times for several enzymes have been correlated with the time of replication of the corresponding gene locus (*4*). However, while the uninduced rate of enzyme synthesis shows cyclic changes, the change does not necessarily occur at the time of replication. Several mathematical models which suggest that the synthesis of enzymes is entrained to DNA replication by concomitant RNA synthesis and which contend that the quantities of RNA and enzyme oscillate under the control of a repressor have been proposed. These periodic events are considered to be part of the timekeeping mechanism of the bacterial cell (*5*). While no such precise system of control for mammalian cells has yet been demonstrated, we have observed that the quantities of several enzymes change periodically through the cell cycle and that these changes are paralleled by net increases in mean cell volume and total protein. Bursts of ribosomal RNA synthesis also occur in G_1 and mid-S phases. Possibly, the bursts in enzyme activity are causally related to the synthesis of ribosomal RNA.

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Composite Membranes: The Permeation of Gases through Deposited Monolayers

Abstract. *We have measured permeabilities of deposited monolayers of stearic acid varying in thickness from 8 to 48 deposited layers. These results are compared with permeabilities of polymeric films and insoluble monolayers. The technique may have applicability in the fabrication of synthetic membranes.*

We have measured the rate of permeation of various gases through built-up monolayers of stearic acid. These multilayers were deposited onto permeable support films by using the conventional Langmuir-Blodgett method (*1*). By means of tracer techniques we were able to observe both the regularity and the concentration of the deposited films. In all, permeabilities have been measured for CO_2, N_2, and He diffusing through multilayers varying in thickness from 8 to 48 deposited layers, a range of 200 to 1200 Å. Our results indicate that this technique may be useful in fabricating composite films for a variety of applications, including model systems for the study of natural membranes (*2, 3*).

The support film used in these tests was an experimental silicone polymer (XD-1) (*4*). Properties which the support film must have are: it must be mechanically manipulatable; it must have suitable deposition characteristics for the monolayer which is to be deposited on its surface; and it must have a high permeability. Of the materials tested XD-1 provided the best combination of these properties (*5*).

The film was prepared immediately before use by casting a 0.02-cm-thick layer of XD-1 (10 percent by weight) in chloroform onto a clean glass surface. After allowing for solvent evaporation the film was removed from the glass and stretched over a solid support consisting of a disc of porous stainless steel (4.4 cm outside diameter) mounted in a stainless steel ring. Two layers of film were necessary to guard against possible pinholes in the film. The properties of the silicone polymer are such that it readily adheres to itself or a clean metal surface; simply stretching the film over the edge of the support gave a uniform, tightly held surface. The thickness of the resulting double layer of film varied from 0.8 to 2×10^{-3} cm. Stearic acid-1-C^{14} (9.19 mc/mmole) (*6*) was used for the depositions. All data reported here are for stearic acid deposited at a constant surface pressure of 29 dyne/cm with oleic acid used as a piston oil (*7*). The substrate solution used for the deposition was $10^{-4}M$ $BaCl_2$, $2 \times 10^{-4}M$ $KHCO_3$, and $10^{-7}M$ $CuCl_2$ with a *p*H of 7.5 and temperature less than 22°C. Under these conditions the deposited monolayer is not simply stearic acid but a mixture of stearic acid and stearate soaps formed with the various substrate cations (*8*).

For the depositions two supported films were mounted back to back in a dipping device which enabled us to lower and raise the films through the water surface at a uniform and reproducible rate. The change in concentration of the monolayer during the deposition was measured by comparing the corrected count rate of the stearic acid on the support film to that above the monolayer on the substrate solution. For this comparison the necessary background, dead time, and backscatter corrections were made (*5*). The ratio of the corrected count above the film to that over the water surface is defined as the deposition ratio. Allowing for geometric and statistical errors in the counting, the precision of the deposition ratio is approximately ±5 percent. In addition, the uniformity of the deposited layers was checked by autoradiography.

The mass transfer equipment consisted of two identical mass transfer cells. The film was placed in one cell, an impermeable plate was placed in the second cell. The film divided the mass transfer cell into two parts. To initiate an experiment a piston device was used to increase stepwise the pressure in both cells. The resulting gas pressure difference between the high pressure side of the two constant-volume cells was measured with a sensitive, variable-reluctance pressure transducer (*9*). From the transient pressure response the mass transfer coefficient, or a related quantity, the permeability, could be calculated. For simplicity, the mass transfer coefficient has been defined as $k_p = N/\Delta p$, where N is the mass flux through the film in milliliters (STP) per square centimeter per second under Δp, the pressure difference of the diffusing gas in centimeters of mercury. Consistent with this definition, k_p here has the units of: ml(STP)/cm²-sec-cm-Hg.

To determine the mass transfer characteristics of the deposited multilayers it was necessary to perform two experiments, one with the silicone support film alone, and a second with the composite film. From these results, the resistance of the multilayer could be calculated. The resistance to transfer, that is, the reciprocal of the mass trans-

Table 1. The effect of thickness on the permeability of stearic acid multilayers.

Film No.	No. of layers	$k_{pm}\left[\frac{10^5 \text{ ml (STP)}}{\text{cm}^2\text{-sec-cm-Hg}}\right]$		
		CO_2	N_2	He
10–2	8	68	7.5	14
27–3	8	78	5.8	14
27–4	8	42	3.8	8.5
4–1	12	26	2.6	7.0
4–2	12	20	2.9	7.6
7–3	18	11	1.2	3.3
7–4	18	17	3.4	6.1
76–3	48	3.39	0.378	1.16
76–4	48	3.27	0.425	1.19

fer coefficient, is an additive property. Therefore, the sum of the support film resistance, $1/k_{ps}$, plus the multilayer resistance, $1/k_{pm}$, is equal to the composite resistance, $1/k_{pt}$. The precision of the data obtained was approximately ±1 percent. Thus k_{pt} had to be less than 0.98 k_{ps} before meaningful resistances for the multilayer could be distinguished. Since the composite mass transfer coefficient, k_{pt}, for four deposited monolayers was on the order of 5 percent smaller than that of the support film alone, k_{ps}, our present study was limited to at least four deposited monolayers.

Both the initial layer deposition and subsequent depositions were so-called "Y" depositions (*3*) in which monolayer was deposited as the film was dipped into the liquid and also as the film was withdrawn. The deposition ratio for the initial dipping was always different from the ratio for subsequent layers. For the nine experiments reported here the average deposition ratio for the initial dippings was 1.81, with extreme values of 1.76 and 1.86. For all subsequent additions the average value was 1.87, with a maximum of 1.94 and a minimum of 1.78. There was no noticeable trend in the ratio after the initial layer was deposited.

Table 2. Comparison of multilayer and polymeric film permeabilities.

Substance	Permeability $\left[\frac{10^{10} \text{ ml (STP)-cm}}{\text{cm}^2\text{-sec-cm-Hg}}\right]$	
	CO_2	N_2
Silicone rubber (*12*)	3200	280
Stearic acid multilayers	4–16	0.5–2
High-density polyethylene (*13*)	3.5	0.25
Polyvinyl fluoride (*13*)	0.090	0.0015

The results for the permeation experiments are listed in Table 1. The greater scatter in the results for the thinner multilayers is to be expected, since in these cases the difference between the transfer coefficients for the composite film and the support film is quite small. It should be noted that the CO_2 rate is consistently greater than that for either He or N_2, even though CO_2 has the largest molecular diameter. This indicates that the permeation process is not simply that of a molecular sieve but that it is dependent on both the solubility and diffusivity of the permeating molecule in the multilayer. Though we do not have solubility data for these gases in stearic acid, data on solubility in various organic solvents (*10*) would indicate that CO_2 should have the greatest solubility and He the smallest. The fact that the permeation rate for He is greater than that of N_2 is probably due to a greater diffusivity for He. As to the use of terms such as "solubility" and "diffusivity," it should be noted that in dealing with monolayers or multilayers which are but a few molecules thick, a detailed explanation of the permeation process is not to be found in terms of macroscopic concepts.

The usual gas permeability (defined as the product of solubility and diffusivity) can be calculated from the mass transfer coefficient by multiplying the coefficient by the layer thickness. Taking the monolayer thickness as 25 Å yields permeabilities, in [ml(STP)-cm/cm²-sec-cm-Hg], of 4 to 16×10^{-10} for CO_2, from 1 to 3×10^{-10} for He, and 0.5 to 2×10^{-10} for N_2. The fact that the permeability is not constant with increasing multilayer thickness may be a facet of the transfer mechanism or it may be caused by imperfections in the initial deposited layer (which would tend to be covered over with subsequent depositions). The multilayer permeabilities are compared with several polymer permeabilities in Table 2. It appears that the stearate layers have a permeability comparable to that of high-density polyethylene.

Blank (*11*) has measured the permeability of various insoluble monolayers spread at the gas-liquid interface. Using his data we have calculated (*5*) from his results a mass transfer coefficient for CO_2 diffusing through a stearic acid monolayer maintained at a surface pressure of 24 dyne/cm and a temperature of 18°C. Compared with our results the resistance of the monolayer is comparable to that of 20 to 40 deposited monolayers. There is evidence for considerable molecular motion in the deposited monolayers (*3*). One would probably not expect as much motion in a stearic acid monolayer at a surface pressure of 24 dyne/cm. The fact that the deposition ratio observed in the deposition of monolayers onto the support film was less than one per layer seems to indicate that the density of the deposited monolayers is less than that of monolayers on the water surface. Therefore, it appears reasonable to find that the stearic acid multilayers are more permeable than a monolayer of stearic acid on the water surface.

Although our measurements to date have been limited to gases, there is no reason to expect that these composite membranes would not operate successfully in a liquid environment. We selected gases for our preliminary studies solely because of the ease of the permeability determinations. Likewise, the fact that we used but a single substance in the deposition does not mean that the technique is limited in this way. Indeed, it would appear that the more interesting applications of this type of membrane would entail using more than one type of monolayer in the composite layer.

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