adhesion of cells to homologous monolayers for cells grown in normal serum and cells grown in delipidated serum plus elaidic acid. Within experimental error (±5%, see Fig. 7) these data appear to be identical. Similar observations (data not shown) were also made with cells enriched in oleate. We would conclude from these observations and the lack of correlation of the absolute value of microviscosity (Figs. 2 and 3) with the change in rate of adhesion, that the change in adhesion rate with temperature appears to be independent of membrane microviscosity and is not affected by changes that we have been able to introduce in the fatty acid composition of the membrane phospholipids. Thus, for example, the apparent microviscosity at 25° C of plasma membranes from cells grown in fetal calf serum is the same as that of plasma membranes from cells grown in delipidated serum and elaidic acid at about 37—40° C.

Shear force and temperature dependence of adhesion

In order to try to obtain additional insight into parameters that influence the temperature dependence of cell adhesion we examined other possible variables including changes in shear force during the assay. The data in Fig. 5A show that

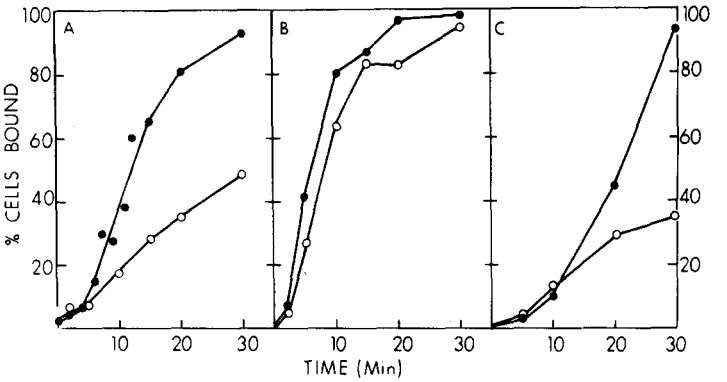

Fig. 5. Effect of shear force on rate of cell adhesion. Panel A shows the rate of adhesion of B103 cells to homologous monolayers at 37°C and measured at either (○) 100 rev./min or (●) 64 rev./min. Panels B and C show the rate of adhesion of B65 cells to homologous monolayers measured at 64 rev./min (●), or 100 rev./min (○), at 37°C (panel B) or 17°C (panel C).

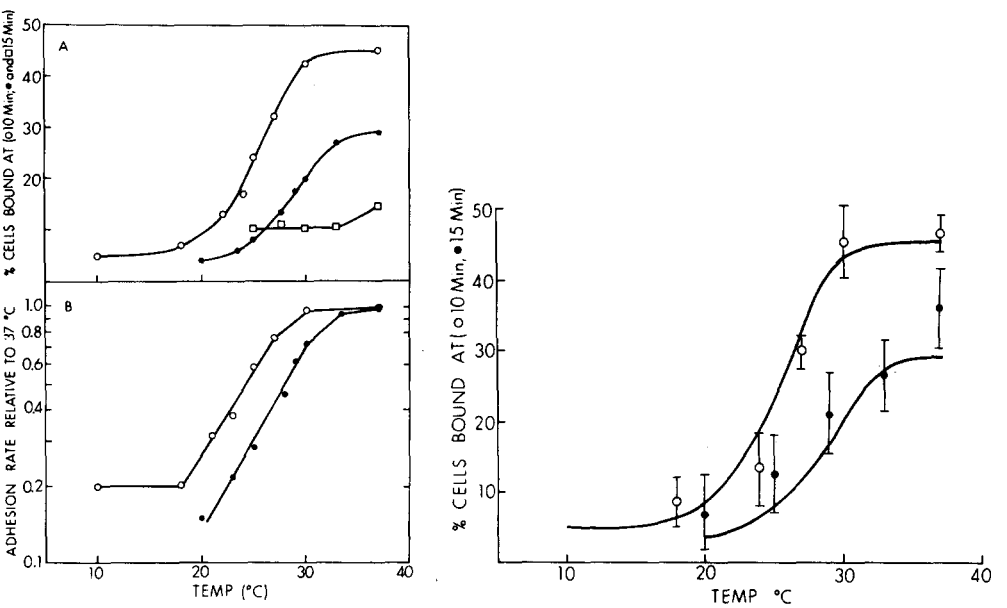

Fig. 6. Effect of temperature and shear force on rate of cell adhesion. The figure summarizes the results of 3 experiments such as those in Fig. 5 in which the rate of adhesion of B103 cells to homologous monolayers was measured at 64 rev./min (○), 100 rev./min (●), and 120 rev./min (□) as a function of temperature. (S.D. are less than ±5.0%.) Part B is a replot of the same data (at 64 and 100 rev./min) where the rate at 37°C is taken as 100% and shows more clearly the effect of shear force on the temperature dependence of the rate of adhesion.

Fig. 7. Effect of temperature and shear force on rate of adhesion of cells grown in elaidic acid. B103 cells were grown in delipidated serum + elaidic acid and their rate of adhesion to homologous monolayers was measured. The points are the average of experimental rates observed in 3 separate experiments, and the lines are the data for cells grown in fetal calf serum shown in Fig. 6. 64 rev./min (○), 100 rev./min (●). The bars on each point indicate the standard deviation.

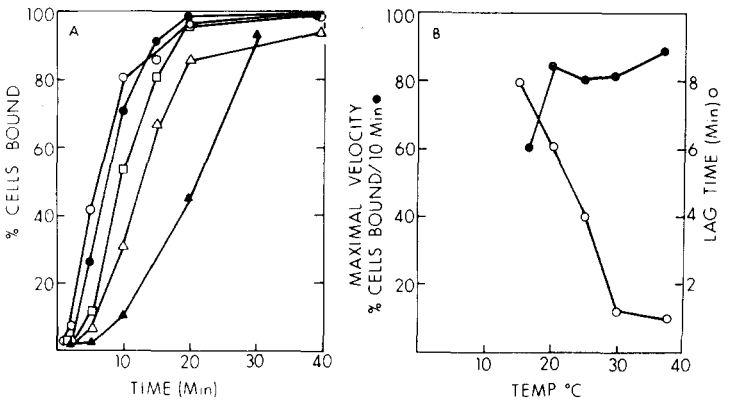

Fig. 8. Rate of adhesion of B65 cells to homologous monolayers. Experiments were carried out by standard methods at 64 rev./min. Panel A shows the rate of adhesion of cells to monolayers measured at various temperatures: (○) 37°C; (●) 30°C; (□) 25°C; (△) 21°C and (▲) 17°C. Panel B shows the change in maximal velocity of adhesion and the lag times with temperature.

the rate of adhesion of B103 cells to homologous monolayers at shaker speeds of 64 rev./min and at 100 rev./min at 37°C. Clearly the rate of cell adhesion is dependent on shear force. When we examined the temperature dependence of the rate of adhesion as a function of shear force, we obtained the data shown in Fig. 6. As the shear force is increased, the rate of adhesion decreases and the midpoint of the transition temperature from a high rate of adhesion to a very low rate shifts to a higher temperature. A possible interpretation of these observations will be presented in the Discussion. Between 35 and 64 rev./min the rate of cell adhesion at 37°C is independent of shear force. Once a stable adhesion is formed it can no longer be dissociated at high shear force, thus for example switching from 64 rev./min to 120 rev./min in an adhesion assay results in a decrease in the rate of adhesion but not in the release of any of the cells that have already adhered to the monolayer. Similarly, decreasing the temperature during the assay decreases the rate of adhesion but does not result in the release of cells already stably bound to the monolayer.

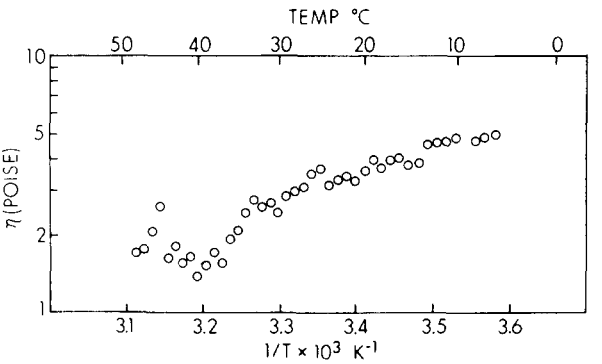

Fig. 9. Microviscosity of B65 plasma membranes. The microviscosity of B65 plasma membranes was measured with 1,6-diphenyl-1,3,5-hexatriene as described under Materials and Methods. Results of 2 experiments agree ±0.2 poise.

The data in Fig. 4 comparing the adhesion of cells grown in normal serum and cells grown in the presence of elaidic acid were obtained using an adhesion assay at 37°C, and 100 rev./min, a speed at which the rate of adhesion is limited by shear force. A more extensive comparison of the rate of adhesion of cells grown in the presence of elaidic acid compared to normal cells is shown in Fig. 7, which shows that the two cell types are indistinguishable at assay speeds of 100 or 64 rev./min and at all temperatures between 20 and 37°C.

We were interested in the generality of this phenomena and have examined a second neural cell line B65 [30]. At 37°C the rate of adhesion of these cells to homologous monolayers is independent of shear force, but becomes dependent on shear force at 17°C (Fig. 5, panel c). The kinetics of cell adhesion of B65 cells are, however, very complex (Fig. 8). Adhesion proceeds with a lag, the length of which is temperature dependent. In addition, the maximal rate of adhesion of cells to the monolayer after the lag is almost temperature independent down to 17°C when it also becomes dependent on shear force (Figs. 6C and 8). Increases in shear force below 17°C affect the steady-state rate of adhesion but not the lag phase. The lag cannot be abolished by preincubating either the probe cells or the monolayer cells or both at 37°C before initiating the experiment at the specific assay temperature. The changes in maximal rate of cell adhesion with temperature or the change in the duration of the lag with temperature fail to correlate with apparent changes in microviscosity as measured with 1,6-diphenyl-1,3,5-hexatriene (Fig. 9).

Discussion

This investigation was started with the notion that the sigmoidal dependence on temperature of the rate of cell adhesion reflected the requirement for the interaction of many adhesive molecules on adjacent cell surfaces in order to form a stable bond. A reasonable model which can be used to discuss the results in this paper is shown in Fig. 10.

This model suggests that initial cell to cell contact may involve the binding of two cells at a single or a very limited number of sites. This attachment is very weak and is readily reversible. A stable bond is formed in a subsequent step which requires the formation of multiple attachments which for simplicity are assumed to involve the same ligands as are used for initial binding. These multiple attachments between cells make the adhesion essentially irreversible under the assay conditions. We assume that the rate of this second step is strongly temperature dependent. In this model the formation of multiple attachments may require the movement of adhesive molecules in the plane of the membrane, but such movement if it occurs appears not to be limited by the viscosity of the lipids in the plasma membrane, perhaps because this motion is triggered by the initial adhesion event and is an active process. Alternatively, one may assume that the adhesive components reside in a specialized lipid environment in the membrane and depends on transitions occurring outside the bulk of the fluid bilayer. The model assumes as has been done by Umbreit and Roseman [6] that cell adhesion involves an initial reversible association of cells followed by an irreversible step. It is also in agreement with the finding (in a different system) that adhesion of liver cells to a carbohydrate-containing acryl-

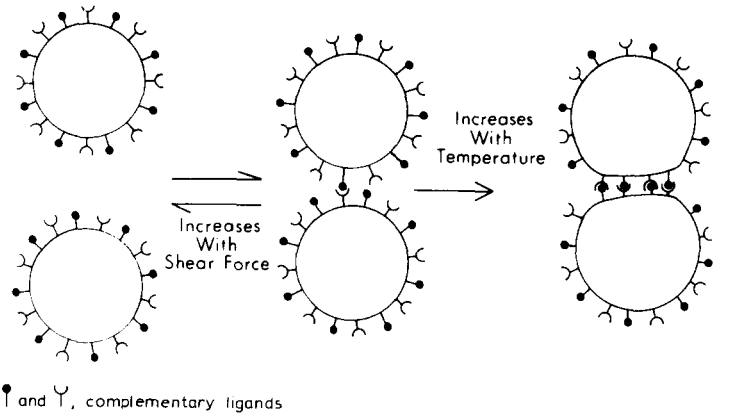

Fig. 10. Model of cell to cell adhesion. Adhesion is presumed to occur through complementary ligands. Initial reversible binding involves only one or two bonds, and can be reversed by shear force. This initial binding is followed by a second step the rate of which is temperature dependent and which results in the formation of multiple cell to cell bonds. This process is irreversible under the experimental conditions used to measure cell to cell adhesion. As the density of adhesive sites increases, then the probability of a stable bond being formed before shear force can separate the cells is increased. Thus, for cells with a high density of adhesive sites, shear force will only affect the rate of stable cell adhesion at very low temperatures.

amide matrix requires a minimum density of carbohydrate molecules/unit surface area before adhesion will take place, after which maximal adhesion is observed [31].

The conclusion that the formation of multiple adhesive sites between B103 cells is not dependent on membrane viscosity is supported by several observations. (a) The rate of adhesion of B103 to a monolayer of B103 cells is clearly not dependent on the fatty acid composition of the membrane phospholipids. (b) The rate of adhesion is the same at any given shear force in cells with different plasma membrane microviscosities, and no drastic changes in microviscosity occur at temperatures where the rate of cell adhesion is highly temperature dependent. (c) Within the interval 18—30° C in which the major adhesion rate change occurs, the fusion activation energy for membranes of cells growing in 10% fetal calf serum, as calculated from the data in Fig. 2A is 6.4 kcal/mol, while for the rate of adhesion the activation energy calculated from data in Fig. 1 is 24.4 kcal/mol, suggesting that fluidity of the lipid bilayer is not the rate limiting factor of the adhesion rate under the assay conditions used. We therefore conclude that if the model of Fig. 10 is a reasonable representation of the adhesive events, then these events are not limited by the rate of lateral diffusion of membrane adhesive components under the conditions of our assay and with the cell lines used. The observations with cell line B65 are in general agreement with this notion, but the complex adhesion kinetics shown by this cell line require some comment.

The adhesion of B65 cells to a monolayer of B65 cells occurs much more rapidly than the corresponding adhesion of B103 cells to a B103 monolayer. In the context of the model shown in Fig. 10, this could be interpreted to indicate that the density of adhesive sites on this cell line is very high and it would therefore not be surprising that the adhesion of B65 cells to monolayer only

becomes dependent on shear force at a much lower temperature (17° C) than that of B103 cells, because it is only at this low temperature that the second step becomes rate limiting.

The pronounced lag phase which is also temperature dependent and cannot be abolished by preincubation of the cells suggest that in this cell preliminary cell to cell contact is also required for the 'induction' of adhesion. While such an assumption seems to fit the data, it is clear that further work will be required to explain these observations in detail and to provide a molecular basis for the induction phenomenon. If this mechanism applies, then the 'induction' of adhesion is itself a temperature dependent process (Fig. 8).

Several other investigators have examined the relationship between fluidity of membranes and cell adhesion or agglutinability of cells with lectins. Horwitz and coworkers [15,32] found a striking correlation between one of the discontinuities in the temperature-dependent partition of 2,2,6,6-tetramethyl piperidine-*t*-oxyl into elaidic acid-enriched membranes (from 3T3 cells) and agglutinability with concanavalin A, but no such correlation was found with wheat germ agglutinin. It is possible that this effect represents a specific interaction of a elaidate-containing phospholipid with a concanavalin A receptor rather than a general effect of membrane fluidity. A similar correlation has been made by Ueda et al. [33] for the binding of BHK cells to collagen-coated dishes in the presence of Ca^{2+} but not in the presence of Mg^{2+}. These data are based on temperature dependence of the ESR signal of a spin-labeled stearic acid, using whole cells, and it is unclear whether this label measures a property of the surface membrane or of some internal membranes. Finally, Hoover et al. [34] have measured the effect of addition of polyunsaturated fatty acids on adhesion of BHK cells to plastic and to homologous monolayer. Small changes were observed which however do not reflect the rate of adhesion, but only the extent of adhesion. The data suggest that, in a population of cells in which the lipids are enriched with polyunsaturated fatty acids, after harvesting a fraction of the cells no longer can adhere to either plastic or other cells. The data of Hatten et al. [32] do not deal directly with cell to cell adhesion but only with lectin-induced agglutination. Hoover et al. [34] have attempted to measure cell to cell adhesion in cells with altered lipid composition, and like us they found no effect of enrichment with oleic acid (or palmitic acid) on the rate of homologous cell adhesion at either 4 or 37° C. The effect they observed with polyunsaturated fatty acids seems to reflect the creation of a non-adhering cell subpopulation rather than a direct effect on cell adhesion. Curtis et al. [35] have measured the adhesion of retinal cells to each other in a Couette Viscometer, after incorporation of fatty acids into cellular lipids. Saturated fatty acids increased the rate of adhesion, and unsaturated fatty acids decreased the rate of adhesion. Unfortunately, no physical measurements of the membrane lipids were carried out and the absolute degree of substitution of fatty acids into membrane lipids cannot be calculated from this data, making comparison with our results very difficult. Schaeffer and Curtis [36] measured the rate of adhesion of mouse L929 cells onto plastic dishes after incorporation of fatty acids to membrane lipids. Some changes in the rate of adhesion were noted, but no data are available on either the degree of fatty acid substitution or changes in the physical properties of the membrane lipids. An attempt to mea-

sure 'fluidity' by the rate of antibody capping observed in these experiments is too indirect to give any information regarding the physical properties of the membrane, since capping could be related to many other cell functions. In addition, the adhesion assays and the capping experiments were carried out with cells incubated with fatty acids under different conditions. In any case, the 'fluidity' determined by this indirect method did not correlate with the rate of adhesion. In general, our experimental findings deviate from the generalization of Curtis and coworkers [35,36] that unsaturated fatty acids decreases the rate of adhesion, since increases in oleic acid content of our cells did not affect cell adhesion. It should be pointed out that the assay system is radically different in the two sets of experiments *. Thus, at the moment, with the exception of the results with retinal cells [35], there is no evidence that the lipid composition of the plasma membrane can affect cell to cell adhesion. To the contrary the results presented here suggest that fluidity of the lipid bilayer is not the rate limiting factor for adhesion as measured in a monolayer assay.

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* It is possible that measurements of the rate of adhesion with other cell lines, or even adhesion of B103 to other cells [12], may show effects of lipid substitution.

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