Review–Hypothesis

An osmotic model for the fusion of biological membranes

Jack A. Lucy and Quet F. Ahkong

Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine, University of London, Rowland Hill Street, London NW3 2PF, England

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A molecular model for fusion-fission reactions in membranes is proposed that is based on data from studies on artificially induced cell fusion and on the behaviour of phospholipid bilayers: it is put forward as a framework for further investigations into this fundamental property of biological systems.

Membrane fusion *Cell fusion* *Osmotic pressure* *Myoblast fusion* *Sendai virus*

1. INTRODUCTION

A variety of lipid-soluble and water-soluble substances can artificially induce cells to fuse [1], and exposure of cells to an electrical breakdown pulse also causes cell fusion [2]. Although phospholipid vesicles can be fused with a phospholipid bilayer by the application of an osmotic gradient [3] and there is evidence that osmotic swelling is important in exocytosis reactions [4,5], early indications that osmotic swelling might be a significant feature of chemically induced cell fusion [6] proved difficult to substantiate. However, recent observations have shown that osmotic swelling can drive the fusion of hen erythrocytes after the cells have been treated with ionophore A23187 and Ca^{2+} to activate proteolysis of the membrane skeleton, and that osmotic forces are involved in the fusion of human erythrocytes by poly(ethylene glycol) and by electrical means [7]. Consideration is therefore given here to a general molecular model for the fusion of biological membranes that involves osmotic phenomena, since earlier models have been based only on the structures and physical properties of the molecules and ions concerned. In addition, some published observations on virally induced membrane fusion and the fusion of myoblasts are re-interpreted in terms of the new model.

2. MEMBRANE APPOSITION

Fig.1 illustrates 6 stages that are proposed as key events in the fusion reactions of biological membranes. On the basis of this model, 6 requirements for fusion may be defined, and each of them normally needs to be met if a fusion-fission reaction is to be completed successfully.

A first requirement in membrane fusion is some means of bringing two membranes very close together by overcoming the short-range forces of repulsion (fig.1a). This has been discussed in detail by others with respect to the physical properties of phospholipid bilayers [8,9]. Suffice it to say here that: (i) the initial close apposition of the two membranes may involve Ca^{2+}, acting either alone or in conjunction with specific proteins, e.g. synexin [10]; (ii) interactions involving membrane-bound receptors are also important, as in the fusion of Sendai virus particles with the plasma membranes of cells and probably in the fusion of myoblasts to yield myotubes; (iii) in cell fusion induced by poly(ethylene glycol), the dehydrating effect of the polymer is believed to force ap-

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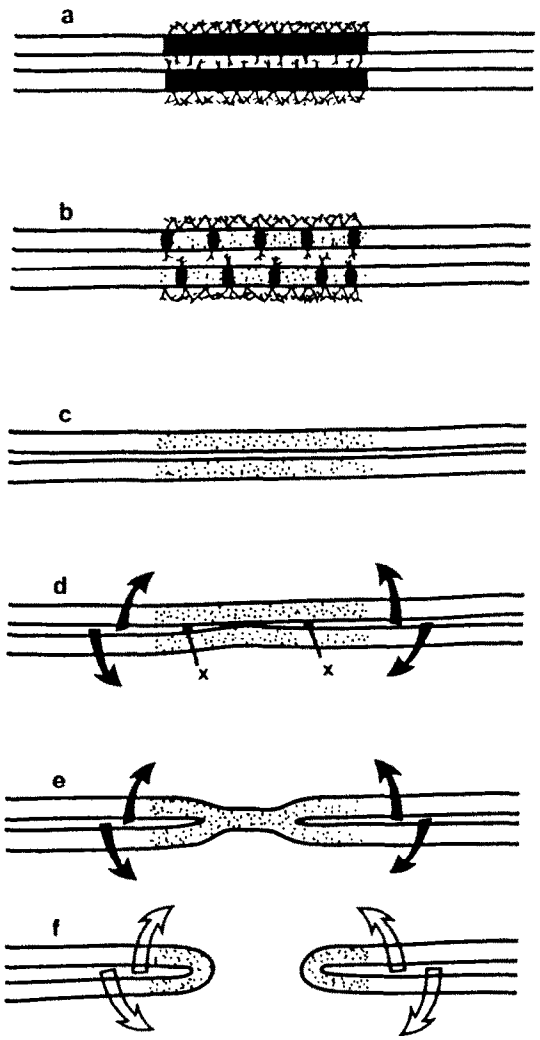

Fig.1. A schematic diagram that illustrates 6 proposed events in the fusion-fission reactions of biological membranes. (a) The initial close approach of two membranes. (b) The phospholipid bilayers of the membranes are perturbed at the potential fusion site. This is illustrated diagrammatically by the change from solid to hatched shading. (c) The membrane proteins are perturbed. The absence of integral membrane proteins, membrane-skeleton and cytoskeleton proteins from the potential fusion site is intended to indicate that the perturbed lipid bilayers are now relatively unrestrained by membrane proteins, and it does not imply that the lipid bilayers are totally free from all polypeptides at the developing site of fusion. (d) Solid arrows represent the entry of water into one, or both (as illustrated), of the two membrane-bound compartments, which causes swelling either because osmotically active particles have accumulated in one or both compartments or because functionally impermeable membranes that previously prevented swelling now have an increased permeability. The regions marked 'x' are indicative of structures or mechanisms that hold the membranes together adjacent to the fusion site as swelling proceeds. (e) Under the influence of the pressure generated by the osmotic gradient, the two phospholipid bilayers are forced together at the fusion site and they form an intermediate, single bilayer that is common to both of the fusing membranes. (f) Osmotic swelling imparts a stretching force that ruptures the intermediate, single bilayer at one or both of the points where three bilayers meet. The original bilayers then re-seal; continued entry of water (open arrows) will cause further swelling, and may cause lysis.

proaching membranes together; (iv) with erythrocytes that are fused by treatment with lipid-soluble fusogens, the treated cells become adhesive [11] and this may result from the perturbation of membrane phospholipids that is discussed below; (v) in the electrically induced fusion of cells that are aligned by dielectrophoresis, an a.c. field of sufficient strength to flatten adjacent cells against one another has to be applied before exposure of the cells to an electrical breakdown pulse [2].

3. PERTURBATION OF MEMBRANE PHOSPHOLIPIDS

A second requirement for membrane fusion reactions is a perturbation of the phospholipid bilayers of two approaching membranes (fig.1b). This may be achieved in several different ways. In fusion induced by viruses, a highly conserved hydrophobic sequence in a viral polypeptide is believed to be inserted into the bilayers. Other, non-viral proteins and peptides are also known to be fusogenic, and it has been proposed [12] that hydrophobic polypeptides, which are functionally comparable to the fusogenic proteins of viruses but which are produced in cells by the endogenous proteolysis of membrane and cellular proteins, may similarly be involved in some of the fusion reactions that occur in vivo for which the fusogenic stimulus is presently unknown. It has also been suggested that the effectiveness of lipid-soluble fusogens in perturbing membranes is related to

their fluid nature since, for example, oleoylglycerol and oleic acid are fusogenic while stearoylglycerol and stearic acid are not [11]. *cis*-Unsaturated fatty acids (but not *trans*-unsaturated, saturated or esterified *cis*-unsaturated fatty acids) fuse chromaffin granules aggregated by synexin [10]. Simple hydrocarbons (e.g. tetradecane, hexadecane and octadecane) will also fuse erythrocytes [13].

Any molecular species that interacts with phospholipids to yield a non-bilayer configuration, such as a micellar structure [14], a hexagonal phase [15], or inverted micelles [16], will be potentially fusogenic because of its ability to perturb phospholipid bilayers, but it does not necessarily follow that such non-bilayer structures are actually intermediates in membrane fusion reactions. The dehydrating action of poly(ethylene glycol) may be sufficient to de-stabilise phospholipid bilayers. However, an additional perturbation by very small quantities of lipid-soluble fusogens enhances the fusion of erythrocytes by the polymer [17] and in some biological systems uncharacterized impurities appear to be necessary for it to have fusogenic properties [18]. In electrically induced cell fusion, the energy of the breakdown pulse provides yet another means of perturbing the phospholipids of membranes.

4. PERTURBATION OF MEMBRANE PROTEINS

A third requirement in many biological systems is perturbation of the protein structures that are associated with membranes (fig.1c). If the proteins are not perturbed, membrane fusion may be inhibited or totally prevented for one or more reasons. Thus, negatively charged sialic acid residues on plasma membrane surfaces may prevent the membranes from coming into very close contact, and integral proteins within the phospholipid bilayers may sterically prevent bilayer-bilayer interactions. In addition, membrane-skeleton and cell-skeleton proteins can restrain cell swelling [19] and thus interfere with osmotically driven cell fusion. Proteinases are important in chemically induced cell fusion [20–22], and the fusion of erythrocytes by some chemical fusogens involves the proteolysis of ankyrin by a serine proteinase [23] and the proteolysis of band 3 by a calcium-activated proteinase [23,24]. Inhibitors of these proteolytic activities inhibit fusion. With poly(ethylene glycol), the presence of the polymer inside the treated cells [7] may precipitate the proteins of the membrane skeleton and cell skeleton to release the plasma membrane from restraining influences, and it is relevant to note that the precipitation of spectrin and actin by protamine sulphate in leaky erythrocyte ghosts allows membrane vesicles to be extruded [25]. There is evidence, however, that proteinases are also important in fusion induced by poly(ethylene glycol) [26]. It has been suggested that proteolytic activity associated with the Sendai virus is an integral feature of its ability to fuse cells [27], and a metalloendoprotease is important in the fusion of myoblasts into myotubes [28]. In electrically induced cell fusion, the breakdown pulse appears to damage the membrane skeleton, since an unusually high diffusion constant for dipoles in the plane of the membranes of electrically fused cells has been observed. This has been attributed to a diminished interaction of the skeleton with the membrane [29]. Finally at the subcellular level, the clathrin coat of coated vesicles inhibits fusion of the vesicle membrane with that of lysosomes [30], and a metalloendoprotease appears to be involved in exocytosis in mast cells and in adrenal chromaffin cells [31].

The inhibitory effects of proteins on membrane fusion reactions may be contrasted with the fact that phospholipid vesicles are fused with a phospholipid bilayer simply by applying an osmotic gradient [3]. It therefore seems logical to conclude, as suggested previously [32], that membrane fusion in biological membranes occurs in regions of lipid bilayer from which endogenous, integral polypeptides have been largely removed, and which are also freed from the restraining influence of skeletal networks. Many electron microscope studies have indicated that, before exocytosis, the plasma membrane overlying secretory granules is denuded of intramembranous particles, and this has been interpreted to mean that fusion occurs in lipid-rich domains that are cleared of proteins. However, quick-freezing experiments have indicated that the clearing of intramembranous particles is an artefact [33]. Membrane fusion is thus likely to involve rearrangements that affect only relatively few lipid molecules [34] and,

in the present model, the protein-free region of lipid bilayer is only some 30 nm in diameter. This is comparable with the size of the initial openings (20–50 nm) observed by rapid freezing in plasma membranes during exocytosis [35,36], and is far smaller than the possibly artefactual cleared areas of bilayer.

5. THE OSMOTIC GRADIENT

In fig.1d, the arrows are intended to indicate the change in membrane permeability or transport that occurs, at sites which are different from the fusion site itself. This results in the osmotic swelling that may drive many fusion-fission reactions. The swelling of two membrane-bound compartments is shown in fig.1 but, in the fusion of phospholipid vesicles with a planar phospholipid bilayer under the influence of an osmotic gradient [3] and in exocytosis that involves vesicle swelling [4,5,40], only one compartment swells. The regions marked 'x' in fig.1d are intended to indicate that the membranes must be firmly held together by surface interactions as osmotic swelling proceeds. This may involve, among other possibilities, interactions of Ca^{2+} with membrane phospholipids (as in the osmotically driven fusion [3] of phospholipid vesicles with a planar bilayer) or specific surface proteins. Adjacent swelling cells that are not held together will lyse separately rather than fuse. For example, when erythrocytes are treated with chemical fusogens or are exposed to an electrical breakdown pulse, some of the cells lyse without fusing. In early work we reported that: "As we have never observed fusion between the oval-appearing, unswollen erythrocytes, and as every chemical found to induce cell fusion in this laboratory also causes cells to become spherical, we conclude that cell swelling by colloidal osmosis plays an essential role in cell fusion" [6]. It was also noted that colloid-osmotic lysis is closely associated with the fusion of erythrocytes by lipid-soluble chemicals and that, in the absence of Ca^{2+}, the cells lyse more readily than they fuse [37].

The possible role of osmotic swelling in exocytosis has been of much interest since it was shown that solutions of high osmolarity can inhibit the exocytosis of chromaffin granules in adrenal medullary cells [4,5], and phospholipid vesicles were induced to fuse with a planar phospholipid bilayer by osmotic means [38]. Chemiosmotic hypotheses of exocytosis are based mainly on the presence of an ATP-dependent proton pump in the membranes of many secretory vesicles but, on the basis of a review of available evidence, it has been concluded that the vesicle pump is very unlikely to play an essential part in exocytosis [39]. Nevertheless, the recent demonstration that exocytosis of cortical granules in sea urchin eggs is inhibited by high osmolarity [40] lends further support to a central role for osmotic swelling in exocytosis reactions.

There is evidence that the electrical breakdown of cell membranes, as in electrically induced cell fusion, results from a decrease in membrane thickness due to electrical compressive forces. Thus, the breakdown potential decreases with increasing cell turgor pressure in algae [41] and, at a sufficiently high hydrostatic pressure, the intrinsic membrane potential of erythrocytes is apparently sufficient to induce membrane breakdown [42]. Also, erythrocytes in a hypotonic medium are fused more readily by a breakdown pulse than in isotonic medium [7]. This may indicate that osmotic and electrical pressures combine to stress membranes in electrically induced cell fusion.

6. AN INTERMEDIATE, SINGLE BILAYER

The penultimate event in the proposed model is fusion of the phospholipid bilayers into an intermediate single bilayer, as a consequence of osmotic swelling that forces the two bilayers together (fig.1e). For this to occur the cohesive interactions between phospholipids within the two bilayers must be sufficiently and appropriately perturbed. If they are not, the osmotic force will be insufficient to accomplish the (otherwise very energetically unfavourable) movement and reorganisation of phospholipid molecules that is involved in the local formation of a single bilayer. Also, it is envisaged that the phospholipids of the original bilayers are likely to be randomly arranged both in the new single bilayer (approx. 10 nm in diameter) and in the perturbed region of its immediate vicinity. Asymmetrical distributions of phospholipids in the original bilayers would thus not be preserved at the site of membrane fusion.

The availability of an extrinsic source of energy in the form of an osmotic gradient offers a possible

solution to a fundamental problem that is inherent in all models that postulate a non-bilayer organisation of phospholipids as an intermediate in the fusion reaction. Namely, if a non-bilayer structure is formed spontaneously when the phospholipid bilayers are perturbed by a fusogenic agent, what causes it to disintegrate in the final fission process? It is relevant that three recent papers [43–45] have supported an earlier proposal [46] that a new bilayer is formed when phospholipid bilayers are pushed together with a sufficient force to overcome their mutual repulsion. When a very large force was applied to two mica surfaces, which were each coated with a bilayer of phosphatidylcholine, the mica surfaces pinched-in to a separation that corresponded to a single bilayer [44]. The pinched-in region then rapidly enlarged until the single bilayer occupied an area of 30 μm in diameter.

It therefore seems that, if a sufficient force is applied, a new bilayer is formed which is so stable that it can distort the mica surfaces. Significantly, the formation of a single bilayer occurred more often with egg phosphatidylcholine than with dilaurylphosphatidylcholine, and when hexane was present. Its formation was thus apparently governed by the degree of molecular cohesion within the bilayers [44]. It is also relevant that, in the fusion of phospholipid vesicles with a phospholipid bilayer induced by the application of an osmotic gradient, it was not possible to be certain that the bilayer membrane was absolutely hydrocarbon-free [47]. Furthermore, the addition of decane to a 'hydrocarbon-free' bilayer resulted in a marked increase in fusion rate [47] and, as noted above, hydrocarbons can induce erythrocytes to fuse [13].

Comparable experiments have shown that the new structure produced by the merging of two bilayers has the electrical properties of a single bilayer. Capacitance measurements have been used to monitor the interaction of bilayers of monooleoylglycerol (containing hexadecane) which were pushed together mechanically, and which fused when water was removed from between them; it was concluded that there was no doubt that a single bilayer had been formed, and that the membranes did not remain as two closely adhering bilayers [45]. Similar data and conclusions have been reported with experiments on phospholipid bilayers that were pushed together by being inflated [43].

Interestingly, an early report [48] on vascular endothelia described a single membrane (20 nm in length) that was common to both the plasma membrane and a vesicle membrane, and which separated the contents of the vesicle from the extracellular fluid prior to their discharge by exocytosis. More recently a model for membrane fusion in exocytosis, based on electron microscope observations, has been proposed that also involves the formation of a single bilayer at the fusion site [49].

7. MEMBRANE FUSION

The final event in the proposed scheme is membrane fission. It is envisaged that this results from membrane stretching, caused by osmotic swelling, and that fission occurs at the fusion site because the region at which three bilayers meet is a weak point (fig.1f). This will give rise to a hole, which is initially of only about 10–15 nm in diameter, but which will rapidly widen as swelling proceeds. If, in the case of cell fusion, swelling continues after the fission reaction, the fused cells will become rounded and then they will lyse.

We suggest that in biological membranes the restraining influence of membrane-skeleton and cell-skeleton proteins that are adjacent to the site of fusion may restrict the surface area of new single bilayer, and that it will not enlarge as in the model lipid systems discussed above. Conversely, it has been commented that the resistance to rupture of single bilayers formed by the fusion of two bilayers in model systems, compared with fusion-fission reactions in biological systems, could be due to hydrocarbon solvent in the bilayer [45]. Such bilayers are, nevertheless, ruptured by an electric breakdown pulse [43,45] or with a needle [43]. The frequency of spontaneous rupture is increased when a single bilayer is formed in model experiments from bilayers that contain different proportions of charged and neutral lipids [43], and this point may be significant in relation to exocytosis.

Although phospholipid vesicles have been much more intensively studied than bilayers as models for membrane fusion, their fusion-fission reactions are accompanied by extensive rearrangements which have no direct parallel in the fusion of biological membranes. For example, (i) the

addition of Ca^{2+} to sonicated vesicles of phosphatidylserine induces the formation of large, multilamellar, cochleated, cylinders which then become large, closed, spherical vesicles following the addition of excess EDTA [50]; (ii) sonicated vesicles of phosphatidylserine treated with polylysine give rise to large, multilamellar structures [51]; (iii) multilamellar vesicles of phosphatidylcholine, phosphatidic acid and cholesterol fuse on treatment with poly(ethylene glycol) into much larger vesicles that are themselves multilamellar [52]. It has nevertheless been shown that unilamellar proteoliposomes containing phosphatidylserine, which cease to fuse in the presence of Ca^{2+} after the liposomes have grown to diameters of about 100 nm, can be induced to fuse further by an osmotic gradient (internal osmotic pressure higher than external) and form single-walled liposomes with diameters exceeding 1 μm as well as multilayered vesicles [53]. This demonstrates that the osmotically driven fusion of phospholipid membranes is not solely a feature of systems in which one or both of the membranes is constrained within an orifice or attached to a substrate.

The scheme proposed in fig.1 is thought to be applicable to most cell fusion and exocytosis reactions although, depending for example on the degree of force applied to the membranes at the fusion site, the intermediate single bilayer may be very short-lived. Furthermore, when an electrical breakdown pulse is applied to cells that are already flattened against each other by an a.c. field, it seems possible that there is no stable intermediate structure since cell fusion is instantaneous with the breakdown pulse [7]. Thus, we suggest that the electrical compressive force (postulated in the electro-mechanical model for membrane breakdown [42]) firstly causes the outer phospholipids of the two closely apposed bilayers to interdigitate and then, in a continuous process, induces extensive thinning that leads directly to membrane rupture. Any applied osmotic or hydrostatic pressure will supplement the electrical compressive force. In this connection it is noteworthy that cell fusion generally occurs when the field strength is 1.5–2-times greater than the minimum necessary for membrane breakdown [2]. This might be a consequence of fusion proceeding by the proposed pathway which, by comparison with mechanisms involving the individual breakdown of the membranes of closely apposed cells [2], will require an additional compressive force to achieve the initial interdigitation of the phospholipids of the two membranes.

The model proposed in fig.1 appears not to apply fully to membrane fusion processes in which one membrane-bound compartment gives rise to two compartments, as in endocytosis, in the pinching-off of vesicles extruded from cells, and in cell division. It is therefore proposed that only the first three requirements for fusion reactions outlined here apply in such instances. The closely apposed bilayers may then rupture (and subsequently reseal) as a consequence of the stretching force that is applied when, for example, dividing cells move apart and endocytic vesicles are pushed away from the cell periphery. It remains conceivable, however, that some osmotically driven cell shrinking and vesicle shrinking may assist in stretching the membranes at the fusion-fission site.

8. SOME APPLICATIONS OF THE MODEL

The following observations indicate that the fusion of erythrocytes by haemolytic Sendai virus is essentially similar to the fusion of these cells by lipid-soluble fusogens. (i) The viral membrane is permeable and its incorporation into the erythrocyte plasma membrane (by virus-cell fusion) therefore causes colloid-osmotic swelling and lysis of the cells [54]. (ii) The time lag that precedes the increased permeability of cells treated with haemolytic Sendai virus is not due to a lag in virus-cell fusion but to the development of a threshold level of membrane damage, e.g. a sufficient number and/or size of permeability pores [55]. (iii) With large numbers of virus particles, Ca^{2+} or other divalent cations are required for cell fusion: otherwise cell lysis rather than fusion occurs [56,57]. (iv) When fusion occurs before lysis, either polycells or polyghosts are formed depending on the experimental conditions [56]. These observations, coupled with the presence in the virus of both a hydrophobic peptide that is believed to perturb lipid bilayers [58] and a protease [27], are consistent with the proposed osmotic mechanism for cell fusion. It is therefore suggested that erythrocytes are permeabilised by the virus, and that osmotic swelling drives the fusion of

erythrocytes with one another and with other types of cell (fig.2a–e), in accordance with our general model. Colloid osmotic lysis of erythrocytes may thus precede or follow fusion with chemical fusogens and with Sendai virus. It is particularly significant in relation to the model that high concentrations of some divalent cations, e.g. 6 mM Mn^{2+}, inhibit both haemolysis and cell fusion induced by the virus [59,60].

Hitherto, it appears not to have been suggested that Sendai virus may fuse cells by two independent mechanisms, even though there has been controversy over whether fusion occurs directly between the membranes of the treated cells [59,61] or via virus particles that fuse simultaneously with two cells and thus provide bridges between them [62,63]. We propose that the latter mechanism, in fact, represents a completely separate pathway for cell-cell fusion that applies to all cells having receptors for the virus (fig.2f,g), including erythrocytes fused by early-harvested, non-haemolytic, Sendai virus [64] which does not cause cell swelling and which cannot act by the mechanism proposed above. (As a corollary, haemolytic Sendai virus and other viruses that cause cells to swell will induce cell fusion not only directly between erythrocytes, as proposed above, but also via virus particles that act as bridges between cells.) Ca^{2+} is not required when virus particles fuse with two cells to form an intracellular bridge [65], and it has been shown that subsequent cell swelling (induced by exposure to a hypotonic medium) will cause rounding of already-fused cells which may be utilised to allow fusion to be detected with a light microscope [66].

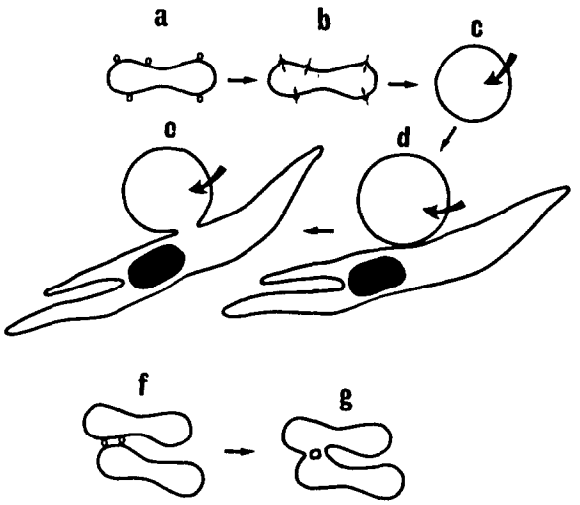

Fig.2. Schematic diagrams that illustrate two independent mechanisms which are proposed for virus-induced cell fusion. In (a–e) cell fusion introduced by haemolytic Sendai virus is illustrated, and this involves direct fusion [59,61] between the membranes of the cells. In (f,g) cell fusion via virus particles that act as cytoplasmic bridges [62,63] is illustrated. (a) Sendai virions attach to the membrane of an erythrocyte. (b) The virions fuse with the erythrocyte, and the cell begins to swell as water enters via the permeable viral membranes (indicated by arrows). (c) Viral membranes are incorporated into the erythrocyte membrane, and the cell continues to swell as water enters the permeabilised cell (arrow). (d) The swelling erythrocyte is attached to a different (nucleated) cell type (or another erythrocyte: not shown). (e) Continued osmotic swelling of the erythrocyte drives cell fusion. (f) Virus particles attach simultaneously to two adjacent erythrocytes or to other cell types. (g) Simultaneous fusion of the virions with both cells allows the virus particles to act as cytoplasmic bridges between the two cells, thereby mediating cell fusion.

How does Sendai virus fuse with the plasma membranes of cells and how do other viruses, e.g. influenza virus, fuse with the endosome membrane? This may be explained on an osmotic basis if the virions swell after attachment to their target membrane. The fusion reaction will then be similar to that in exocytosis, and topographically identical to the osmotically driven fusion of a phospholipid vesicle with a bilayer [3]. In this connection, it seems possible that conformational changes in viral membrane proteins which expose hydrophobic fusogenic peptides may simultaneously open channels through the viral membrane, which close again (except in haemolytic virions) after membrane fusion-fission is completed. Sendai virions are roughly spherical but, when they attach to an erythrocyte, their surfaces develop a striking, grooved appearance (fig.3a) that is essential for fusion with the cell [67]. This change in morphology is apparently consistent with viral swelling that is restricted by a structural framework, as with an over-inflated rubber balloon that blebs out between the longitudinal elements of a spherical wire cage (fig.3b). Thin sections of Sendai virus have revealed tightly folded, parallel nucleocapsid strands just beneath the viral membrane (fig.3c), and it has been proposed that viral M protein and

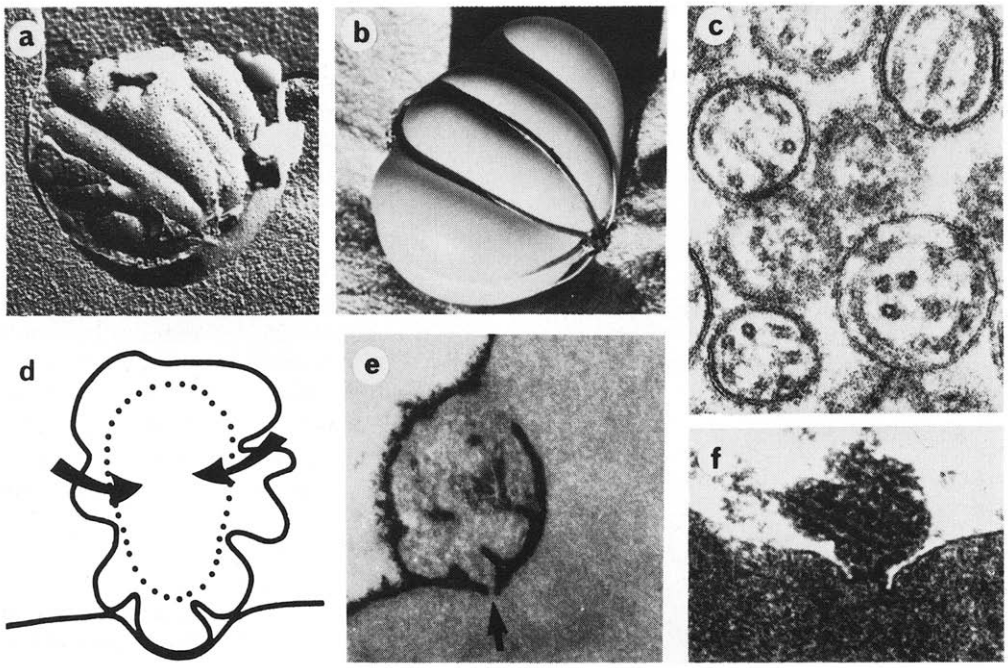

Fig.3. (a) A freeze-fracture replica [67] (× 35500) of a Sendai virion showing the grooved, convoluted appearance which develops at 37°C when the (previously spherical and unconvoluted) virus particle is attached to an erythrocyte. (b) A model constructed from a balloon, which is over-inflated within a wire cage and blebs out between the framework, to illustrate the possibility that the grooved appearance of the virus may result from viral swelling that is restricted by a structural framework. (c) An electron micrograph of a thin section of Sendai virion showing folded, nucleocapsid strands that lie beneath the membrane of the virus [68] (× 100000). [Reproduced with kind permission.] (d) A diagram illustrating the proposed general role for viral swelling in fusion of the viral membrane with a plasma membrane, or with the membrane of an endosome. The shape of the virus particle shown is like that of a freeze-fractured replica [67] of a Sendai virion with the grooved, convoluted appearance which is essential for fusion with an erythrocyte. Arrows represent the entry of water into the virion which causes it to swell, and the dotted circle represents approximately the proposed shape and size of the initial virion before its cell-mediated change in morphology. The virus is attached to a plasma membrane by two lobes. Between them, a third lobe is shown expanding against the membrane and it is proposed that osmotic swelling drives the fusion of this lobe with the cell. (e,f) Electron micrographs of thin sections showing the fusion of Sendai virions with erythrocytes [67,71] (× 55000 and 60000). Micrographs (a,e,f) were kindly provided by Dr S. Knutton, and are reproduced with permission.

nucleocapsid strands may together be regarded as a 'viroskeleton' [68]. We suggests that the wire framework of the model in fig.3b 'corresponds' to the viroskeleton and that, in general, the osmotically driven fusion of a virus particle with a plasma or endosome membrane may occur when a region of viral membrane that is not attached to the viroskeleton bulges out against the cellular membrane to which the virus is bound (fig.3d–f).

Finally, we suggest a modification of our general scheme that may apply to the fusion of cells, e.g. differentiating myoblasts, which do not swell when they fuse. For cells to fuse osmotically without cell swelling, it is necessary to isolate a small portion of cytoplasm that is adjacent to the plasma membrane which can then be subjected to osmotic swelling independently of the rest of the cell. This may be achieved by an osmotically driven, exocytosis-like reaction (in which a swelling subcellular vesicle fuses with plasma membrane) that is temporarily arrested prior to the final stage of membrane fission. Such a phenomenon would allow osmotic forces to be applied to a highly localised region of plasma membrane (the new bilayer shared by the vesicle and the plasma membrane that corresponds to fig.1e) without the cell

swelling as a whole. It is proposed that cell fusion with an adjacent myoblast involves a further fusion reaction of the new single bilayer with the plasma membrane of the second myoblast. This process (driven by the swelling vesicle) will yield a single bilayer that is shared by the vesicle and by the plasma membranes of the two myoblasts (fig.4c). Continued swelling of the vesicle will then rupture the common membrane at one or both of the points where four bilayers meet, and this will establish continuity between the cytoplasm of the two myoblasts. The suggested mechanism may enable a fusion-sensitive membrane, which could be present in specialised subcellular vesicles, to be inserted into the plasma membrane immediately prior to myoblast fusion (like the insertion of the glucose transporter into fat cell membranes in response to insulin). In relation to this proposal, it is also interesting that an increase in the cytoplasmic concentration of Ca^{2+} both precedes the fusion of myoblasts [69] and serves as a 'trigger' for the initiation of exocytosis reactions in other cells.

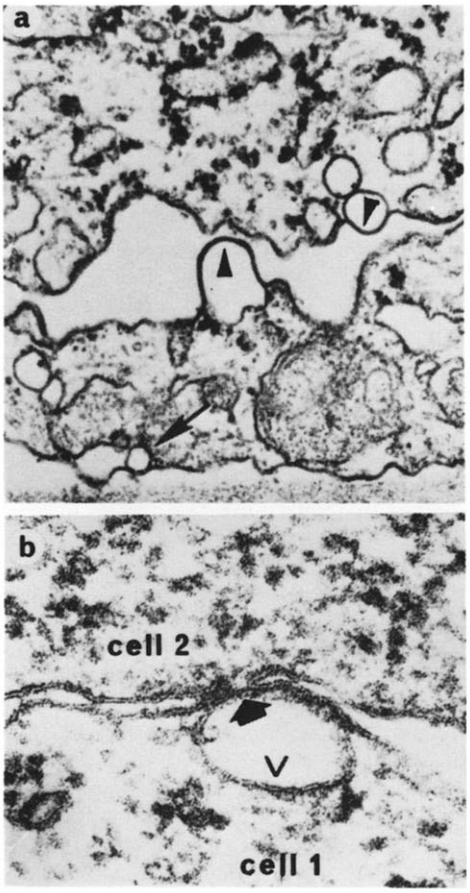

Fig.4. (a) An electron micrograph [70] of a thin section of fusion-competent myoblasts showing apparently swollen, cytoplasmic, unilamellar vesicles and their abutment against the myoblast plasma membranes (× 46000). (b) An electron micrograph [70] of a thin section of the fusion of two myoblasts showing the participation of a vesicle in the fusion of the plasma membranes (× 110000). The membrane of an intracellular vesicle (V) and the plasma membranes of cell 1 and cell 2 are each fused into a single bilayer (arrowhead). (c) A diagrammatic illustration [70] of the fracture planes in a replica of two fusing myoblasts that have also fused with a cytoplasmic vesicle. (The micrographs and diagram were kindly provided by Dr N. Kalderon and Dr N.B. Gilula, and are reproduced with permission; copyright of the Rockefeller University Press.) The following explanation, based on osmotically induced, membrane fusion-fission, is proposed for the appearance of these membranes. Firstly, an intracellular vesicle in a myoblast initially fuses with the plasma membrane of the cell as a consequence of osmotic swelling of the vesicle, and a new, single bilayer (common to the vesicle and to the plasma membrane) is formed at the fusion site. Next, the new, single bilayer fuses osmotically (as a consequence of continued swelling of the vesicle) with the plasma membrane of a closely apposed, second myoblast to produce a single bilayer that is common, as in (b,c), to the vesicle and to the plasma membranes of the two cells. Finally, the common membrane then ruptures at one of the points where four lipid bilayers meet, due to continued vesicle swelling. This establishes continuity between the cytoplasms of the two cells and thereby completes the process of myoblast fusion.

A process like that proposed here may have been identified in chick myoblasts. Small unilamellar vesicles, free from intramembranous particles, have been described which are present in fusion-competent but not in fusion-arrested cells (fig.4a) [70]. In places, two myoblasts and a vesicle were observed to be mutually fused and connected by a single bilayer (fig.4b), as illustrated diagrammatically in fig.4c which is based on a freeze-fracture micrograph and shows the fracture planes in the replica. When they reported their findings, Kalderon and Gilula [70] proposed that myoblast fusion occurs where the vesicles fuse with the plasma membrane but they did not discuss the possible involvement of osmotic swelling in the behaviour of the vesicles or in myoblast fusion.

In conclusion, the central argument in this article is that many fusion-fission reactions of biological membranes essentially depend on perturbed phospholipid bilayers being forced into a single bilayer, which is then ruptured, both events being driven by osmotic forces. A 6-stage molecular model for fusion-fission reactions that embodies this idea has been proposed, and it appears that application of the model may clarify some existing observations on fusion phenomena in biological systems. Much of our discussion has been concerned with colloid osmotic swelling in permeabilised membrane systems. It is hoped, however, that the model may stimulate investigations on the possible roles in fusion reactions of membrane transport processes that establish ionic gradients which could lead to swelling.

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