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CELL MEMBRANE FLUIDITY: MOLECULAR MODELING OF PARTICLE AGGREGATIONS SEEN IN ELECTRON MICROSCOPY

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Summary

The first simulation of the fluid mosaic model is reported. Intra-membrane particles, initially "placed" randomly in a membrane with fluid properties, are allowed to diffuse in the plane of the membrane and to interact with one another, in a model using molecular parameters. The resulting particle aggregates are very similar to those observed in freeze-fracture electron microscopy.

The elucidation of the structure and function of membranes remains one of the most important problems in biology. The fluid mosaic model [1] of membranes, based on thermodynamics [2] is strongly supported by a wide range of evidence, from the seminal experiments demonstrating macroscopic movement of antigenic determinants in heterokaryons [3], antigen-induced "capping" in lymphocytes [4] and cell fusion [5] to translocation of particles observed in electron microcopy by the freeze-fracture technique in chloroplast and other membranes [6] and the diffusion of rhodopsin molecules in the retina [7]. There are several excellent reviews [8,9]. The fluid mosaic model stresses that protein-associated elements of the membrane are not held rigidly in particular locations, but may be free to move in the plane of the membrane.

Studies of the diffusion constant D would provide an essential parameter for applying the fluid mosaic model to the biological function of membranes of different cell types, and could also be of clinical importance in dysfunction. To measure the D of particles in an intact membrane, in principle one need only (i) label them (for biochemical identification), (ii) start them off in an identifiable pattern, and (iii) measure a distance x moved with time; then $\overline{x^2} = 4Dt$, where the bar denotes an average in time. Unfortunately, this

measurement is feasible under physiological conditions only in a limited number of cases [8]. The movement of fluorescent labels on heterokaryons [3] and myotubes [10], and of light-bleached rhodopsin molecules can be measured by optical means [7], but a method of wide ultramicroscopical applicability is not at hand partly because of the technical difficulties of laying down an identifiable pattern of membrane entities. (For lipids there are methods for measuring the mobility of a typical lipid molecule surrounded by its neighboring lipids [8].)

However, with the electron microscope there is clear evidence of aggregation of the particles observed by freeze-fracture [6,11] as well as of antibody trans-membrane induced aggregation of colloidal iron hydroxide sites by antibodies [12]. There is evidence that the particles are proteinaceous [13, 9]. These particles can be called IMP (intra-membrane particles), MAP (membrane-associated particles), PPPPP (peripatetic, presumably predominantly protein particles), or simply particles. Aggregation of particles may well have physiological [6,14] and oncogenic [15] significance. Because so much electron microscopical work has been published on the aggregation of particles, one is challenged to model the morphology of aggregation on a molecular scale. Here a model [16] is used to predict the patterns which would be made by particles aggregating in a membrane, compare the results with those seen in freeze-fracture micrographs of membranes, and good agreement is found.

Following established precedent a simple model is chosen [17]. The model assumes that particles of 8 nm diameter are to be found initially at random in two dimensions, at a packing density of 4300 particles/μm^2 in an effective viscosity of 1 poise [8]. These values ($6.2 \cdot 10^5$ particles per cell [18] of area 145 μm^2 [19]) are typical for mammalian (human) erythrocyte membranes at least. The particles in chloroplast membranes from wild-type chlamydomonas are at a similar packing density but can be larger [6]. The Brownian motion is mimicked, in the spirit of the Monte Carlo technique [20], by moving each particle in turn separately and randomly in distance and direction. The size of the random movements is simply related to the particle motion: the variation of x with time t of a particle is given by the Brownian motion equation of Einstein [21].

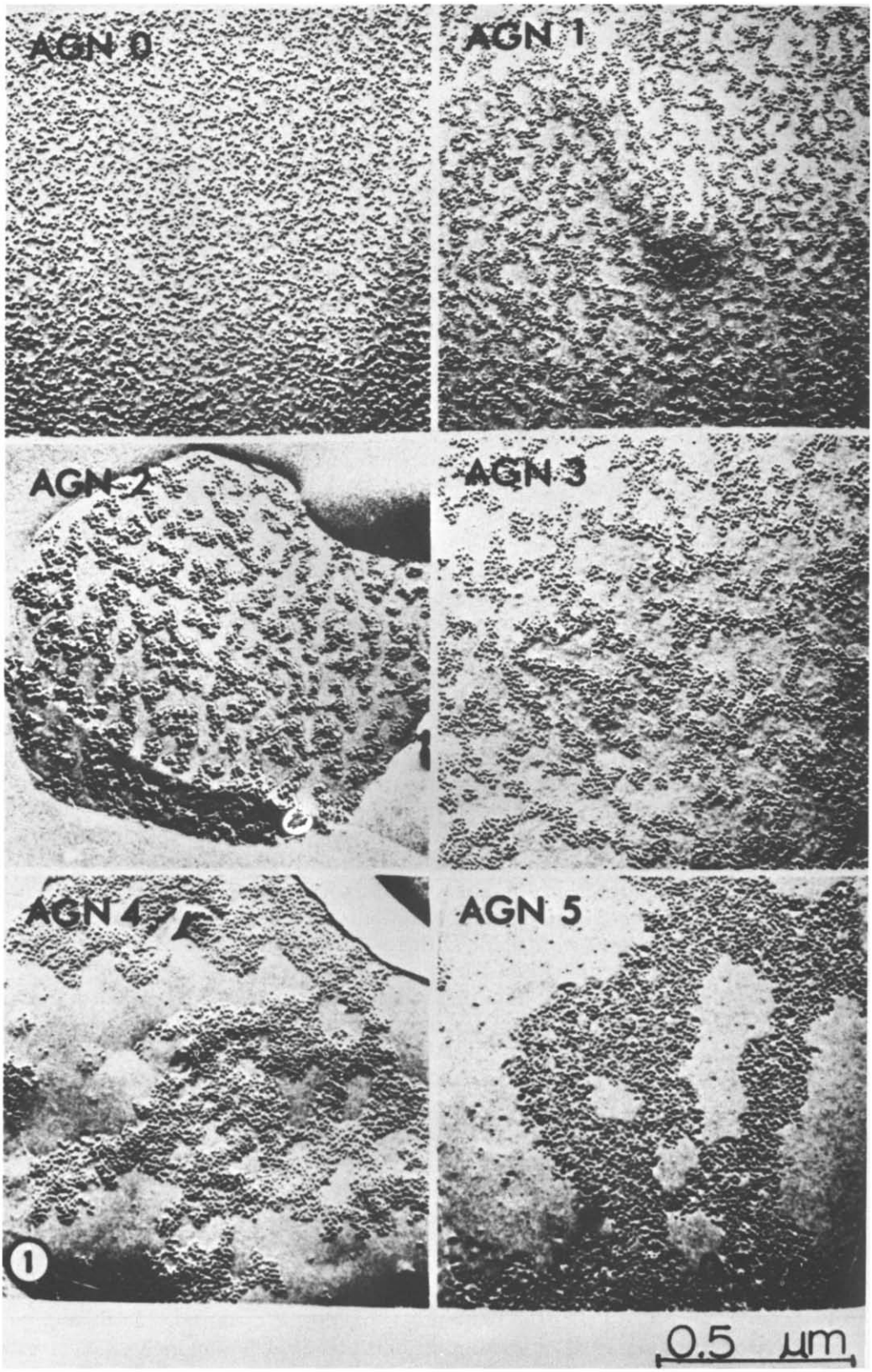
$$\overline{x^2} = \frac{2kT}{6\pi\eta a} \quad 2t$$

The bar denotes average value, k is Boltzmann's constant; the particle, of radius a, is in a medium of viscosity η, at an absolute temperature T. (The use here of the Stokes equation is a simple convenience which is not essential.) When all the particles have been randomly moved once, then the distance between all of them is measured in turn to see if any two are close enough to collide. Since, at present, we do not know the interaction parameters between particles, we have simply assumed that when two particles approach to within the interparticle distance observed in electron micrographs of the aggregated particles, they always bind irreversibly and non-cooperatively, irrespective of direction of mutual approach. Hence steric hindrance limits the number of nearest neighbor particles to six. From micrographs kindly

sent by Professor D. Branton [11], a reasonable interparticle distance is 12.5 nm. In the Monte Carlo approach, it is easy to include later (a) any interparticle interaction, (b) any desired binding-unbinding kinetic rates, (c) any particle asymmetry and valence. Indeed, one of the powers of the method is just that of using statistical mechanics to explore the microscopic world. The computer notes which particles have aggregated in pairs. This completes one machine "cycle", which may be thought of as one picture frame in a cinematograph of the particles on the membrane. The frequency with which pictures are "taken", i.e. frequency of machine cycles, controls the simulated time rate of aggregation. Succeeding machine cycles proceed as before, but each cluster of two or more particles is translated and rotated randomly as a whole about its common centre of mass. Larger clusters both translate (drift) and rotate more slowly: a study of the hydrodynamics involved shows that plausible computable behavior for an n-particle cluster is $\overline{x^2}$ proportional to $1/n^{1/2}$ for translation and the corresponding angular variation proportional to $1/(n-1)^{3/2}$, but the exact variation is relatively unimportant at this preliminary stage. After every particle and cluster has been translated and rotated, again the computer tests for which particles and/or clusters are close enough to combine, and the whole process is repeated cyclically until practically every particle has combined into a cluster, and the clusters have aggregated. After any desired cycle the positions of each of the particles is drawn.

Fig. 1 shows electron micrographs of human erythrocyte membrane particles revealed by the freeze-cleave process [11]; the aggregation was induced by pH and other agents from the unaggregated state under physiological conditions. For comparison, "micrographs" of the aggregation process for a typical computer run of 784 particles are shown in Fig. 2. It is evident that the two series have great similarity, and hence that the morphology of aggregation seen in freeze-etch electron micrographs has been successfully simulated by a computational model. No attempt whatsoever was made to adjust the parameters of the computer model to give better agreement, although computer runs were made at different values of some of the parameters to check questions of sensitivity and "convergence". (A variation of some parameters gives spider-liker pictures which are quite unlike reported micrographs.) Hence the assumptions are quite consistent with, and strongly support, the fluid mosaic model [1]. Moreover, because the induced aggregation experiments [11] are not done kinetically as frames in a time sequence, it appears that the effect of (e.g.) pH is to release the membrane particles (which are relatively immobile in the human erythrocyte ghost membrane under normal conditions) temporarily from constraints. These constraints very probably involve spectrin [12,11] and calcium [9].

Preliminary studies of the model, with a different number density of particles per unit area, show that aggregation patterns similar to those observed in electron microscopy occur only for a relatively narrow range of particle density, which later is found to agree with the observed microscopical density. It is very likely [8,21] that the two-dimensionality of membranes leads to a much higher frequency of interaction of particles (which may include membrane-bound enzymes) than does a three-dimensional system, provided of course


AGN 0
AGN 1
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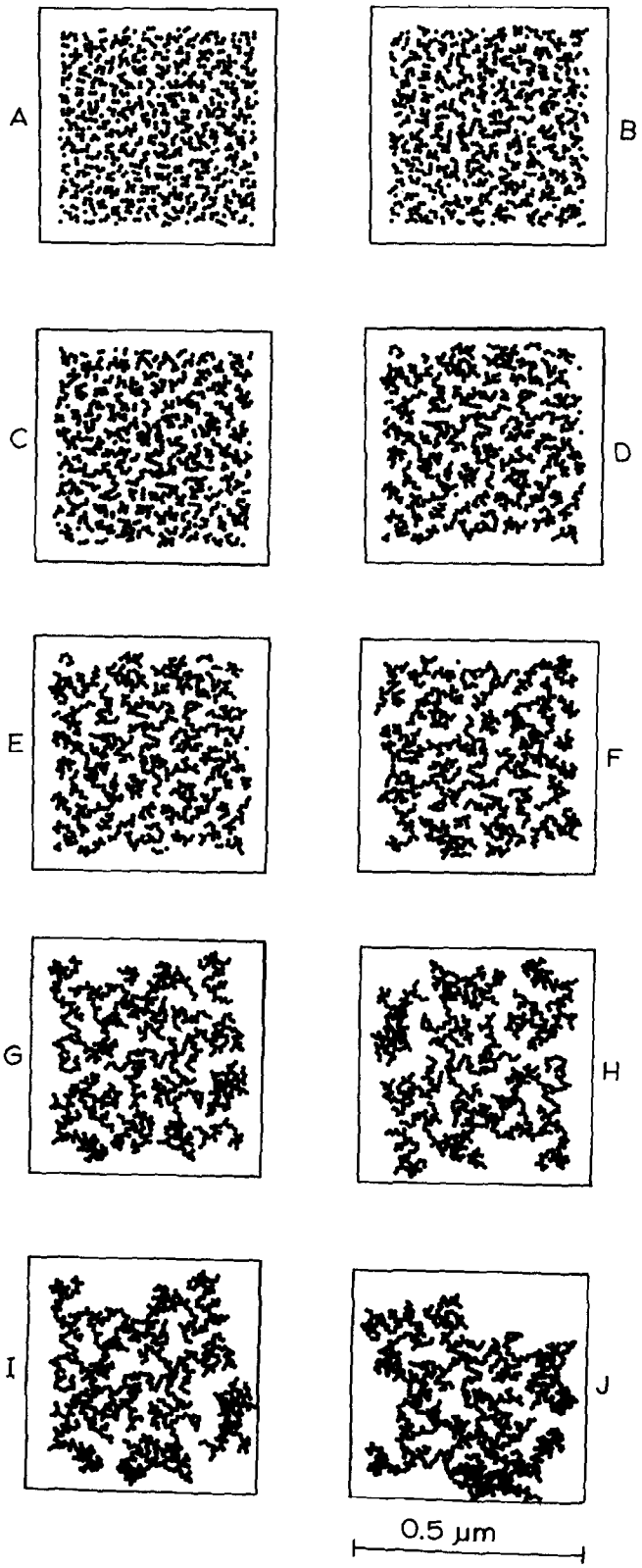
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that the membrane is "fluid". Hence the actual physiological fluidity observed in membranes may be, and may provide, a happy medium for particle interaction. This dependence on particle density/fluidity, which has close similarities to a phase change in two dimensions, is consistent with the many observations made on the melting ranges of lipids and of membrane transport systems: many creatures do appear to maintain a constant membrane fluidity [22].

Hence, by using experimentally-determined data on membrane viscosity and other membrane parameters, in a quite novel combination with computer techniques, morphological computer-generated "electron micrographs" show that the aggregation of membrane particles seen by freeze-cleave electron microscopy is indeed a natural and direct consequence of membrane fluidity.

Refinements in progress will include recent work [23].

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