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RESEARCH ARTICLES

Surface Pressure Relaxation and Hysteresis in Stearic Acid Monolayers at the Air-Water Interface

JAMES W. MUNDEN, DAVID W. BLOIS, and JAMES SWARBRICK*

Abstract □ Time-dependent changes in the surface pressure of stearic acid monolayers were examined using an automated Wilhelmy-type film balance. Different surface pressure-area isotherms were obtained for two different rates of compression. Pressure relaxation from preselected surface pressures was examined as a function of time. The results indicated two types of relaxation which, along with the compression rate effect, may be rationalized on the basis of changes in molecular orientation and redistribution, together with expulsion from the monolayer at areas below the limiting area per molecule. Marked hysteresis effects were also noted when stearic acid monolayers were subjected to compression-expansion cycles. The effect of repeated cycling and the minimum area of compression on hysteresis were investigated. The onset and extent of hysteresis may also be explained on the basis of expulsion and reentry and orientation and redistribution of molecules at the interface.

Keyphrases □ Stearic acid monomolecular films—effect of compression rate □ Surface pressure relaxation—stearic acid monolayers □ Hysteresis—effect of minimum area of compression □ Cycling, requested, effect—hysteresis □ Air-water interface—Surface pressure

In recent years, the study of monomolecular films of biologic materials spread at the air-liquid interface has become increasingly significant in pharmaceutical and medical research. Serving as simulated biologic interfaces, these systems have been used to examine the interactions of such medicinal agents as the phenothiazines, local anesthetics, bactericides, and antibiotics with various cell membrane constituents. The evaluation of such studies is based primarily on the surface pressure-area per molecule (π-A) relationships exhibited by the system and the manner in which these change with time.

There are many factors affecting the shape and type of surface pressure-area diagram obtained for monolayers. Influencing factors such as pH, temperature, and the ion content of the supporting media have received fairly extensive investigation and are well documented in several texts (1–4). The time-dependency of surface pressure has been examined under conditions of slow discontinuous compression (5–7) and more rapid continuous compression (8–11). With the exception of the work of Rabinovitch *et al.* (11), the treatment of the time effects in these papers is limited. Examination of the literature shows that monomolecular film studies have been undertaken using a wide range of compression rates and methods of compression. This arises, presumably, from the lack of appreciation of the time-dependent properties of monomolecular films. Accordingly, investigations were undertaken to examine the effects of compression rate on the surface pressure-area isotherms of stearic acid monolayers and to study surface pressure relaxation, *i.e.*, the decrease in surface pressure with time when compression is stopped and the film held at constant area.

The property of surface hysteresis was also examined since it appeared there might be a link between the time-dependency of surface pressure and this property. Surface hysteresis or the significant separation seen between compression and expansion surface pressure-area isotherms has been examined in the case of the biological surfactants obtained from lung tissue, the so-called pulmonary or lung surfactants (12–15); however, the ability of simpler compounds such as stearic acid to exhibit similar behavior has been almost ignored. Ries and Walker (16) have stated, without presenting any evidence, that compression-expansion isotherms for stearic acid show hysteresis.

As a result of studies on the monolayer properties of biologic amphiphiles currently under investigation in this laboratory, the authors wish to report their observations on (*a*) the effect of compression rate on the π-A isotherm; (*b*) pressure relaxation following compression to preselected film pressures; and (*c*) surface hysteresis as affected by repeated cycling and minimum area of compression.

EXPERIMENTAL

Materials—Stearic acid[1] was dissolved in *n*-hexane[2] and used as the film-forming material. Double distilled water containing less than 0.1 p.p.m. as sodium chloride equivalents was used as the subphase.

Film Balance—Preliminary studies were carried out with a dynamic surface tension accessory.[3] This instrument was found to be unsatisfactory due to the distance between the two movable barriers varying in a nonlinear manner as a function of time. In addition, the reversal of the barriers at maximum and minimum area was not instantaneous, giving rise to exaggerated hysteresis loops. Accordingly, an automatic recording Wilhelmy-type film balance of the authors' design and fabrication was used in the present work. This consisted of a Teflon trough, an electromicrobalance,[4] and a 25.40-cm. (10-in.) recorder.[5] The trough, 20.2 cm. wide and 27.3 cm. long was jacketed on five sides by a water bath maintained at 25°. The trough and water bath were enclosed in a Plexiglas chamber saturated with the vapor of the subphase. Two solid Teflon barriers, one stationary and the other motordriven, rested on top of the trough and served to change the area available to the monolayer spread between them. The rate of compression and expansion could be varied by changing the gears which powered the movable barrier. Instantaneous reversal of the movable barrier was also possible. The minimum and maximum surface areas were 60 and 424 cm.^{2}, respectively.

A nichrome hangdown wire attached to one pan of the balance was used to suspend the sensor, a thin mica plate of known perimeter, in the trough between the barriers. Due to the dimensions of the sensor and the null-balance principle of the electrobalance, which prevented vertical movement of the sensor, bouyancy effects could be neglected. The balance was operated so as to give a sensitivity of 0.1 dyne/cm.

Procedure—The film balance was calibrated with the mica sensor in air, after which the trough was filled with water to a predetermined level. The surface was repeatedly swept and cleaned until reducing the area to a minimum gave no loss in weight, indicative of a surface free from contamination. The solution of stearic acid in *n*-hexane was pipeted onto the surface of the water with the barriers set for maximum area. The film was allowed to spread and equilibrate for 10 min., at which time compression was begun. The mass changes observed with changing area were converted to surface pressure (the difference between the surface tension of the pure subphase and the surface tension of the film covered subphase) using the following equation:

$$\pi = gdM/p$$

where π is the surface pressure in dynes/cm., dM the mass change in gram, g the acceleration due to gravity, and p the perimeter of the sensor in centimeters. Following completion of a run, the surface was cleaned by suction, the entire subphase removed, and the trough rinsed several times with distilled water before being refilled. At the same time the mica sensor was thoroughly cleaned.

Surface pressure-area isotherms were obtained with the movable barrier operated at 2.54 and 0.24 cm. (1.0 and 0.1 in.)/min. With a monolayer on the surface these rates corresponded to compression rates of 4.4 and 0.44 Å.^{2}/molecule/min., respectively.

Surface pressure relaxation studies were conducted by compressing a monolayer to a given surface pressure at both compression rates, halting compression at the preselected surface pressure, and then following the decrease in initial surface pressure, π_0, as a function of time until equilibrium was attained. The equilibrium surface pressure, π_{eq}, was taken to be that pressure at which the decrease in surface pressure did not exceed 0.1 dyne/cm./min. This normally occurred in not more than 10 min. A new film was used for each relaxation study.

Compression-expansion studies were first carried out by repeatedly cycling a stearic acid monolayer between 34.7 and 18.2 Å.^{2}/molecule. These cycling experiments were done at a rate of 4.4 Å.^{2}/molecule/min. and five complete compression-expansion cycles were run for each film examined. One-cycle compression-expansion experiments were also conducted to determine the effect of the minimum area of compression on hysteresis. Successively smaller minimum areas from 23.4 to 18.2 Å.^{2}/molecule were examined at a compression rate of 4.4 Å.^{2}/molecule/min.

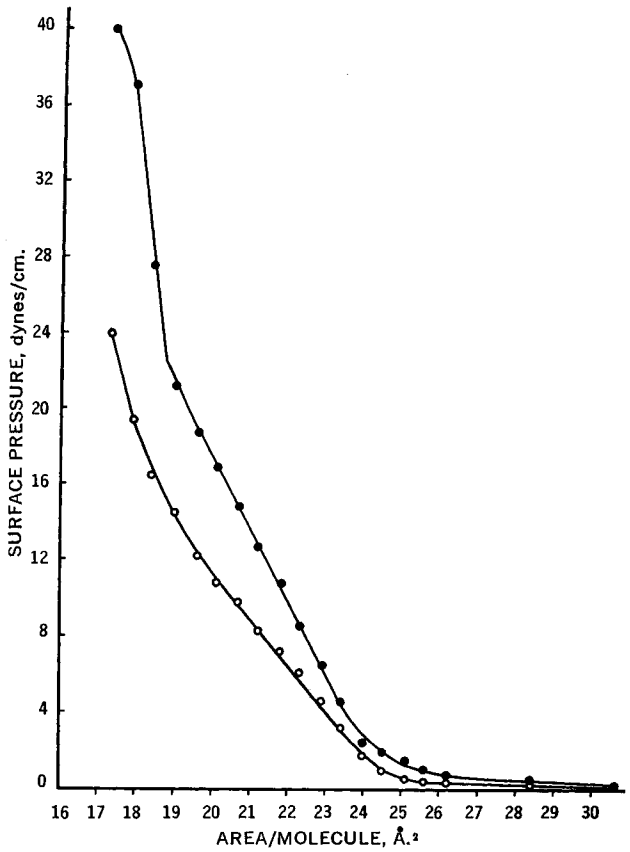


Figure 1—*Effect of compression rate on the surface pressure-area per molecule isotherm of stearic acid at 25°. Key: ●, 4.4 Å.^{2}/molecule/min.; ○, 0.44 Å.^{2}/molecule/min.*

RESULTS

Effect of Compression Rate—A significant difference was noted between the surface pressure-area isotherms of stearic acid compressed at two different rates. Figure 1 shows the average isotherms for three monolayers compressed at each rate. The variation between the three isotherms was such that the surface pressures at any one area per molecule were all within 1 dyne/cm. of the mean values plotted in Fig. 1. In most cases, the scatter was considerably less than this value. The isotherm generated by more rapid compression gave higher surface pressures at equivalent areas than slower compression. The isotherm may be conveniently considered in two portions. At the lower areas per molecule there is a linear low compressibility region. Extrapolation of this linear portion of the more rapidly compressed isotherm gave 20.3 Å.^{2}/molecule for the limiting area of stearic acid. At higher areas per molecule there is a second linear region of the surface pressure-area isotherm; this region is more compressible and extrapolation gives 24.8 Å.^{2}/molecule.

Surface Pressure Relaxation—The decline in surface pressure as a function of time from preselected initial surface pressures is shown in Fig. 2. These data obtained using a rate of compression of 4.4 Å.^{2}/molecule/min. represent the average of at least three separate experiments for each initial surface pressure. That the observed decreases in surface pressure were not due to leakage of film past the barriers was confirmed by placing the sensor behind the movable barrier while compressing a film between the barriers to an area equivalent to that which previously gave a surface pressure of 40 dynes/cm. After 10 min. the movable barrier was reversed, thereby compressing any material behind the barrier. The sensor showed no change in weight even at the minimum area, indicating that no material had leaked past the barrier. Plots of initial surface pressure

[1] Nutritional Biochemical Corp., Cleveland, Ohio.
[2] Eastman Organic Chemical, Rochester, N. Y.
[3] Cahn Instrument Co., Paramount, Calif.
[4] EMB-1, Research and Industrial Instruments Co., London, England.
[5] x-t, Beckman Instruments, Inc., Fullerton, Calif.

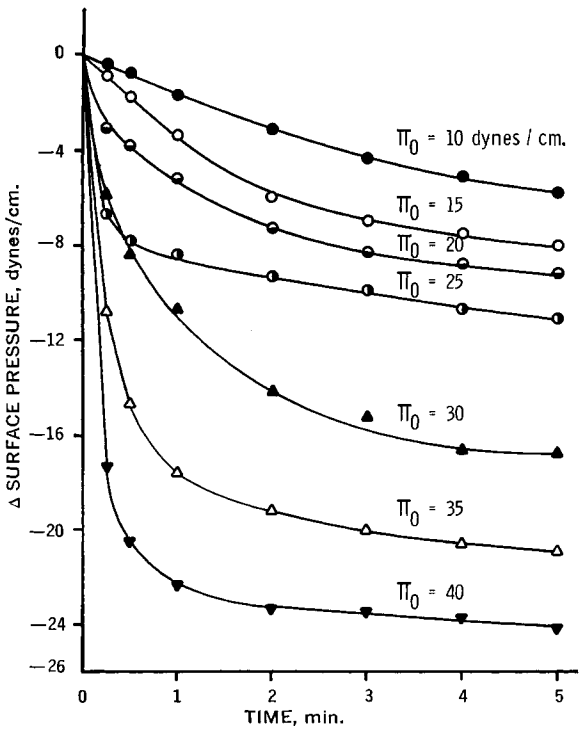


Figure 2—*Decrease in initial surface pressure* versus *time from preselected surface pressures. Compression rate—4.4 Å.²/molecule/min.*

versus area per molecule gave curves which coincided with that shown in Fig. 1 and the equilibrium surface pressure-area diagram was common for both rates of compression.

The curves in Fig. 2 may be classified into two groups. In the first, obtained at initial surface pressures of 20 dynes/cm. and below, there is a gradual decrease in surface pressure with time. The second group of curves are found at initial surface pressures of 25 dynes/cm. and above. These are characterized by an initial rapid decrease in surface pressure followed by a more gradual decrease which parallels that of the first type. The extent of the rapid fall-off increases with increasing inital surface pressure. The type of relaxation curve changes at an area per molecule of approximately 20 Å².

Compression-Expansion Studies (Hysteresis)—Two types of compression-exapansion studies were carried out.

Effect of Repeated Cycling—Table I shows typical data for the first three compression-expansion cycles of a stearic acid monolayer compressed and expanded between 18.2 and 34.7 Å.²/molecule. Hysteresis was noted consistently in these studies and a definite pattern was observed with regard to area of hysteresis, extrapolated area per molecule, maximum film pressure at the minimum area of compression, and the extent of recycling. The area of hysteresis decreased markedly between the first and second and the second and third cycles. Maximum surface pressure and the extrapolated

Table I—Effect of Recycling on Parameters of Stearic Acid Film[a]

Cycle	Extrapolated Area/Molecule, Å.²	Area of Hysteresis, Planimeter Units	Maximum π, dynes/cm.
1	20.2	248	37.3
2	19.9	100	24.2
3	19.8	82	21.4

[a] Rate of compression equal 4.4 Å.²/molecule/min.

area per molecule behaved in a like manner. Data for the fourth and fifth cycles were virtually the same as the third cycle and the compression and expansion isotherms nearly superimposable.

Effect of Minimum Area of Compression—The results for compression and expansion to successively smaller areas per molecule are shown in Fig. 3. Each hysteresis loop represents an average of three, first-cycle, experiments with a new film being used for each experiment. It can be seen that the area of hysteresis increases as the minimum area of compression is decreased. This is shown graphically in Fig. 4 where the area of hysteresis is plotted against the minimum area of compression. A noticeable break occurs in this curve at approximately 21 Å.²/molecule.

DISCUSSION

As a film is progressively compressed, the movement and rotation of molecules at the interface becomes increasingly restricted. In the case of stearic acid, Vold (17) has calculated that cylindrical close packing occurs at 25.2 Å.²/molecule. At this area however, the molecules are free to rotate. Vold (17) has further calculated that rotation ceases at 20.5 Å.²/molecule at which point maximum packing has presumably been achieved. Examination of Fig. 1 shows that extrapolation of the two linear portions of the isotherms gives areas per molecule of 24.8 and 20.3 Å.², in good agreement with the limiting values derived by Vold. Under the conditions used in this study, the linear increase in surface pressure observed between these two values is due presumably to the progressive restriction of free rotation as the closest packed configuration is approached (11).

As mentioned earlier, the relaxation profile observed with stearic acid films also changes at approximately 20 Å.²/molecule, which is close to the limiting area calculated by Vold (17). At areas in excess of this value and up to 25.2 A.²/molecule, one finds the gradual type of relaxation occurring. Although molecular orientation, such as the flipping of hydrocarbon tails from a horizontal to a vertical position, following compression may play a part in this relaxation, it is difficult to conceive of this process being wholly responsible for the observed relaxation due to the theoretical closeness of cylindrical packing. Relaxation may, in part, be due to the redistribution of unequally distributed molecules, a state resulting from the disturbing effects of compression, *per se*. The latter explanation could account for the observed differences in the surface pressure-area per molecule isotherms obtained at different compression rates. At slower rates of compression there would be less disturbance of the surface layer and

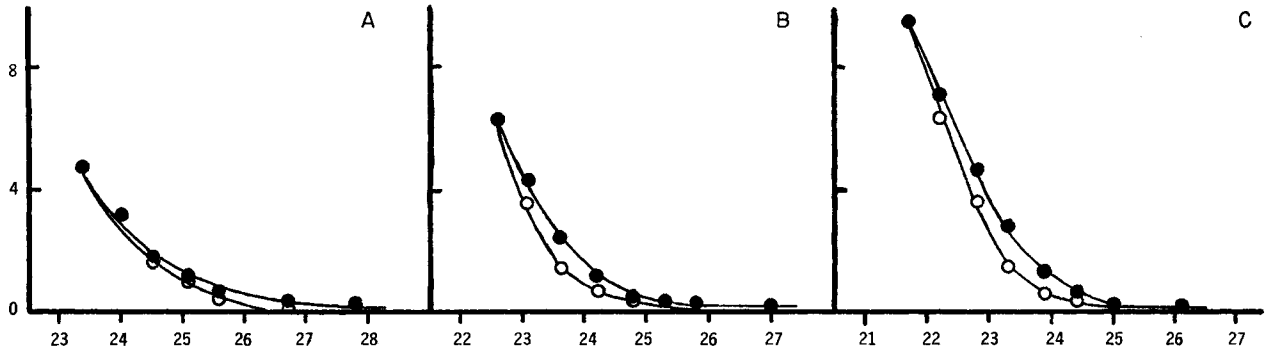
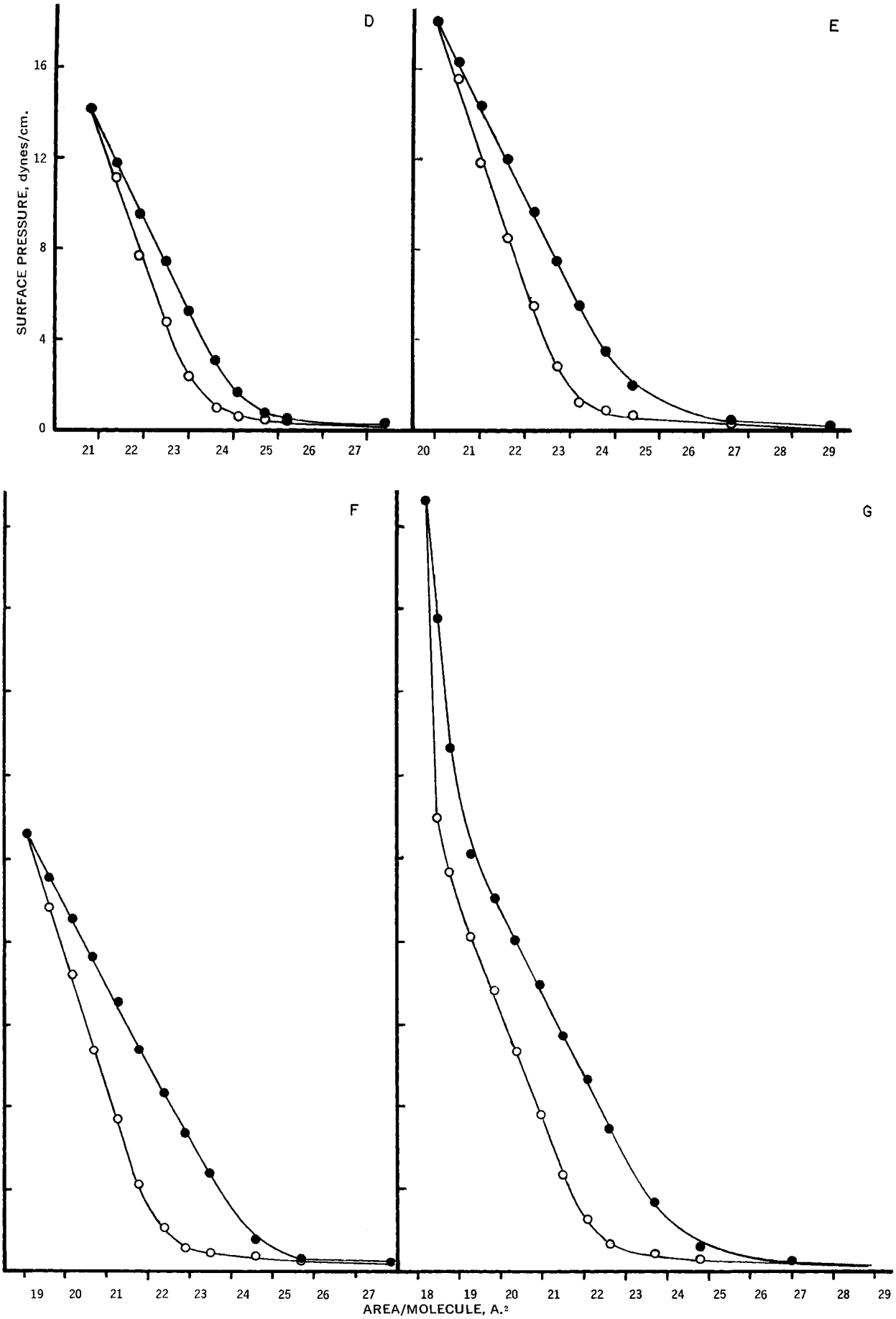


Figure 3—*Compression-expansion isotherms (first cycle) of stearic acid monolayers*



compressed to decreasing areas per molecule. Key: ●, compression; ○, expansion.

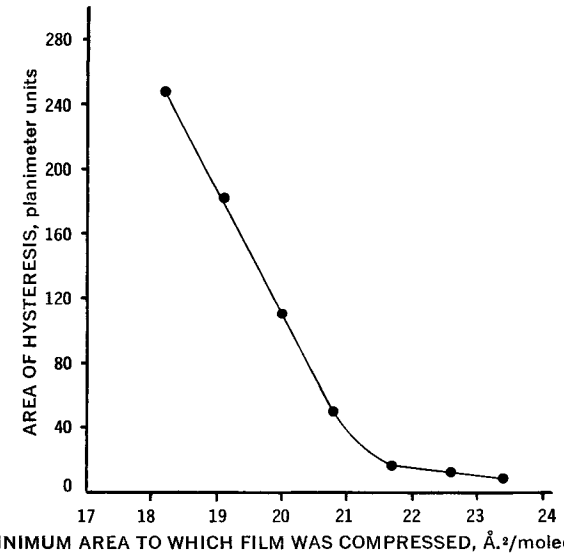


Figure 4—*Effect of minimum area of compression on area of hysteresis for stearic acid monolayer on water at 25°.*

consequently a more uniform distribution of molecules. Thus, lower surface pressures would be seen at equivalent areas per molecule when compared with the more rapidly compressed film. Rabinovitch (11) has shown a similar effect of compression rate.

Once compression is stopped in the range 20.5 to 25.2 Å.²/molecule, a finite period of time passes before the processes of redistribution and orientation are complete and the monolayer attains the equilibrium state. It is interesting in this regard that the equilibrium surface pressure-area isotherm was common for both rates of compression examined. It appears likely that the lower the compression rate, the closer one approaches the equilibrium state.

At areas per molecule below 20.5 Å.²/molecule, the compressibility of the molecules is presumed to be such that only a small reduction in area per molecule is possible before molecules are expelled from the monolayer. At higher compression rates this expulsion process apparently lags behind the decrease in area as evidenced by the sharp increase in surface pressure at low areas per molecule (Fig. 1). This lag may be a result of the London dispersion forces existing between adjacent alkyl chains, which would resist expulsion of those molecules in excess. It thus appears that the rapid initial relaxation noted in Fig. 2 is due to expulsion of residual excess molecules. When this process is complete, it is followed by the slower relaxation described previously at areas in excess of 20.5 Å.²/molecule. The magnitude of this initial decrease increases with increasing surface pressure since there are more molecules in excess at the lower areas of compression. At slower rates of compression, the sharp increase in surface pressure is not seen at comparable areas, presumably due to the expulsion rate of excess molecules now being more nearly equal that of compression rate.

Although no leakage of film past the barriers was detected in the authors' studies of repeated compression and expansion, the apparent extrapolated area per molecule is reduced, indicating that the monolayer becomes, in part at least, a multilayer due to expulsion and overlapping of the stearic acid molecules. Both the reduction in area of hysteresis and maximum surface pressure at minimum area in Table I are attributed to expulsion of stearic acid molecules. Reduction in area of hysteresis with repeated cycling has been reported for monolayers more complex than stearic acid (13, 15). Apparently the expulsion process is reversible to some degree since increasing the time at maximum area between cycles rather than immediate recompression was found to bring subsequent compression-expansion isotherms closer together (18). Under conditions of rapid recompression, expelled material does not have time to reenter the monolayer.

Rabinovitch *et al.* (11) have proposed that when the stearic acid monolayer is rapidly recompressed to areas below 21 Å.²/molecule, an inversion process occurs which is responsible for the change in compressibility seen in successive cycles. This process can be described as an inversion of molecules out of the plane of the interface to form a bilayer with associations between nonpolar portions of the molecules, in which case the second layer is above the interface; or with associations between polar portions, in which case the second layer is below the interface. Prior to this point, Rabinovitch *et al.* (11) have proposed that the planar configuration of the head groups at the interface should give way to a multilayer one, as head groups are forced to a position either above or below the original plane. Since, however, the cross-sectional area of the head group and the alkyl chain are probably quite similar, this latter process would not serve to accommodate more molecules at the interface. It seems likely, therefore, that complete expulsion of an increasing number of molecules from the interface occurs as compression proceeds below about 21 Å.²/molecule. It is also doubtful whether the expelled molecules would have the high degree of orientation proposed by these workers.

Of major significance is the observation that large areas of hysteresis are not obtained until this process of expulsion sets in. As shown in Fig. 4 by the break in the curve, expulsion occurs at an area per molecule of 21 Å.²/molecule, which is in reasonable agreement with that value obtained in the relaxation studies for the onset of expulsion. The fact that hysteresis is noted, although to a much lesser extent, at areas in excess of 20.5 Å.²/molecule is apparently due to the orientation and redistribution processes suggested earlier to explain the slow relaxation seen at comparable areas. Similar behavior, *i.e.*, increasing area of hysteresis with decreasing minimum area of compression, has been reported previously by Galdston and Shah (19) for dipalmitoyl lecithin films undergoing intermittent compression and expansion.

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