havior of the chloroplast population during light-induced development. There are some observations that young chloroplasts can divide (*14*) and a few studies of chloroplast numbers in mature cells (*15*). Fasse-Franzisket (*16*) showed that in normal development of the light-grown leaf of *Agapanthus umbellatus* the plastids replicated several times. Plastids in etiolated leaves multiplied very slowly, but, if the etiolated leaves were exposed to light, plastids divided very rapidly until the normal number was reached. Glydenholm (*17*) demonstrated that there is no increase of chloroplast numbers in 16-day-old etiolated bean leaves during "greening." In our own material the plastids replicate at least twice. Replication starts immediately, and the shape of the curve indicates that this division is regular and asynchronous, whereas growth lags by about 24 hours and does not progress linearly.

We now have a method of specifically inhibiting chloroplast growth without permanently damaging the plastids. We can separate chloroplast growth from replication and thus explore the regulation of chloroplast development. In order to understand the action of FUDR on chloroplast growth, it must be shown that FUDR acts on DNA synthesis. There is a report (*3*) that it also affects RNA synthesis and that the effects are reversible with uridine. The effect of FUDR on higher plants has always been reversible with thymidine (*4, 18*) and RNA synthesis in tobacco callus was not affected by FUDR (*4*).

An important question is whether DNA synthesis during chloroplast growth occurs in the nucleus or in the plastid. Theoretically, it would be enough to show that chloroplasts treated with FUDR contain less DNA than those in the control, but since differences would not be more than 25 to 50 percent, it would be virtually impossible to purify the plastids enough to produce significant results. The autoradiographic data show that DNA synthesis occurs during plastid recovery from FUDR inhibition. Most of it is localized in the cytoplasm, so it seems likely that it is indeed this DNA synthesis that is involved in plastid growth.

The number of plastids increases considerably during greening, so it is not surprising that inhibiting DNA synthesis should influence chloroplast development. It is, however, most surprising that this inhibition has no effect on normal chloroplast replication. One explanation for this phenomenon is that the plastids contain more than one copy of their DNA, so that they can divide a few times even without additional DNA synthesis, but that DNA redundancy is necessary for the chloroplasts to attain their normal size. This hypothesis demands that plastid replication would come to a halt after a few division cycles, and the kinetin data show that this is so. 5-Fluorodeoxyuridine did inhibit kinetin-induced plastid division. Another consequence is that FUDR would have no influence on plants in which no plastid replication occurs.

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6 June 1969; revised 11 August 1969

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## Calibrated Membranes with Coated Pore Walls

Abstract. *Extremely uniform small-radius pores, formed in thin mica membranes, have been coated with mono- and multimolecular layers of fatty acids. Stearate monolayers orient themselves on the pore walls in layers 25 to 28 angstroms thick. The resulting membranes, with radii of the order 100 angstroms or less, may have applications in simulating certain features of biological membranes and in fabricating highly selective membranes for differential dialysis or ultrafiltration.*

Price and Walker (*1*) described a technique for producing uniform holes of molecular dimensions in certain nonmetallic materials. Holes are produced by exposing the material to massive, high energy particles which traverse the solid and leave tracks along which the material undergoes radiation damage. The damaged material along the tracks can be removed in a chemical etching solution which creates fine, hollow channels while the rest of the solid remains unaltered. By controlling the radiation exposure and etching time, holes smaller than 50 Å in diameter and hole densities up to $10^{11}$ holes per square centimeter have been produced (*2*).

Starting with thin mica sheets containing holes formed by this irradiation-etching technique, we coated the channel walls with stearate multilayers (*3*). Stearate deposited onto the surface of the sheet migrates into the pores, forming oriented layers on the pore wall. We summarize here our procedure for preparing coated holes in mica membranes and for measuring the thickness of the built-up layers.

Our membranes were made from clear muscovite (*4*), a natural mica, cleaved to thicknesses of 7 to 8 $\mu$m and cut into circular disks approximately 5 cm in diameter. The mica was irradiated by exposure to fission fragments resulting from the neutron-induced fission of $U^{235}$. Both the mica sheets and the uranium source were mounted in an evacuated collimating device placed in the thermal column of a nuclear reactor (*5*). After irradiation, holes were etched in a 20 percent HF solution (*1*).

Recrystallized, radioactive stearic acid (*6*) was used for the depositions by the Langmuir-Blodgett technique

(*7*). The present data are for fatty acids deposited at a constant surface pressure of 29 dyne/cm; double-distilled oleic acid was used as a piston oil. The substrate solution used for the deposition was $10^{-4}M$ $BaCl_2$, $2 \times 10^{-4}M$ $KHCO_3$, and $10^{-7}M$ $CuCl_2$. The *p*H of the solution was adjusted with a small amount of $NH_4OH$ so that depositions could be carried out at a *p*H of 7.20 or 7.35. Under these conditions, the deposited mono- or multilayers consisted of a mixture of stearic acid and stearate soaps formed with the various substrate cations (*8*). Substrate temperature was maintained between 14° and 16°C.

To coat the mica membranes, the sheet was placed on a Teflon support with one face exposed for deposition. The support was fixed in a dipping device which could be raised and lowered through the water surface at a uniform, reproducible rate. The first dipping resulted in only one deposited layer, and this first layer was attached as the mica was removed from the substrate. Subsequent dippings yielded two layers, one as the mica entered the substrate and one as it was removed. The resulting odd-numbered multilayers were the so-called Y-layers (*9*, p. 338). To monitor the transfer of the stearic acid from the substrate surface to the mica surface, we compared the corrected count rates of the $C^{14}$-labeled stearic acid on both surfaces. The mica has excellent deposition characteristics, and in all cases count rates on the mica were within ± 5 percent of the corrected rates over the solution. Uniformity of the depositions was checked by autoradiography.

It is well known that molecules in deposited monolayers are not held in a rigid, immobile state; rather, they exhibit considerable molecular motion. Evidence for this motion includes phenomena such as overturning, surface migration, and the ease with which deposited monolayers can be transferred between solid surfaces (*9*, p. 338; *10*). In our experiments, surface mobility resulted in rapid migration of deposited molecules from the basal plane into the pores. Molecules on the curved surface of the pore are more tightly bound than those on the cleavage plane for two reasons. First, the cleavage surface consists of a chemical arrangement [in its main part, a layer of oxygen atoms (*4*)] different from that of planes perpendicular to the basal plane. The stearic acid forms a more stable chemical combination on the perpendicular planes, approaching a chemisorbed state as opposed to a van der Waals type of adsorption on the cleaved surface (*11*). Second, for pores with very small radii capillary considerations dictate that chemical equilibrium will be characterized by a surface concentration on the pore walls greater than that on the plane (*12*).

We measured pore radii, with and without deposited layers, by measuring the rate of transport (Knudsen flow) of various gases through the membrane. After stearate was deposited onto the surface of the mica, the membrane was dried for several hours. Presumably, it was during this drying period that water evaporated from the channels and stearate from the plane surface diffused onto the pore walls. For measuring the rate of gas permeation through the membrane, the mica was mounted in a modified filter holder (*13*) with the coated side exposed to the high-pressure (1 atm) gas. The low-pressure side was then isolated, and the increase in pressure resulting from permeation through the membrane was recorded with a capacitance manometer (*14*). With small transfer rates, conditions were constant, and the flux was directly proportional to the pressure drop, $\Delta P$, across the membrane. For sufficiently small pores (ones for which the pore diameter is smaller than the mean free path of the permeating gas molecules) the flux (moles per area per time) can be calculated from kinetic theory (*15*)

$$N = \frac{4}{3} \left( \frac{2\pi}{M R_0 T} \right)^{1/2} \frac{n R^3 \Delta P}{L}$$

where $n$ is hole density (number of holes per square centimeter), $L$ is hole length, $R$ is the radius of a hole, $M$ is the molecular weight of the permeating gas, $R_0$ is the gas constant, and $T$ is the absolute temperature. With this equation, pore characteristics can be calculated from measured permeation rates (Table 1).

The quantity of most interest in Table 1 is the relative change in hole radius as monolayers are built up on the bare mica. There is, of course, some indefiniteness in the hole density, radius, and so forth. These uncertainties are minimized by reducing the permeation data to a ratio of pore radii. The first set of results, designated by subscript $\alpha$, were all taken with the same membrane. After the irradiated membrane was etched ($n \sim 10^6$) to a hole radius of 110 Å [this radius was determined from the calibration data of Price and Walker (*1*)] the permeation rate through the bare membrane was measured. A single stearate monolayer was next deposited, and the permeation rate through the coated membrane was measured. In all rate measurements the three gases, helium, nitrogen, and carbon dioxide, were used. The values listed for each gas represent the average of at least three separate determinations. From the data on the bare and the coated membrane, together with the radius of the bare holes, we can

Table 1. Relative radii of coated pores. The subscripts $\alpha$, $\beta$, or $\gamma$ designate the membrane; 0, 1, or 3 refer to the number of deposited layers; and S, A, or B indicate stearate, arachidate, or behenate layer.

| Ratio of pore radii | He | $N_2$ | $CO_2$ | Hole radii |
|---|---|---|---|---|
| | | | | $R_{\alpha o} \simeq 110$ Å |
| $(R_{\alpha o} - R_{\alpha 1})/R_{\alpha o}$ | 0.234 | 0.242 | 0.230 | $R_{\alpha' o} \simeq 140$ Å |
| $(R_{\alpha' o} - R_{\alpha o})/R_{\alpha o}$ | 0.282 | 0.257 | 0.262 | $(R_{\alpha o} - R_{\alpha 1})_{av} = 26$ Å |
| $(R_{\alpha' o} - R_{\alpha' 1})/R_{\alpha' o}$ | 0.173 | 0.171 | 0.184 | $(R_{\alpha' o} - R_{\alpha' 1})_{av} = 25$ Å |
| $(R_{\alpha' o} - R_{\alpha' 3})/R_{\alpha' o}$ | 0.533 | 0.522 | 0.522 | $(R_{\alpha' o} - R_{\alpha' 3})_{av} = 74$ Å |
| | | | | $R_{\beta o} \simeq 240$ Å |
| $(R_{\beta o} - R_{\beta 1})/R_{\beta o}$ | 0.100 | 0.121 | 0.118 | $(R_{\beta o} - R_{\beta 1})_{av} = 27$ Å |
| $(R_{\beta o} - R_{\beta 3})/R_{\beta o}$ | 0.330 | 0.341 | 0.342 | $(R_{\beta o} - R_{\beta 3})_{av} = 81$ Å |
| | | | | $R_{\gamma o} \simeq 200$ Å |
| $(R_{\gamma o} - R_{\gamma 1S})/R_{\gamma o}$ | 0.130 | 0.144 | 0.151 | $(R_{\gamma o} - R_{\gamma 1S})_{av} = 28$ Å |
| $(R_{\gamma o} - R_{\gamma 1A})/R_{\gamma o}$ | 0.138 | 0.164 | 0.162 | $(R_{\gamma o} - R_{\gamma 1A})_{av} = 31$ Å |
| $(R_{\gamma o} - R_{\gamma 1B})/R_{\gamma o}$ | 0.174 | 0.169 | 0.178 | $(R_{\gamma o} - R_{\gamma 1B})_{av} = 35$ Å |

calculate the change in hole radius resulting from one stearate layer. Averaged over the three gases, this value, $(R_{\alpha 0} - R_{\alpha 1})_{\mathrm{av}}$, is approximately 26 Å.

This same membrane was cleaned with ether to dissolve the stearate and was then rinsed successively with warm, concentrated KOH solution; water; concentrated chromic acid; water; and, finally, ethyl alcohol. The membrane was then etched further to increase the hole radius ($R_{\alpha' 0}$) to 140 Å (as determined by comparing Knudsen rates with the original 115 Å radius). A single layer on this enlarged radius gave a thickness $(R_{\alpha' 0} - R_{\alpha' 1})_{\mathrm{av}}$ of 25 Å. The single layer was removed, and three layers were deposited, giving an equivalent thickness for the three layers $(R_{\alpha' 0} - R_{\alpha' 3})$ of 74 Å. Intermediate runs with the bare mica showed that within the precision of our experiments the cleaning procedure did not alter the hole size.

The next series of runs, designated by subscript $\beta$, refer to a hole of larger radius, approximately 240 Å. Here, as with the $\alpha$ membrane, one and then three stearate layers were deposited. The equivalent thicknesses were 27 and 81 Å. The last data, designated by subscript $\gamma$, are measurements made on a single membrane for monolayers of stearic, arachidic, and behenic acids (the fatty acids of chain length 18, 20, and 22 carbon atoms). These layers reduced the hole radius by 28, 31, and 35 Å, respectively.

The thicknesses of the layers calculated from the permeation rates show rough agreement with thicknesses of multilayers calculated for fatty acid soap films oriented with the fatty acid chain perpendicular to the solid surface. A typical value for the thickness of a calcium stearate layer as determined from x-ray diffraction measurements is approximately 25 Å (*16*). The fact that the triple layers display a thickness three times that of the monolayer suggests that the perpendicular orientation is maintained in the multilayers. Also, the increment in thickness for the arachidate and behenate layers is of the order one would expect for the increased chain length.

In the preceding discussion, the pores in the mica have been considered to be circular. Actually, they are rhombi with angles of 60° and 120°, resulting from the basic crystalline structure of mica (*17*). Treating the rhombi as equivalent circles results in little error as far as the Knudsen determinations are concerned because the Knudsen conductance for a tube of uniform cross section depends on the ratio of the square of the cross section to the perimeter (*18*), a factor which differs by less than 2 percent for a 60° rhombus and the equivalent circle. That is, the circle and the rhombus of equivalent conductance have side and diameter which differ by less than 2 percent.

In certain respects the thicknesses of the layers are more uniform than one would expect, especially those of the tri-layers. Obviously, with the smaller inner perimeter of each additional layer, the head-to-head and tail-to-tail spacing cannot be the same for each layer. Also, the layer spacing in the corners of the rhombi—where the radii of curvature are greatest—cannot be completely regular. The precision of our present determinations is reflected in the range of thicknesses, 25 to 28 Å, measured for a single stearate layer. The scatter of the data appears to be random, with equivalent results for the small diameter and the large diameter pores.

Although our measurements have been restricted to the permeation of gases, there is no reason to expect that these membranes would not operate successfully in an aqueous environment. Also, the wall coatings are not limited to fatty acids.

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16 May 1969

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# Thymus and Reproduction: Sex-Linked Dysgenesia of the Gonad after Neonatal Thymectomy in Mice

Abstract. *Neonatal thymectomy of mice, when no ectopic thymus existed, constantly resulted in developmental arrest of the ovary but not of the testis; it also caused sterility in the female. The ovaries of thymectomized mice were extremely small and were characterized by absence of follicles and corpora lutea. Such an ovarian dysgenesia was observed when the mice were thymectomized at 3 days of age, but not at 7 days or later; it was prevented by thymus grafting.*

The essential role of the thymus in immunology has been established in many species of animals (*1*). Although the thymus has long been considered to be an endocrine organ somehow related to sexual physiology, no evidence has yet been presented. Thymectomy at 3 days of age in C3H/HeMs and (C3H/