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MEMBRANE MOBILITY AGENTS

II. ACTIVE PROMOTERS OF CELL FUSION

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Summary

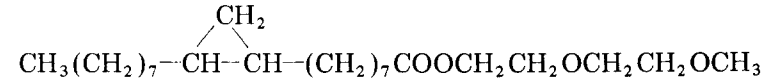
The membrane mobility agent, A_2C, actively promotes the fusion of hen erythrocytes under conditions similar to those used by Lucy et al. for glyceryl monooleate.

Intracellular membrane fusion is a common biological event, examples being exocytosis (e.g. neurotransmitter release) and endocytosis (e.g. phagocytosis). Intercellular membrane fusion (cell fusion) is less common, but can be promoted by certain agents and conditions. Experimental cell fusion, originally obtained with certain viruses [1—4], was later achieved by the use of chemical agents. Lucy et al. [5—9] have shown that lysophosphatidylcholine and a variety of other (usually) long chain molecules (e.g., unsaturated fatty acids and certain esters, polyols, dimethyl sulfoxide) can induce cell fusion for different types of cells. Other means of inducing cell fusion include the use of pederine [10], high pH with high $[Ca^{2+}]$ [11], and heat [6]. The obvious importance of lipid unsaturation, the effect of heat and the fusion promoting role of the appropriate vesicles have focused attention on membrane 'fluidity' and increased membrane 'disorder' as being involved in membrane fusion [8, 9, 12—15].

We have recently introduced a new class of biologically active compounds called membrane mobility agents, and have demonstrated their effectiveness in enhancing the mobility of anti-Ig·Ig complexes in lymphocyte membranes [16] and in promoting the redistribution of lectin sites in mastocytoma cell

Abbreviation: A_2C, 2-(2-methoxy)-ethoxyethyl 9,10-metholeneoctadecanoate [16].

membranes concomitant with a decrease in the agglutinability of the cells [17]. Since the membrane mobility agents were designed to promote local disorder within the membranes of cells, and since the practical expression of their activity was consistent with such disordering, it was logical to examine the effect of the agents on cell fusion. We now report that the membrane mobility agent, A_2C (formula below), actively promotes the fusion of hen erythrocytes under conditions similar to those utilized by Lucy and co-workers for the same cell type.



A_2C

Blood from the brachial vein of adult hens was mixed with heparin (as anticoagulant), centrifuged and the buffy coat removed. The erythrocytes were washed twice with 150 mM NaCl and once with buffer containing 76 mM sodium acetate, 70 mM NaCl, 1 mM $CaCl_2$ and 100 mg/cc Dextran (M_r 70 000 Pharmacia, Uppsala, Sweden) (pH 5.7). The buffer is similar to but simpler than that used in a similar procedure by Lucy et al. [7]. The cells were resuspended in the same buffer and mixed with an equal volume of a suspension of the mobility agent A_2C dispersed in 150 mM NaCl by sonication (approx. 2 min). No special precautions are required during sonication and the suspension remains active for several hours. The final concentrations of A_2C used ranged between 0.06μl to 0.5 μl/cc containing $2-3 \cdot 10^8$ cells, 0.5 mM $CaCl_2$, 38 mM sodium acetate, 110 mM NaCl and Dextran (50 mg/cc). Cell suspensions were incubated at the desired temperature, mixed gently every 5—15 min and aliquots removed at intervals for viewing and photography at room temperature.

Addition of A_2C in amounts of 0.125 to 0.5 μl/cc of cell suspension led to rounding and swelling of the cells within 10—30 min after mixing and incubation at 37°C. Contacts between the rounded and swollen cells were noted, followed by increasing degrees of fusion, starting with binucleated, trinucleated and tetranucleated cells and proceeding to multinucleated cells. The fusion process eventually involved most of the erythrocytes. The results are illustrated in Fig. 1. Little or no lysis occurred at the binucleate and trinucleate stage but did eventually ensue for multinucleate cells (e.g. as in Fig. 1e) 30—60 min after their formation.

The rate and extent of fusion observed in suspensions containing $3 \cdot 10^8$ cells/cc were dependent upon the amount of A_2C used. Amounts below 0.1 μl/cc led to only a small degree of fusion (10%) after 2—3 h. Quantities of 0.12 μl/cc gave a satisfactory degree of fusion (25—40%) after 1 h but amounts of 0.25 μl/cc gave a high proportion of fusion (70—90%) usually within 45 min. Further increases in the amount of A_2C did not improve the rate or the extent of fusion in a significant way. Lucy et al. [7] have noted a similar rapid increase in the rate of fusion of hen erythrocytes with glycerol monooleate.

Temperature changes affected the rate of fusion induced by A_2C very greatly. At 37°C, a high degree of fusion was noted between 45—70 min,

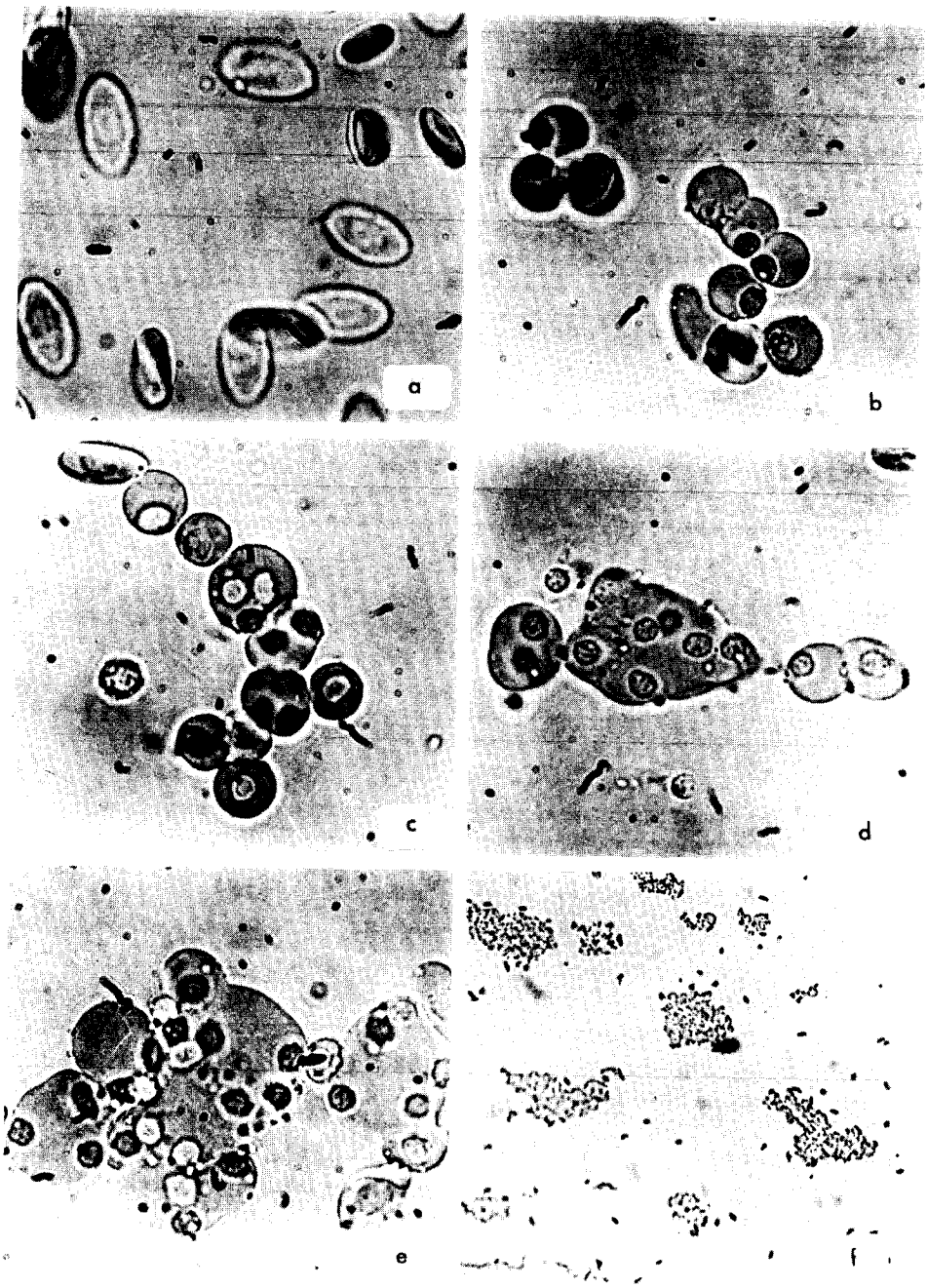


Fig. 1. Fusion of hen erythrocytes by the membrane mobility agent, A_2C

The preparation of the incubation mixture is described in the text. A_2C 0.25 μl/cc. Temperature of incubation 37°C. Frames a—e × 800. Frame f ×80. Viewed with Leitz light microscope. (a) 5 min, cells are similar in shape and size to control cells. (b) 25 min, rounding and contact between cells may be seen. (c) 40 min, fusion to binucleated and trinucleated cells. (d) 60 min, multinucleated cells. (e) 80 min, extensive fusion with many cells involved. (f) 80 min, high degree of involvement of cells illustrated.

but 100—180 min were required at 27°C for a similar degree of fusion to occur. All stages (rounding and swelling as well as fusion) were slowed down by lower temperatures. Incubation at a temperature of 17°C resulted in a very small percentage (10% or less) of fused cells after 3—4 h of incubation. Fusion could be arrested if the sample were placed in ice, or diminished by washing or mixing with bovine serum albumin solution (1% final concentration) at the beginning of incubation.

The mechanism(s) of fusion are not understood. Some general knowledge about the factors which contribute to the fusion of cells is available. Certainly the nature of exogenous trigger (catalyst) is lipophilic although Ca^{2+} is also required in most cases. (glycoproteins have occasionally been implicated [23,24]. The viruses capable of inducing cell fusion belong to groups of lipid-containing viruses. Kohn assumed that the integrity of viral phospholipids was required for cell fusion since he found that lipid hydrolysis was the only treatment which abolished the ability of the NDV virus to induce fusion [2,4]. It seems likely that the "fluidity" of the membrane of the cell which undergoes fusion contributes to the ease of fusion, as shown by the promotion of fusion by higher temperatures. Pasternak and Micklen have shown that no turnover or degradation of cell membrane phospholipids or other membrane components occurs during fusion [18]. Thus, fusion results from physical rather than chemical processes. Studies on vesicles [12--15] suggest that altering the state of the bilayer may promote fusion. A role for membrane protein mobility is suggested by the work of Loyter et al. [19] who found that close contact between fusing cells occurs in areas denuded of virus particles in fusions promoted by virus. Bächi et al. [25] have shown that redistribution of protein in the membrane occurs after virus attachment and that viral protein is absent from sites of membrane fusion; however, the presence of virus at such sites has been reported [26]. Redistribution of membrane sites in fused cells, but not in unfused cells of the same type, was demonstrated by Okada et al. [20]. Redistribution is of course well-known from the work of Frye and Edidin [21] but the precise relationship of this phenomenon to fusion is not clear. Haywood [22] has found Sendai viral "patches" on liposomes.

In view of our evidence that membrane mobility agents promote motion through cell membranes [16,17], it seems reasonable to suppose that some increase in local "fluidity" favours cell fusion. We hope that further experiments with other membrane mobility agents will provide additional insight into the fusion process.

The obvious practical importance of cell fusion for such purposes as somatic cell genetics, therapy for genetic defects, and hybridization of plant cells could be enhanced by the introduction of well defined stable agents which do not contain nucleic acid or protein. Membrane mobility agents, which are easy to convert to reasonably stable suspensions, may be useful in advancing the study and application of cell fusion.

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