controlled, it should be possible to identify the vulnerable components by determining how the rate of repair is affected when various parts are supplied in excess.

It will be important to extend the electrorotation experiments to other bacterial species. Washizu et al., working with *S. typhimurium,* did not encounter the resistance to reversed rotation or the motor breakage noted by Berg and Turner. While it is often assumed that the motors of *E. coli* and *S. typhimurium* are identical in all important respects, that might not be true. Likewise, experiments with motile *Streptococcus* cells will be invaluable, because they can be starved and reenergized by artificial proton gradients.

The torque-speed characteristic of the flagellar motor, now known much better than before, must be accounted for by any viable model for torque generation. In contrast to an earlier suggestion that motor torque decreases steadily with increasing speed (Lowe et al., 1987), we now can say that the motor torque remains nearly constant up to about 100 Hz, and decreases more or less linearly above that speed, until more complex behaviors are encountered at very high speeds. The question is, what causes the torque to decrease in this way? The new data, and analogous data obtained using D_2O or motor mutants, should furnish some clues to the number and character of the processes that become limiting at high speeds. Why is the torque constant across such a broad range? Berg and Turner suggest the existence of a mechanical stop in the motor that limits the torque. Alternative explanations appear possible. The torque in this range might just reflect the total amount of energy available from the proton gradient; to explain the constancy, one would only have to assume that internal processes are fast enough not to dissipate significant amounts of energy across this range of speeds. In any event, the new data place severe constraints on plausible mechanisms, and more than ever it will be essential that new mechanistic proposals include calculations of steady-state performance that will permit them to be evaluated quantitatively.

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To Fuse or Not to Fuse?

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That was the question addressed by David Siegel in his paper "The Energetics of Intermediates in Membrane Fusion: Comparison of Stalk and Inverted Micellar Intermediate Mechanisms." This theoretical analysis of the fusion of pure phospholipid bilayers, and of the mechanisms underlying this process, poses a legitimate question: should one spend one's time on this problem? Is it a *jeau d'esprit* of a mathematician, or has it some bearing on physical chemistry or on real biological phenomena? We believe that answer is "yes" to all three questions.

The cellular membrane evolved as a wall separating the cell's contents from the external medium. This barrier function was conveniently assigned to a lipid bilayer, an ingenious biological invention combining the remarkable property of self-organization with unhindered lateral movement of membrane components. This leakproof shell maintains its integrity even in the toughest situations; rupture of the membrane is a catastrophic event leading to the loss of important cellular components and potentially to a cell's death.

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Despite the importance of membrane integrity, a number of crucial physiological events require the breakdown of two apposing membranes and their subsequent reconnection to one another. Examples include fertilization, cell division, endocytosis, exocytosis, and the entry of enveloped viruses into cells. Although membrane fusion has long been a focus of attention, even recent reviews inevitably come to the conclusion that "the physical and molecular mechanisms of membrane fusion remain obscure."

There is no doubt that, in many important cases of membrane fusion, specific proteins initiate the process. Then why study fusion of pure phospholipid bilayers? This approach is appropriate because any structural rearrangement of membranes must involve lipid bilayers. Besides, membrane fusion can be observed in pure phospholipid systems which have important practical applications per se and hence deserve special attention. These model systems have taught us important lessons about fusion (Rand and Parsegian, 1986) by defining the different stages of this process: membrane apposition, triggering, contact, local destabilization, membrane coalescence, and final restabilization. Different physical forces are involved at various stages of the overall process. One can assign various priorities to these stages, but the most crucial are breakdown of the apposing membranes and their reconnection. The intermediate structures involved in these processes stimulated intensive discussion in the literature and led to the emergence of two models. The first proposal, that fusion proceeds by way of inverted micelles, was initially advanced and strongly advocated by Verkleij and his colleagues (Verkleij et al., 1979a, b). The opposing model, which favors a stalk as the intermediate structure, was analyzed in detail by Russian biophysicists led by Chizmadzhev (Markin et al., 1984; Chernomordik et al., 1985, 1987; Leikin et al., 1987; Kozlov et al., 1989).

The theoretical analysis of the intermediate structures exploits a simple physical concept, the elasticity of membranes. Biological membranes resist

not only stretching, but bending as well. Any membrane conformation is accordingly endowed with a certain energy, and a change in a membrane's conformation means a change in its energy. These facts suggest a way to predict the most probable conformation and the most probable course of membrane transformations.

One of the first applications of the concept of the bending elasticity to a biological problem was conducted by Canham (1970) in the analysis of red blood cell shape. He postulated that the density of elastic energy in membrane is proportional to the square of the membrane's mean local curvature, $(C_1 + C_2)^2$, and found a shape which minimizes the elastic energy. This analysis was refined by Helfrich (1973), who introduced the concept of a membrane's spontaneous curvature, C_0, and added to the analysis the Gaussian curvature, C_1C_2. His final equation for elastic energy, w, had the form:

$$w = \tfrac{1}{2}\kappa(C_1 + C_2 - C_0)^2 + \kappa_1 C_1 C_2, \quad (1)$$

in which the kappas denote stiffness. This equation was extensively applied in the analysis, not only of red blood cell shape, but also of a variety of other phenomena: osmotic lysis, vesicle shrinkage, electroporation, membrane fusion, lipid phase transitions, and formation of bicontinuous structures.

In the instance of membrane fusion, the basic strategy of analysis is to calculate the energy of an initial state, which might be planar or spherical, of the intermediate state, and then of the final state. The change of energy from state to state determines the probable evolution of the fusion process. This program was meticulously carried out by Siegel in his recent paper. He compared the two most popular models of intermediate structure, those based upon inverted micelles and upon stalks, and concluded that the latter has the better chance for realization. Developing the earlier model, Siegel provided a more detailed geometrical description of the stalk, accurately matching the shapes of *cis*- and *trans*-monolayers. He explicitly introduced void spaces at the extremes of the stalk, bringing to light a possible pressure dependence of membrane fusion.

Models of physical processes usually develop through a dialectical process that gradually elevates our understanding of the physical reality. But one must bear in mind that there is always a price for such progress. Simple and rough models have their own spell of naiveté; they embody prevailing ideas (and prejudices) and usually aspire to only a semiquantitative agreement with the experimental data. Attempts to refine simple models usually provoke a number of questions related to the fact that analysis becomes strongly model-dependent. Is the more detailed model quite unique, or could it be modified somehow? What would happen to numerical results in that case? Would they support the conclusion of the analysis or rather contradict it? *Et cetera, et cetera!*

We believe that Siegel's analysis is sound and the results are very convincing. He presented an interesting development of an important problem. The author was not only able to select between the inverted-micelle and stalk mechanisms, but also found that traces of apolar lipids in membranes should have a substantial influence on the fusion rate. His explicit proposal of void spaces introduced the idea that fusion might be a pressure-controlled phenomenon and suggested corresponding experiments. Helfrich's concept of spontaneous curvature plays an important role in this analysis, which brings up the question of effective lipid shape (Chernomordik et al., 1985). This issue deserves special attention in conjunction with the possible redistribution of different membrane components in the process of conformation change. Such a redistribution, a local phase transition or small domain formation, could strongly influence the path and the rate of the overall process.

The mechanical approach to the fusion problem can shed some light on a number of phenomena now under intensive study. One of these involves fluctuating fusion pores, which can exist for milliseconds but eventually close without membrane fusion. The existence of *exocytosis interruptus* implies that a system of two membranes can find itself in a minimum of elastic energy as a function of a pore radius, and that this minimum is broad enough to ensure rather large fluctuations in pore size (Nanavati et al., 1992). The study of mechanical effects should be especially important for exocytosis, a process in which the controlled swelling of the secretory granule matrix probably plays a key role (Nanavati and Fernandez, 1993).

The problem of membrane fusion is by no means closed; considerable work remains to be done even with Siegel's approach. For example, in the existing models the shape of a stalk was not calculated but rather postulated. Although the configuration assumed seems quite reasonable, it is unlikely to represent the absolute minimum of elastic energy. It would therefore be valuable to determine explicitly the stalk shape with minimal energy.

Another important question is the applicability of Helfrich's simple equation to the cases of very high membrane curvature; in a fusion stalk, for example, the radius of curvature would be of a few nanometers. Although anybody could have reservations about the physical reality of such a curvature, this model is the best available at present. Solution of the problem might involve the methods of molecular dynamics, which in the same time could answer a number of other queries.

A final important question is how to incorporate this mechanical analysis of the intermediates into our understanding of the total course of the fusion process. The preceding and the following steps might have important effect on membrane fusion, making it a cooperative process rather than a chain of independent events (Leikin et al., 1987). But before moving exploring this possibility, we need a clear understanding of single steps even if they must be considered in isolation from each other. And this is exactly what David Siegel's paper provides.

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A Closer Look at How Membrane Proteins Move

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The lateral diffusion of many plasma membrane proteins is impeded and a significant percentage of those proteins appear virtually immobile. This restricted mobility is commonly believed to result directly or indirectly from interactions of proteins with the underlying cytoskeleton (Zhang et al., 1993). Ultrastructure studies have shown that in red blood cells as well as other cells a regular spectrin lattice is attached to the membrane (Pumplin and Bloch, 1993). In spectrin-deficient red blood cells, a large increase in the diffusion rates of membrane proteins was observed (Sheetz et al., 1980). These findings, together with other support, led to the view that proteins are corralled within membrane domains by interactions of their cytoplasmic moiety with cytoskeletal lattices (Sheetz, 1983; Tsuji and Ohnishi, 1986; Tsuji et al., 1988; Saxton, 1990). However this model has not been confirmed by direct experiment.

Single Particle Tracking (SPT) is a recently developed technique, in which membrane proteins or lipids are specifically labeled with antibody-coated submicron colloidal gold or fluorescent latex particles and the trajectories of the individual labeled molecules are followed by digital imaging microscopy (Zhang et al., 1993; Cherry, 1992; Ghosh and Webb, 1990). By direct observation of individual protein motions, the method should provide detailed information on submicroscopic membrane structures, although careful data analysis is required in order to distinguish trajectories of nonrandom motions from those of random motions (Saxton, 1993). Kusumi et al (1993) applied this new technique to study the mobility of E-cadherin, a Ca^{2+}-dependent cell adhesion molecule, in cultured epidermal cells. Trajectories were carefully analyzed with the help of computer simulations. The results give a strong evidence for the notion that some membrane proteins are confined within certain regions.

The results of Kusumi et al. (1993), as well as earlier work on the low-density lipoprotein receptor (Ghosh and Webb, 1990; Anderson et al., 1992), indicate that the SPT method can, indeed, provide more detailed information about membrane protein mobility. The more conventional method, fluorescence recovery after photobleaching, typically divides the labeled protein population into mobile and immobile fractions. For E-cadherin, 25% was immobile and the remaining 75% was characterized by a diffusion coefficient of 2.6×10^{-11} cm^2/s. On the other hand, the SPT method revealed that four different modes of the membrane protein mobility were operant. More than half the proteins tracked (64%) showed diffusion confined to regions on the order of 500 nm or less. 6% of the population was virtually stationary, while 2% showed directed motion. The remaining 28% of the population exhibited random diffusion.

To further characterize random diffusion, "microscopic diffusion coefficients," which reflect the mobility on the tens of nanometer scale, were calculated from individual protein trajectories. These values were distributed over a surprisingly large range of 4.6×10^{-12} to 1×10^{-9} cm^2/s, but it is not yet known how much of this variation is real and how much is statistical. Directed motion has been observed previously and is presumably due to the interaction with the dynamic cytoskeleton system (De Brabander et al., 1991; Sheetz et al., 1989). However, what is most striking is that many of the tracked proteins appear to be trapped within small regions. The size of these regions was variable, ranging between 0.3 and 0.6 μm in diameter. This domain size is slightly smaller than that previously estimated by Edidin et al (1991) (0.6 μm) and larger than the estimated size of the spectrin mesh in red blood cells (approximately 0.1 μm).

Do all membrane proteins experience such confined diffusion in the plasma membrane? Kusumi et al. (1993) found that epidermal growth factor and transferrin receptors do, which is plausible because these proteins, as well as E-cadherin, possess relatively large cytoplasmic domains. Indeed, truncation of the cytoplasmic domains of E-cadherin results in much less confined diffusion (Sako et al., 1992). Whether proteins with smaller cytoplasmic domains experience such direct cytoskeletal interactions or more indirect effects is not yet known (Zhang et al., 1993). Laser trap evidence suggests that glycosylphosphatidylinositol-anchored

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