Pediplain in the Juan de Morales region in Northern Chile might have occurred during the Oligocene and Miocene epochs in a period of 30 million years. Because pediplains are common in deserts, such an erosion surface supports Brüggen's idea that northern Chile has been arid since the Oligocene Epoch. The uplifting of some relatively high fault blocks of the Choja Pediplain at the same time as the other Andean tectonic blocks precluded deposition of younger orogenic materials that were transported from eastern source areas, which indicates that the aggradation of the block basins is younger than the north-trending block-fault system associated with the Andean uplift.

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References and Notes

1. R. J. Dingman and C. Galli-Olivier, *U.S. Geol. Surv. Bull. No. 1189* (1965).
2. C. Galli-Olivier, thesis, University of Utah (1964).
3. C. Ruiz-Fuller, *Geología y yacimientos metaliferos de Chile* (Instituto de Investigaciones Geológicas, Santiago, 1965). Ruiz, on the contrary, concluded that the diastrophism that resulted in uplift of the Andean Range in northern Chile began after the deposition of the orogenic sediments and the ignimbrites.
4. R. J. Dingman and C. Galli-Olivier, *ibid.*, p. 34; C. Galli-Olivier, *ibid.*, p. 26; W. Zeil, *Geologie von Chile* (Borntraeger, Berlin, 1964); C. Ruiz-Fuller, *ibid.*, p. 87. The ignimbrites form one of the largest ignimbrite areas of the world, extending from southern Peru to northern Argentina and Chile.
5. Young canyons and eastward migration of nickpoints are indications of an active second-cycle erosion that followed the Andean uplift.
6. K. Segerstrom, *Bull. Geol. Soc. Amer.* **74**, 513 (1963). Segerstrom described the old surface or matureland in northern Chile as a gently rolling surface of deeply decayed rocks, partly incised by deep valleys and canyons and partly buried by mantling gravels and piedmont deposits. He mentions that the higher parts of the old surface exhibit oxidation, leaching, and supergene enrichment of sulfide mineral deposits as a result of deep weathering.
7. J. Brüggen, *Fundamentos de la geología de Chile* (Instituto Geográfico Militar, Santiago, 1950).
8. R. W. R. Rutland, J. E. Guest, R. L. Grasty, *Nature* **208**, 677 (1965).

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Fracture Planes in an Ice-Bilayer Model Membrane System

Abstract. *Experiments with transferred stearate layers were performed to determine the location of fracture planes in frozen ice-lipid systems. Bilayers and multilayers of carbon-14–labeled stearate were frozen in contact with an aqueous phase and then fractured. The distribution of radioactivity on both sides of the fracture showed that the stearate layers were cleaved apart predominantly in the plane of their hydrocarbon tails. Because bilayers split in this manner, it was possible to measure time-dependent exchange of label between the layers. Exchange occurred with a half-time of 50 minutes in the presence of calcium and 25 minutes in the absence of calcium. Since stearate bilayers and multilayers are models of hydrophobically stabilized structures, the strong influence of their hydrophobic region on the fracture plane provides an explanation of how the freeze-etch technique of electron microscopy can expose inner, hydrophobic faces of cell membranes.*

The freeze-etch method of preparing biological specimens for electron microscopy has provided evidence that fractures in frozen tissues split inner, hydrophobic regions of biological membranes (*1*). For freeze-etching, specimens are frozen and then fractured under vacuum. A small amount of ice is allowed to sublime from the fractured face and this etched surface is then shadowed and replicated. Examination of the replica by usual electron microscopic techniques reveals extensive structures which evidently represent membranous portions of cells.

This technique was first applied to biological materials by Steere (*2*) and later developed by Moor *et al.* (*3*). Moor and Mühlethaler (*4*) proposed that fracturing occurs along exterior faces of membranes to expose true membrane surfaces. Branton and coworkers (*1, 5, 6*) have presented evidence for the alternative possibility that fractures in frozen tissues may split membranes in their inner, hydrophobic regions rather than along their hydrophilic surfaces. It is generally accepted that two of the major forces stabilizing lipoprotein membranes in aqueous environments are entropic (hydrophobic) bonding and van der Waals interactions (*7*); it was reasoned (*6*) that upon freezing entropic bonding would no longer be important and only relatively weak van der Waals forces would stabilize membranes in hydrophobic regions. Such regions could therefore represent weak areas in the frozen structure, since strong charge-dipole interactions between hydrophilic portions of the membrane and the surrounding ice would be less affected by freezing.

In the present investigation a model system was employed to test this reasoning. The model consisted of a bimolecular layer of stearate on glass, deposited according to the multilayer technique first described by Blodgett (*8*). The bilayer is composed of two successive monolayers of stearate as shown in Fig. 1. Although this model is not analogous to biological membranes, it is similar in that there exists an extensive hydrophobic region, consisting of hydrocarbon chains, and a hydrophilic region composed of carboxylate groups. If the water (Fig. 1) is frozen and the glass split away from the ice, several fracture modes are possible: (i) there is no preferred fracture plane; (ii) the fracture occurs between the glass and the bilayer; (iii) the fracture occurs between the ice and the bilayer; (iv) the fracture cleaves the bilayer down the middle. Using C^{14}-labeled stearic acid, we determined that the last-mentioned fracture mode is preferred and that approximately half the stearic acid was left on the glass and half on the ice.

Stearic acid (99.8 percent, Applied Science Laboratories, Inc., State College, Pa., 1 m*M* in *n*-hexane) was spread on 1 m*M* $CaCl_2$-$NaHCO_3$ solution, *p*H 8.5, until a lens of hexane solution formed, indicating that the surface was covered with a monolayer. Blodgett (*8*) found that the presence of calcium or barium ions was necessary for production of multilayers. At *p*H 8.5, the spread monolayer is predominantly present as stearate (*9*). Divalent cations apparently link together carboxylate groups of the stearate molecules (*10*) and facilitate multiple transfers of layers from the water surface to the slide. The solutions were contained in a 2-liter Teflon-lined trough with approximately 300 cm^2 of surface area. After spreading was completed, a drop of methyl laurate was placed at one end of the trough to act as a piston oil (*8*). Glass cover slips that had previously been cleaned in chromic acid and immersed in the trough were slowly withdrawn (~ 2 cm/minute) and reimmersed at the same speed by means of a small hydraulic lift (Fig. 1, a and b). This caused a bilayer of calcium stearate to

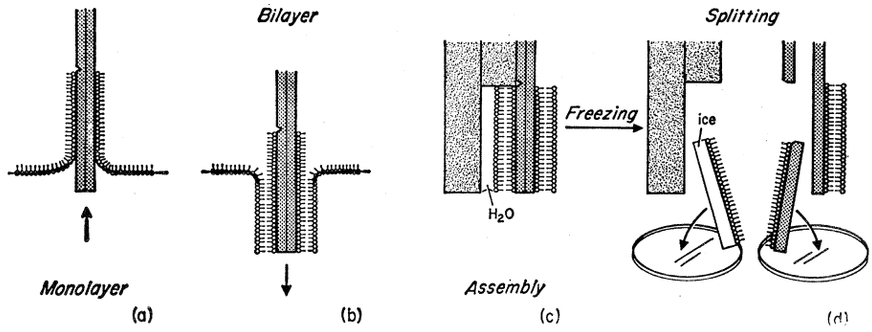

Fig. 1. Bilayer formation and splitting. (a) Monolayers were transferred to two glass cover slips (one of them scored) as they emerged from a stearate-covered $CaCl_2$-$NaHCO_3$ solution in the trough. (b) Bilayers were formed as the cover slips were reimmersed. (c) While immersed, a glass spacer and slide were clamped to the cover slips, and the entire assembly removed from the trough for freezing. (d) After freezing and splitting, the inner cover slip was broken along the score and put in a planchet. The previously apposed ice was partially thawed and, with the upper surface still frozen, pushed into a second planchet. The rest of the assembly was discarded together with the unwanted labeled stearate.

be deposited on the cover slips. Either or both layers of the bilayer were labeled with trace amounts of 1-C^{14} stearic acid (Volk Radiochemical Co., Burbank, Calif.).

While still beneath the surface, the cover slips were fastened to a glass assembly (Fig. 1c). The complete assembly with entrained solution was removed from the trough, rinsed gently for 1 minute in 1 m*M* $NaHCO_3$, *p*H 8.5, and rapidly frozen by placing a small piece of solid CO_2 against the cover slip. With very little force the glass was split away from the ice (Fig. 1d), and the radioactivity of both glass and ice was assayed in a thin-window, gas-flow Geiger counter. Interference fringes commonly appeared beneath the cover slip during freezing, indicating that most or all of the actual splitting was due to stresses arising from differential expansion of the glass, lipid, and aqueous phases as they were cooled.

In preliminary transfer experiments it was found that a monolayer of the labeled stearate on glass gave 440 ± 30 count/min for the experimental area (2.4 cm^2), and only data which fell within that range per monolayer were included in the calculations. No loss of radioactivity occurred if control bilayers were allowed to remain in the buffer solution for periods up to 24 hours.

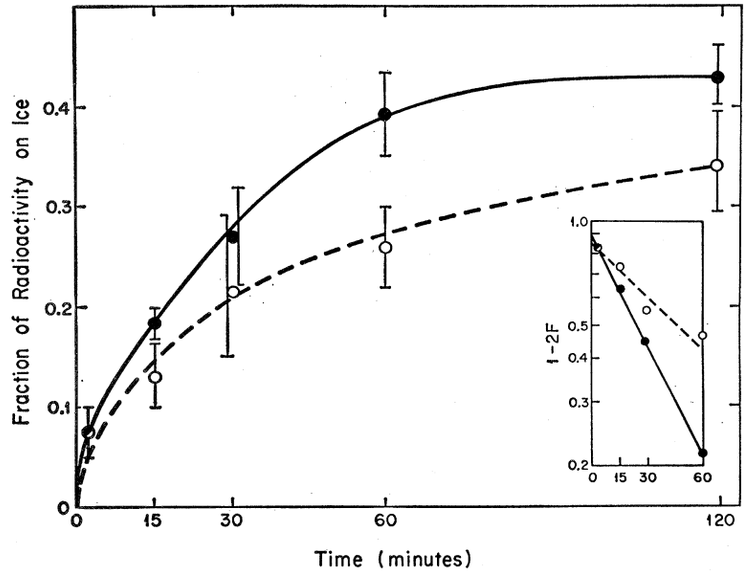

Fig. 2. Exchange kinetics. Means, with standard deviation, for bilayers in $NaHCO_3$ (○) and in $NaHCO_3 + CaCl_2$ (●). Inset, same data plotted according to equation of form $F = 0.5(1 - e^{-kt})$ (see text).

When both layers of the bilayer were labeled, the radioactivity was equally distributed between the subsequently split ice and glass (Table 1, a). When only the layer next to the ice or next to the glass was labeled, over 90 percent of the radioactivity appeared on the ice or glass respectively (Table 1, b). These results provided strong evidence that cleavage occurred preferentially in the hydrophobic region of the model system.

Rothen (*11*) has performed related experiments in which multilayers of barium stearate were stripped away from glass with adhesive tape. Under the conditions of his experiments, only the first layer remained on the glass. This parallels our results in that his fractures followed a hydrocarbon plane, rather than the carboxylate plane, but it did raise the possibility that the glass may be biasing the fracture by weakening the adjacent hydrocarbon plane. We checked this possibility by using four- and six-layer labeled multilayers in our system, and found that under these conditions an amount of label equivalent to at least three monolayers always remained on the glass (Table 1, c). This indicated that the presence of a glass support did not impart a pronounced bias to the split, and, in fact, our results suggest that the hydrocarbon planes closest to the ice are most weakly bonded.

It was never possible to retrieve 100 percent of the radioactivity on the glass or ice alone when only one layer was labeled. This could be accounted for if exchange between the layers had taken place during the 2-minute interval necessary for bilayer production and final freezing. To test this possibility, bilayers were produced with the glass side labeled, but the bilayers were then kept submerged in buffer solutions up to 2 hours before freezing and splitting. Exchange between layers did take place (Fig. 2) with a half-time of 25 minutes at 25°C in 1 m*M* $NaHCO_3$ buffer, *p*H 8.5. Similar results were obtained when the layer next to the ice was labeled. The presence of calcium ion retarded the exchange, and the half-time in the same buffer but with 1 m*M* $CaCl_2$ present was 50 minutes. Both curves can be described by the equation $F = 0.5(1 - e^{-kt})$, where F is the fraction of radioactivity found on the ice, t is time, and k is a rate constant. Rearranging this equation, we get: $-kt = \log(1 - 2F)$. Plotting the averages of experimental

data as $\log(1 - 2F)$ against time results in a straight line for exchange in sodium bicarbonate (Fig. 2, inset). At $t = 0$, the curve passes through F at about 0.05, suggesting that the split was slightly biased for the glass side, with small amounts of bilayer coming away with the glass. A somewhat poorer fit is obtained for exchange in the presence of calcium.

That the exchange illustrated in Fig. 2 may occur even in bilayers of tightly packed stearate molecules is significant in the context of diffusion processes across membranes. For the molecules to exchange in this manner it is necessary that hydrophilic heads be dragged through a hydrophobic layer, a possibility previously suggested by Langmuir (*12*). If this form of exchange can occur in stearate bilayers, it would be expected to take place even more rapidly in biological membranes where hydrocarbon chains are presumably in a highly fluid state due to their unsaturated character (*13*). Thus, it may not be necessary to invoke hydrophilic pores to explain transfer of hydrophilic materials across the hydrophobic regions of a cell membrane.

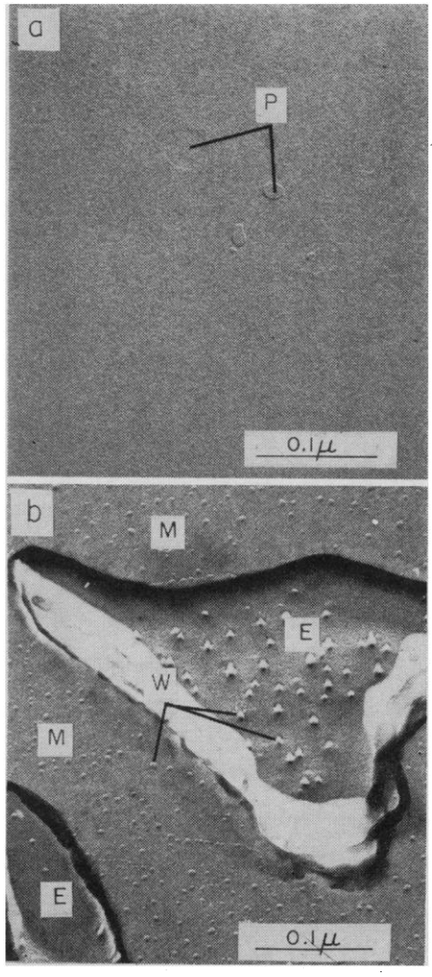

Fig. 3. Replica of glass and ice after splitting. (a) Glass side shows patches (*P*). (b) Ice side shows deeply etched regions (*E*) and large non-etched areas presumably covered with a stearate monolayer (*M*). The warts (*W*) always found on the ice side of the split bilayers may be intrusions of the similarly sized and distributed warts in the underlying etched ice (*3*).

Electron microscopic examination of the ice and glass following a split was also undertaken. The splitting process was carried out in a Bendix-Balzers freeze-etch apparatus by fastening the glass assembly to the stage and pulling away the cover slip with the knife holder in the apparatus. During etching the stage temperature was −100°C, the knife holder was −195°C, and the vacuum was maintained at less than 10^{-5} torr. After the glass was removed the ice surface was etched for 1 minute, shadowed, and replicated with platinum-carbon. The surface of the cover slip that had been split away from a bilayer was also shadowed and replicated.

Although control replicas of clean cover slips were smooth, the replica of glass split away from a bilayer had small patches on its surface (Fig. 3a). These patches probably represented areas of bilayer which did not split, and covered at most 5 percent of the glass. On the other hand, the ice typically showed rather large areas of deeply etched material, interspersed with apparently non-etched patches (Fig. 3b). Results from the labeling experiments led us to expect large areas of smooth surface representing a monolayer of stearic acid on the ice which had retarded etching. Instead, only about half of the surface had the expected appearance.

This anomalous result could in part be explained by the effect of a wind of water vapor which could blow away the stearate layer during sublimation of underlying ice. We tested this by measuring disappearance of radioactivity from labeled bilayers split, and then etched 10 minutes at −100°C *in vacuo*. Controls were maintained at less than −165°C, at which temperature little or no etching occurs (*3*). There was no loss of stearate from the ice control at less than −165°C or from monolayers on glass at −100°C. However, about a fourth of the stearate was lost from the etched ice at −100°C. This result accords with Greaves's suggestion that vapor winds may carry away nonvolatile components of specimens during freeze-drying (*14*), and indicates the possibility of material loss —other than water—in freeze-etching experiments.

Table 1. Distribution of radioactivity after splitting.

Conditions	Fraction* of total radioactivity on ice†
Bilayer	
a. Both layers labeled	0.45 ± .05 (14)
b. Ice layer labeled	.91 ± .02 (8)
b. Glass layer labeled	.07 ± .02 (7)
Four layers	
c. All layers labeled	.26 ± .01 (4)
Six layers	
c. All layers labeled	.23 ± .03 (2)

* Fraction = (radioactivity on ice) ÷ (radioactivity on ice + radioactivity on glass). † Mean ± maximum deviation is given. Number of experiments given in parentheses.

The tracer experiments reported here show that cleavage occurred almost entirely within the hydrocarbon plane of the model system. These observations demonstrate the importance of hydrophobic regions in guiding the fracture of frozen material, and support the view that many of the structures observed in the freeze-etch technique represent inner, hydrophobic faces of cell membranes.

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References and Notes

1. D. Branton, *Proc. Natl. Acad. Sci. U.S.* **55**, 1048 (1966).
2. R. L. Steere, *J. Biophys. Biochem. Cytol.* **3**, 45 (1957).
3. H. Moor, K. Mühlethaler, H. Waldner, A. F. Wyssling, *ibid.* **10**, 1 (1961).
4. H. Moor and K. Mühlethaler, *J. Cell Biol.* **17**, 609 (1963).
5. D. Branton, *Exptl. Cell Res.* **45**, 703 (1967).
6. D. Branton and D. Southworth, *ibid.*, in press; D. Branton and R. B. Park, *J. Ultrastruct. Res.* **19**, 283 (1967).
7. W. Kauzmann, *Advan. Protein Chem.* **14**, 1 (1959); L. Salem, *Can. J. Biochem. Physiol.* **40**, 1287 (1962); D. G. Cornwell and L. A. Horrocks, in *Proteins and Their Reactions*, H. W. Schultz and A. F. Anglemier, Eds. (Avi Publishing Co., Westport, Conn., 1964), p. 117; G. Nemethy, *Cryobiology* **3**, 19 (1966).
8. K. B. Blodgett, *J. Amer. Chem. Soc.* **57**, 1007 (1935).
9. J. Bagg, M. B. Abramson, M. Fichman, M. Haber, H. P. Gregor, *ibid.* **86**, 2759 (1964); D. W. Deamer and D. G. Cornwell, *J. Lipid Res.* **8**, 255 (1967); H. Kimizuka and K. Koketsu, *Nature* **196**, 995 (1962).
10. D. W. Deamer and D. G. Cornwell, *Biochim. Biophys. Acta* **116**, 555 (1966).
11. A. Rothen, *J. Phys. Chem.* **63**, 1929 (1959).
12. I. Langmuir, *Science* **87**, 493 (1938).
13. P. Byrne and D. Chapman, *Nature* **202**, 987 (1964).
14. R. I. N. Greaves, in *Biological Applications of Freezing and Drying*, R. J. C. Harris, Ed. (Academic Press, New York, 1954), p. 125.

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