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# Preparation and Characteristics of Lipid Vesicles

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*Summary.* As determined by electron microscopy, lipid sonicated in buffer initially forms large vesicles which may be multilamellar. Prolonged sonication results in a population of vesicles of smaller, but not uniform diameters. These vesicles are bounded by only one bilayer. The lipid suspension can be partially fractionated according to size by column chromatography. A fraction of the eluate has been selected for further study. The weight-average vesicle weight and average radius of gyration are obtained by light scattering measurements. The volume of buffer enclosed by the vesicles is determined using $^{14}C$- or $^{3}H$-labelled sugars as a marker. These values are in reasonable agreement with the corresponding values calculated from the size distribution of the vesicle fraction obtained by electron microscopy.

Lipids from biological membranes, when suspended in water, most frequently form liquid-crystalline phases or myelin figures in which the lipid molecules are arranged in layers. The hydrophilic groups of the lipid are on both surfaces of the layer and the hydrocarbon portions of the molecules fill the interior. This arrangement is called a lipid bilayer, and such bilayers are thought to play an important role in the structure and function of biological membranes (for a review, *see* Stoeckenius & Engelman, 1969). The properties of such bilayers are therefore of considerable interest to biologists, and attempts have been made to find model systems in which they can be studied (for a review, *see* Thompson & Henn, 1969). The most successful systems so far are the "black lipid films" developed by Mueller, Rudin, Tien and Wescott (1962) and the "liposomes" of Bangham, Standish and Watkins (1965). Both systems have characteristic advantages and disadvantages. In the black lipid film system, the geometry is reasonably well known and the two compartments separated by the film are both directly accessible. However, the composition of the film itself is not known and a solvent, usually a hydrocarbon such as *n*-decane, has to be present. In the liposomes, the composition of the bilayer is much better controlled, but the

geometry of the system has not been well established and may vary considerably. It is especially difficult to determine accurately the size of the inner compartments and the surface area, since these liposomes usually appear to consist of concentric bilayers.

Experiments during 1964 and 1965 on the reconstitution of mitochondrial membranes were carried out in collaboration with E. Racker. A lipid suspension used to restore enzyme activity in reconstituted membranes was examined in the electron microscope and found to consist of vesicles of relatively uniform size which appeared to be bounded by only one bilayer. It was then realized that this preparation might serve as a model system for the study of bilayer permeability to avoid some of the ambiguities of both the black lipid films and the liposomes. Some preliminary studies were carried out in collaboration with W. D. Seufert. This paper characterizes the geometry of the system. A study of the permeability properties of these lipid vesicles is in preparation.

## Materials and Methods

Asolectin is a commercially available complex mixture primarily of phosphatides extracted from soy bean (Associated Concentrates, Inc., Woodside, N.Y.). It was used without further purification. All other chemicals were obtained commercially and were of reagent grade. Water was doubly distilled.

### *Preparation*

In a tissue homogenizer with a motor-driven Teflon pestle, 3 g of Asolectin was suspended in 30 ml of 10 mM Tris buffer containing 0.25 M sucrose, 0.5 mM sodium ethylenediaminetetraacetate, and 7.5 mM $\alpha$-thioglycerol. The pH of the buffer was adjusted to 7.1 at 23 °C with HCl. The homogenate was sonicated at 15 °C with a 10 kHz Raytheon Sonic Oscillator, Model DF-101, operated at maximum output and cooled with tap water. No precautions were taken to exclude molecular oxygen. Sonication was continued until the optical transmission, measured at 770 nm, attained a maximum and constant value of about 60%. This required $\sim$60 min. The suspension was then centrifuged at 7 °C for 30 min at $109{,}000 \times g$. The small brownish-yellow sediment was discarded and the supernatant dialyzed against 1 liter of buffer at 4 °C for 12 hr.

Dry Asolectin contained 3.0% phosphorus by mass. After sonication and dialysis, the lipid suspension was extracted by the technique of Folch, Lees and Sloane Stanley (1957), and the extract was lyophilized. It contained 3.09% phosphorus. Phosphorus determination followed the method of Bartlett (1959).

A 3 to 5-ml portion of the dialyzed suspension was chromatographed at 4 °C on a $2.5 \times 30$ cm column packed with 200–400 mesh Corning CPG-10 glass beads (average pore diameter 1,250 Å). Flow was adjusted to about 8.5 ml/hr, and fractions were analyzed by optical density measurements at 300 nm.

## *Optical Studies*

Fractions obtained from column chromatography and used for light absorption refractometry and light-scattering studies were first dialyzed against buffer, and then centrifuged at $27{,}000 \times g$ for 30 min to remove any particulate contamination. When lower concentrations of lipid were prepared, the buffer used for dilution was either centrifuged under the same conditions or passed through an ultrafine sintered-glass filter. All optical studies were carried out at a room temperature of $23 \pm 0.5$ °C.

The spectrum of the lipid suspension was recorded with a Cary Model 14 spectrophotometer fitted with an expanded scale slidewire. The linearity of the slidewire was checked by optical density measurements at various concentrations of azocarmine G at 512 nm. Matched cuvettes of 1-cm pathlength were used.

Values for the specific refractive index increment of the vesicles at 436 and 546 nm were determined with a Brice-Phoenix Differential Refractometer, Model BP-2000-V The instrument was calibrated with KCl solutions of known refractive indices.

Light-scattering experiments were carried out in cylindrical cells with a Brice-Phoenix Light Scattering Photometer, Model 2000-D-M equipped with a Honeywell Electronik 19 recorder. Unpolarized light of 436 and 546 nm was used. Instrumental constants were determined according to the instruction manual. The angular dependence of light scattering, from 135 to 45 degrees, was analyzed according to Zimm (1948) with an IBM 360 computer programmed for the method of least squares.

## *Electron Microscopy*

For electron microscopy, the lipid suspension was fixed with a 1% osmium tetroxide solution buffered by veronal acetate (Palade, 1952), pH 7, and made isotonic by addition of sucrose. Approximately 1.5 mg of lipid in suspension was added to 3 ml of fixative at 4 °C and kept in the cold for 10 hr. Without washing out any excess fixative, five volumes of a 0.5% gelatin solution were added to prevent close packing; 0.1 ml of this mixture was filtered through a Millipore filter (25 nm average pore diameter) under gentle suction. The filter was then dehydrated in a graded series of ethanol followed by immersion in propylene oxide which dissolved the filter. The use of the filter technique for concentration is an adaptation of that of Baudhuin, Evrard and Berthet (1967). It prevents the non-random spatial distribution usually obtained in pellets of centrifuged material. The resulting gelatin film was infiltrated with epon and polymerized (Luft, 1961). Thin sections for electron microscopy were cut perpendicular to the plane of the filter and stained with uranyl acetate and lead citrate (Huxley & Zubay, 1961; Reynolds, 1963).

Micrographs were taken with a Siemens Elmiskop 1 electron microscope operated at 80 kV and calibrated with a cross-ruled grating. Magnification of the instrument was corrected for differences in specimen position by monitoring the objective lens current.

Vesicle diameters were measured either directly from the glass negatives or from photographic enlargements. Approximately 1,000 vesicles showing a distinct trilamellar wall structure were measured for each experiment. Since vesicle diameters were of dimensions comparable to the section thickness, the vesicles were regarded as opaque bodies in a transparent medium. Hence the probability of observing the true diameter of a vesicle would depend on the section thickness and the vesicle size based on geometrical considerations. Correction of the data for sectioned vesicles followed the treatment of Coupland (1968). Section thickness, determined by the method of Small (1968), ranged from 300 to 700 Å. This method involves direct measurements of upright folds which occur in sections.

For qualitative electron microscopy, the osmium tetroxide-fixed vesicles were negatively stained with a 2% solution of phosphotungstic acid neutralized with potassium hydroxide.

## *Thin Layer Chromatography*

Various lipid preparations were compared qualitatively by thin layer chromatography using Silica Gel G plates and, as the developing solvent, diisobutyl ketone, acetic acid and water in the volume proportions 40:25:3.7 (Nichols, 1963). Spots were detected by charring after they were sprayed with ammonium sulfate.

## *Fatty Acid Analysis*

The fatty acid content of various lipid samples was determined by gas-liquid chromatography. The lipid was treated in 1% sulfuric acid in methanol at 70 °C for 1 hr. The extracted methyl esters were analyzed in a Perkin-Elmer Model F II gas-liquid chromatograph with a 1/8″ × 11′ column packed with 12% ethylene glycol succinate on Chromosorb W.

## *Trapped Volume*

The volume of buffer enclosed by the lipid vesicles was determined with $^{14}C$- or $^{3}H$-labelled sucrose or glucose. First, an equilibrium distribution of the label was established across the vesicle wall, either by preparing the vesicles in buffer containing the label or by addition of the label to the vesicle suspension, allowing sufficient time for penetration into the vesicles. The vesicles were then rapidly separated from the external buffer on a 1 × 10 cm Sephadex G-75 column previously equilibrated with buffer. Two peaks of label appeared in the eluate. The first of these contained all of the lipid. The lipid-associated label required about 10 min for elution. Owing to the relative impermeability of the vesicles to glucose or sucrose, very little label leaked out of the vesicles during separation in the column, as judged by the amount of label trailing the lipid fraction. All label appearing before the second peak was assumed to have been initially trapped by the vesicles. The second radioactive fraction, representing the external label in the original lipid suspension, was collected in 25 ml of eluted buffer. Both fractions were counted in a liquid scintillation spectrometer. The trapped volume was determined from the ratio of the total activity of label from the first and second fractions and can be expressed as the volume percent of buffer trapped per mass of lipid in suspension. Binding of label to the lipid could be estimated by measuring the time course of penetration of the label into the vesicle and extrapolating the results to zero time. Correction of data for binding was found to be insignificant.

## *Hydrated Density*

The hydrated density of the lipid vesicles was determined by density-gradient centrifugation, using an IEC B-60 ultracentrifuge and the SB-405 swinging-bucket rotor. The gradient was formed by mixing buffer either with buffer containing an increased amount of sucrose or with deuterium oxide containing 9% sucrose. Initially, the vesicles were either mixed uniformly throughout the gradient or layered at the extremes. Centrifugation continued for 23 hr at $250,000 \times g$ at 7 °C. Fractions, obtained by drop-wise collection of the punctured tube, were analyzed for density by mass measurements of a filled capillary tube of known volume and for phosphorus content. Subsequently, the fractions containing lipid were fixed with osmium tetroxide and examined in the electron microscope after negative staining.

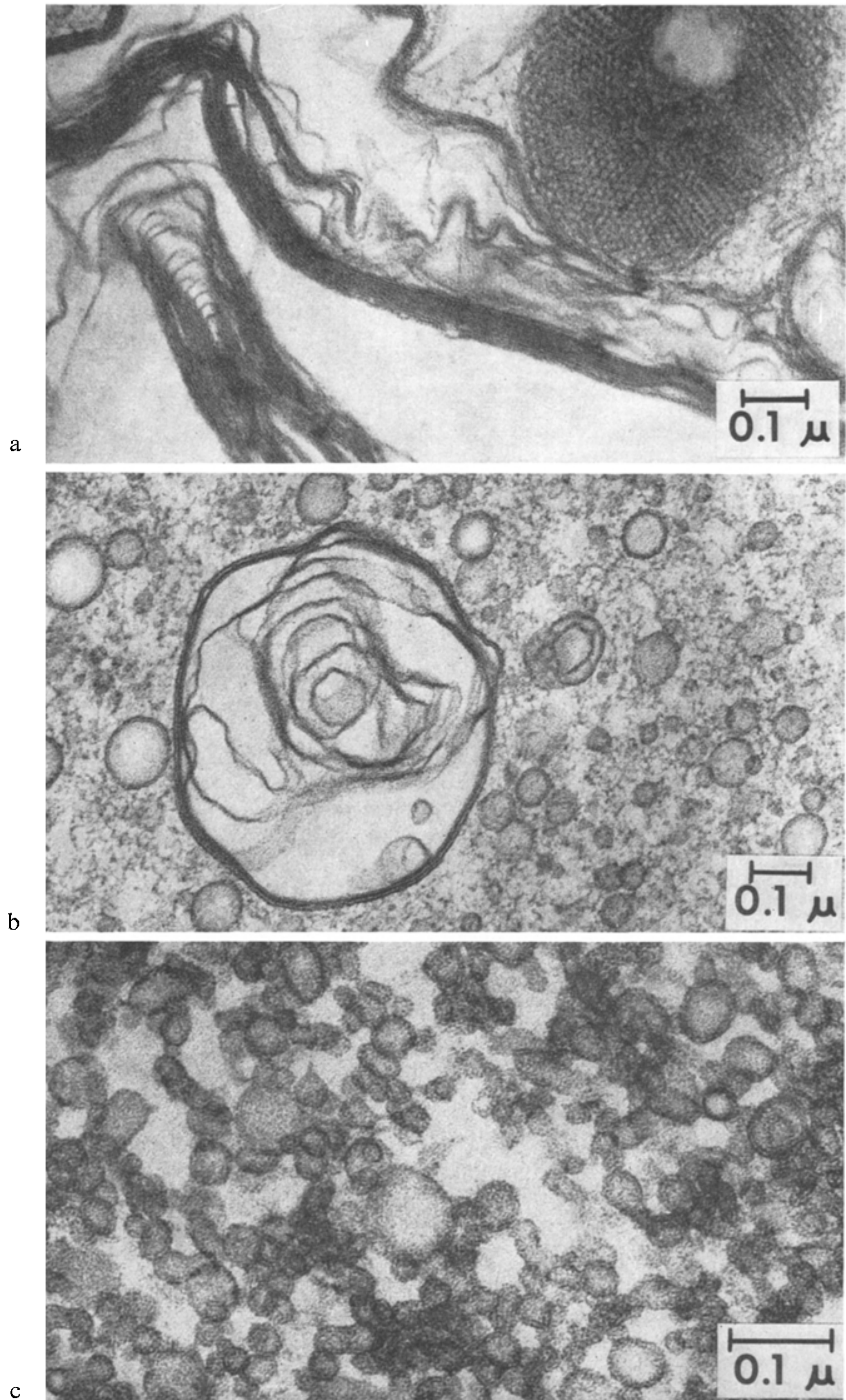


Fig. 1a–c. Thin sections of osmium-fixed lipid at different times during vesicle preparation. (a) After homogenization (× 87,400). (b) 5-min sonication (× 78,000). (c) 60-min sonication (× 140,000)

## Results

Various stages in vesicle formation have been observed with the electron microscope after fixation and sectioning (Fig. 1). Homogenization of lipid in buffer produces large aggregates of multilamellar myelin figures, vesicles, and other more complex structures. Sonication of the suspension results first in the formation of large vesicles, some of which are still multilamellar. Further sonication produces spherical vesicles of smaller diameter. They are bounded by only one triple-layered membrane-like structure (Figs. 1c & 2). The other structures seen in the section can be explained as grazing sections through such vesicles. Densitometry tracing of the vesicle wall gave a peak-to-peak distance of $45 \pm 5$ Å between the two outer dense layers. This is the typical appearance of a phospholipid bilayer after osmium tetroxide fixation (Stoeckenius & Engelman, 1969). Negative staining of fixed suspensions showed the same overall configuration of the vesicles (Fig. 5). Freeze-etched preparations again showed vesicles of the same size, concentrated in small spaces between large ice crystals. The vesicles appeared slightly deformed with no visible surface detail. When a suspension of fixed material is dried and shadowed, the vesicles appear collapsed.

The different preparative techniques used for electron microscopy of the lipid suspension therefore all indicate that the lipid is exclusively or nearly exclusively in the form of vesicles bounded by only one lipid bilayer.

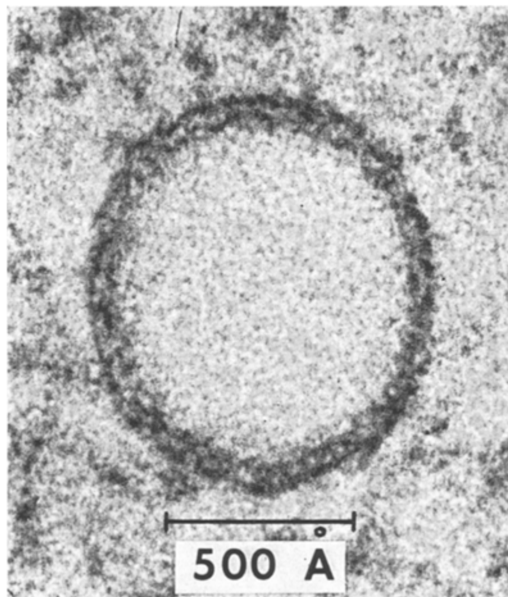


Fig. 2. Thin section of osmium-fixed lipid, showing wall structure of large vesicle $\times$(513,000)

This is further confirmed by the low-angle X-ray scattering studies ‹ A. E. Blaurock (1969, *unpublished results*) and the electrical impedan‹ measurements of Schwan, Takashima, Miyamoto and Stoeckenius (1970).

The size distribution of the unchromatographed vesicles, as determine by electron microscopy, is shown in Fig. 3. The distribution is broad an skewed to larger sizes.

Passage of the preparation through a column containing packing m terial of 1,250-Å pore diameter results in a rather broad elution patte (Fig. 4). Fixed and negatively stained preparations obtained from vario tubes show that the vesicles were eluted according to size (Fig. 5). N

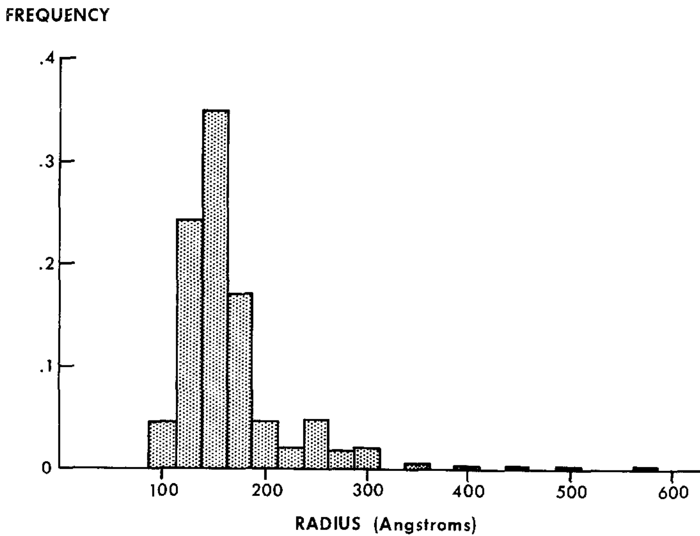


Fig. 3. Size distribution of unchromatographed lipid suspension, as determined by electron microscopy of thin sections of osmium-fixed vesicles

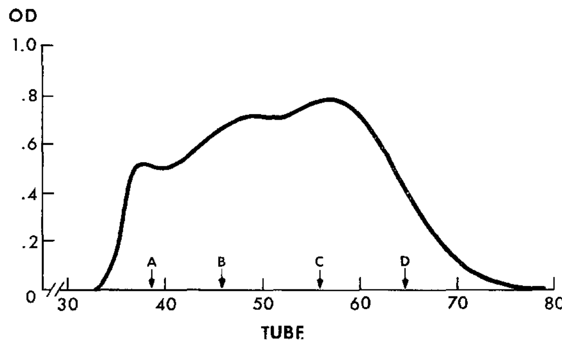


Fig. 4. Elution of vesicle suspension by column chromatography. Optical density meas urements at 300 nm. 1.8 ml/tube. Lipid in first peak is contained in excluded volum Points *A, B, C, D* refer to Fig. 5

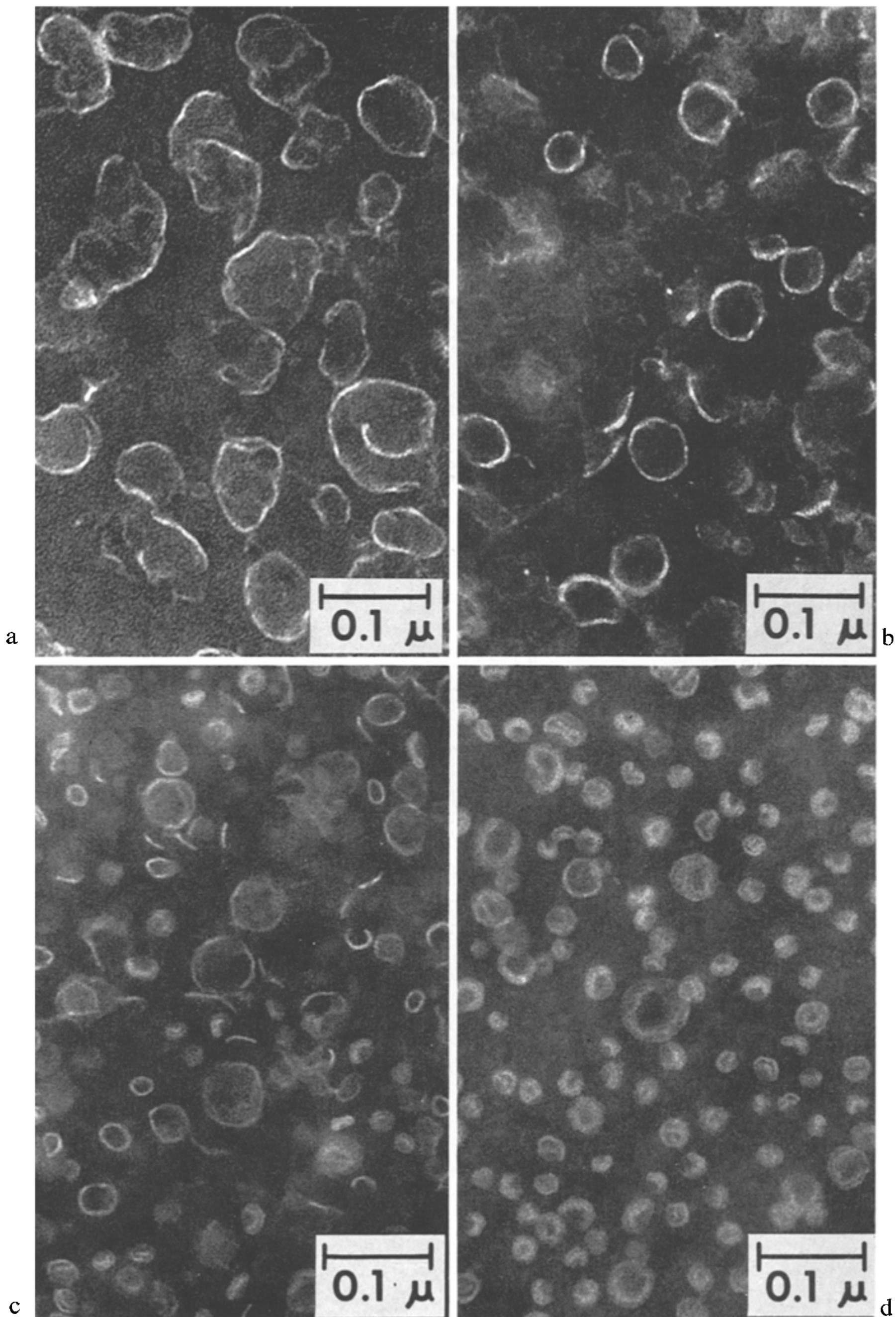


Fig. 5. Negatively stained preparation of osmium-fixed vesicle fractions. Fields *a*, *b*, *c*, *d* refer to vesicles taken from points *A*, *B*, *C*, *D* respectively, in the eluate shown in Fig. 4 (× 142,000)

significant differences among the fractions containing vesicles of variou sizes are found when they are analyzed for fatty acid composition, phos phorus content, or in one-dimensional thin layer chromatography of ex tracted lipids. Specific refractive-index-increment measurements of the vesi cles also failed to reveal any differences. A fraction taken from the ascendin portion of the principal peak of the elution pattern contained the large vesicles and was selected for further studies. This fraction possessed a nar rower volume distribution than the unfractionated vesicles, as evaluated b electron microscopy (Fig. 6). This is desirable because it facilitates the inter pretation of permeability data. The following observations and calculation refer to this fraction.

The vesicle suspension did not exhibit any light absorption from 650 to 300 nm. The turbidity showed an approximately inverse fourth-power de pendency on the wavelength of the incident light, as calculated from the slope of a log-log plot of absorbance and wavelength, shown in Fig. 7.

The effects of fluorescence and depolarization were found to be negli gible in light-scattering studies. Fig. 8 is a Zimm plot of one such experi ment. The weight-average vesicle weight is obtained from the reciproca of the $y$-axis intercept. The $Z$-average of the square of the radii of gyration $\langle R_G^2\rangle_Z$, is obtained from the ratio of the slope of the zero concentration line and the $y$-axis intercept, according to the formula:

$$\langle R_G^2\rangle_Z = \frac{\text{slope}}{\text{intercept}} \, \frac{3\lambda^2}{16\pi^2 n^2} \qquad (1$$

where $\lambda$ is the wavelength of the incident light *in vacuo*, and $n$ is the refrac tive index of the medium.

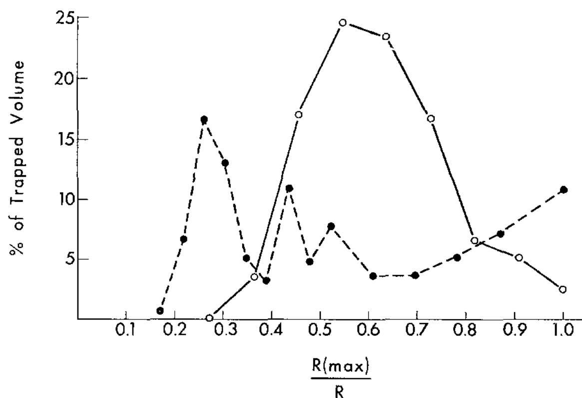


Fig. 6. Percent of volume trapped by vesicles of relative radius $R(\max)/R$. Dashed line values of unchromatographed vesicle preparation, calculated from Fig. 3. Solid line chromatographed preparation, calculated from Fig. 9

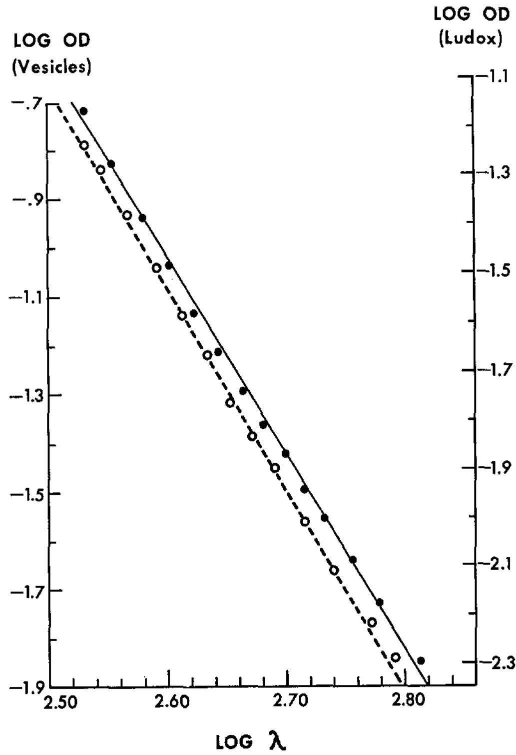


Fig. 7. Wavelength dependence of turbidity. Solid line: vesicle fraction. Lipid concentration ~1.3 mg/ml. Slope = −4.00±0.01. Dashed line: colloidal silica (Ludox IBD-1019-69, obtained from E. I. du Pont de Nemours & Co.). Average diameter, 154 Å. Concentration ~2.3%. Slope = −4.08±0.01

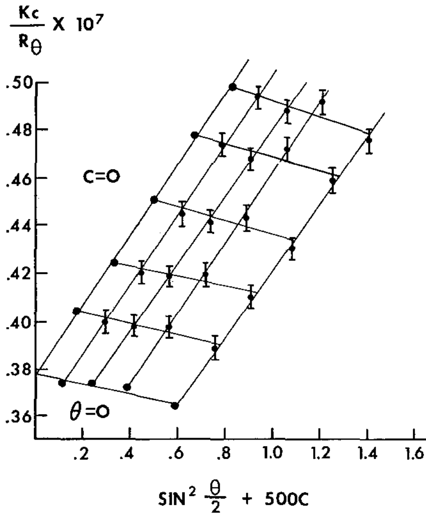


Fig. 8. Zimm plot of light-scattering data. For dimensions, *see*, for example, Tanford (1961). Wavelength, 546 nm. Radius of gyration, 350 Å. Vesicle weight, 26.5 million Daltons

In all cases, both zero-concentration and zero-angle lines converged to the same intercept. The vesicle weights thus obtained using two wavelengths for each experiment agreed to within 5%. The accuracy of the measurement is limited by the value for the specific refractive-index increments, which are 0.142 and 0.132 ml/g ± 7% for 436 and 546 nm, respectively. It is estimated that the error in the radius of gyration measurement is ±15%.

A portion of the sample used for each light-scattering experiment was fixed for electron microscopy. The size distribution for one such experiment is presented in Fig. 9. This is approximately a logarithmic normal distribution with a median radius of 290 Å and a geometrical standard deviation of 70 Å.

The errors for the electron-microscopy results are taken from the percent deviation from the mean of corresponding quantities at either extreme of the radius class range in the bar histograms of the frequency distribution data.

A comparison of the results of light scattering and electron microscopy is presented in Table 1. The average radius of gyration obtained from thin sections (column B) was calculated from the size distribution. The definition of the quantity obtained by light scattering is shown by (Tanford, 1961):

$$\langle R_G^2 \rangle_Z = \frac{\sum_i f_i M_i^2 (R_G^2)_i}{\sum_i f_i M_i^2} \tag{2}$$

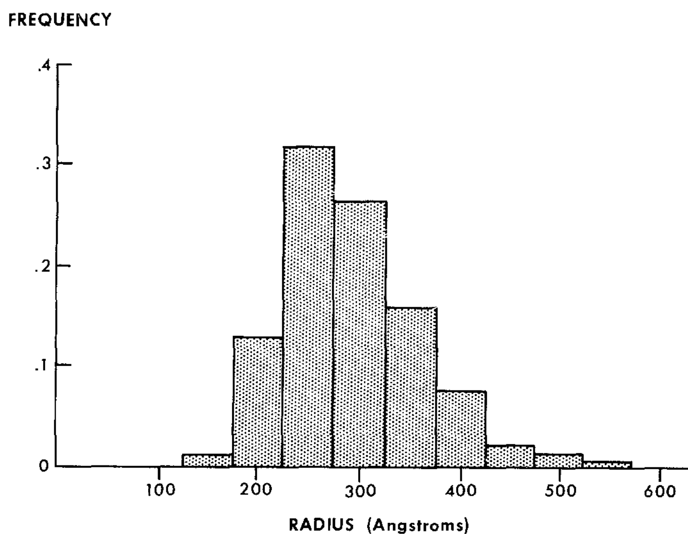


Fig. 9. Size distribution of vesicle fraction, as determined by electron microscopy of thin sections of osmium-fixed vesicles

Table 1. *Summary of vesicle size determinations*

| Sample | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 1 | 288±43 | 202±14 | 0.70±0.15 | 13.2±0.9 | 11.1±1.6 | 0.84±0.18 | 1.1±0.19 |
| 2 | 276±41 | 228±16 | 0.83±0.18 | 12.8±0.9 | 14.1±1.9 | 1.1 ±0.22 | 1.3±0.26 |
| 3 | 363±54 | 358±25 | 0.99±0.22 | 25.4±1.8 | 34.7±4.6 | 1.4 ±0.28 | |
| 4 | 413±62 | 335±23 | 0.81±0.18 | 27.5±1.9 | 29.4±5.0 | 1.1 ±0.22 | |

A: average radius of gyration obtained from light-scattering experiments. B: average radius of gyration obtained from electron microscopy. C: B/A. D: vesicle weight ($\times 10^{-6}$) Daltons by light scattering. E: vesicle weight ($\times 10^{-6}$) Daltons by electron microscopy. F: E/D. G: ratio of trapped volume, calculated from size distribution and divided by value obtained by isotope studies.

where $(R_G)_i$ is the radius of gyration, $M_i$ the vesicle weight, and $f_i$ the frequency of vesicles of radius $R_i$. Assuming that vesicles are spherical shells, the vesicle weight is proportional to the wall volume, as given in the expression:

$$M_i = \frac{4\pi}{3}(R_i^3 - r_i^3)\rho N \quad (3)$$

where $r_i$ is the inner radius of the vesicles, $\rho$ the wall density, and $N$ Avogadro's number. The square of the radius of gyration of a spherical shell is shown by:

$$R_G^2 = \frac{0.6(R^5 - r^5)}{(R^3 - r^3)}. \quad (4)$$

Eq. (5) is obtained after the appropriate substitutions are made:

$$\langle R_G^2 \rangle_Z = \frac{0.6 \sum_i f_i (R_i^3 - r_i^3)(R_i^5 - r_i^5)}{\sum_i f_i (R_i^3 - r_i^3)^2}. \quad (5)$$

A membrane thickness, $R - r$, of 43 Å is assumed. This value is determined from X-ray diffraction studies of Asolectin in the liquid-crystalline lamellar phase by V. Luzzati and T. Gulik-Krzywicki (1969, *personal communication*) and from low-angle X-ray scattering studies of the vesicle suspension by A. E. Blaurock (1969, *unpublished results*).

The size distribution obtained by electron microscopy can be combined with the vesicle-wall density to calculate the weight-average vesicle weight. In addition, since the lipid concentration of the suspension is known, the volume of buffer trapped in the vesicles can be calculated. The computed values for the vesicle weight and trapped volume can then be compared

with the corresponding values obtained by light-scattering and radioactiv‹
tracer studies.

The weight-average vesicle weight is defined as:

$$M_W = \frac{\sum_i f_i M_i^2}{\sum_i f_i M_i} . \qquad (6$$

Combining Eqs. (3) and (6), we obtain:

$$M_W = \frac{4\pi}{3} \rho N \frac{\sum_i f_i (R_i^3 - r_i^3)^2}{\sum_i f_i (R_i^3 - r_i^3)} . \qquad (7$$

The results are presented in column E of Table 1. In this calculation, a wal
density of 1.065 g/cc was used. This value was obtained from density
gradient centrifugation experiments using either $D_2O$ or sucrose gradients
Corresponding values obtained by light scattering are given in column D

The total number of vesicles, $S$, per unit volume, is related to the trappe‹
volume by:

$$V = \frac{4\pi}{3} S \sum_i f_i r_i^3 \qquad (8$$

where $V$ is the trapped volume per volume of suspension. The total volum‹
of lipid per volume of solution, $c/\rho$, is:

$$\frac{c}{\rho} = \frac{4\pi}{3} S \sum_i f_i (R_i^3 - r_i^3) \qquad (9$$

where $c$ is the lipid concentration.

Hence, the trapped volume can be expressed as:

$$V = \frac{c}{\rho} \frac{\sum_i f_i r_i^3}{\sum_i f_i (R_i^3 - r_i^3)} . \qquad (10$$

Two isotopic studies were carried out for the trapped volume. The results,
expressed as the ratio of calculated to observed values, are presented in
column G.

Gas-liquid chromatography of the lipids before and after sonication
revealed no significant changes in the fatty acid composition and no indica-
tion of oxidation. The results are presented in Table 2.

The freshly prepared lipid suspension prior to column chromatography
papears to be unstable (Fig. 10). Even under sterile conditions, the amount

Table 2. *Fatty acid composition of Asolectin and lipid extracted from vesicle suspension*

| Fatty acid | Asolectin (% by mass) | Extract (% by mass) |
|---|---|---|
| 14:0 | 0.1 | 0.1 |
| 16:0 | 18.5 | 18.4 |
| 18:0 | 3.0 | 1.2 |
| 18:1 | 6.5 | 6.4 |
| 18:2 | 66.6 | 64.6 |
| 18:3 | 5.4 | 9.2 |

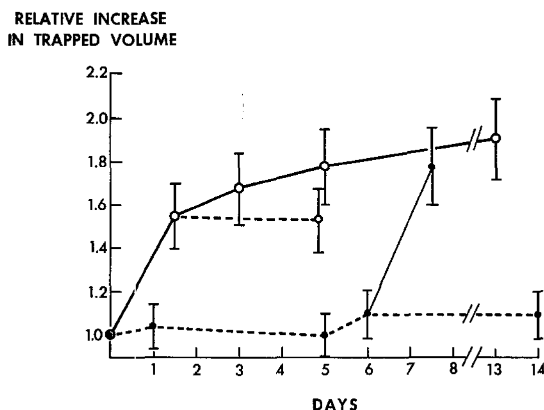


Fig. 10. Relative increase in trapped volume of unchromatographed vesicles. Lipid concentration, 87 mg/ml. $^{14}C$-sucrose added before sonication. Effect of temperature of storage. Solid line: 23 °C. Dashed line: 3 °C

of $^{14}C$-sucrose trapped in the vesicles increases with time after sonication. It is apparent that low temperature retards the increase in buffer volume trapped by the vesicles. One explanation of these observations would be the fusion of vesicles with increase in trapped volume. Such a process would depend on the concentration of vesicles in the suspension. No increase in the trapped volume has been observed in the more dilute suspensions of the partially fractionated vesicles.

## Discussion

The structure of lipid vesicles obtained from electron microscopy is consistent with the assumption that they are bounded by a single bilayer of lipid. The observed effects of sonic irradiation (namely, the formation first of large multilamellar vesicles which are then transformed into smaller unilamellar vesicles) are in agreement with those reported by Papahadjo-

poulos and Miller (1967), Chapman, Fluck, Penkett and Shipley (1968), and Saha, Papahadjopoulos and Wenner (1970).

Because negative staining has been widely used to study the structure of similar lipid suspensions, it is of interest that, in this system, negative staining of *unfixed* vesicles results in a variety of structures, including myelin figures (Fig. 11) and apparently amorphous masses. Since this appearance is inconsistent with all other results, we must assume that these are artifacts of this preparative technique. Presumably, myelin figures are formed when the lipid vesicles are concentrated during drying in the negative stain.

The suspension of unilamellar vesicles presents a two-compartment system of high surface-to-volume ratio. It can be used in permeability studies especially for slowly permeating substances. In contrast to the black lipid films, the vesicles also have the advantages that they are comparatively stable, that all material is in the form of a bilayer, and that no hydrocarbon solvent is present. A disadvantage of our preparation, i.e., its complex and not exactly determined lipid composition, is not an intrinsic feature of this system. Similar vesicles, smaller but very homogeneous with respect to size, have been prepared from egg lecithin and characterized by Huang (1969),

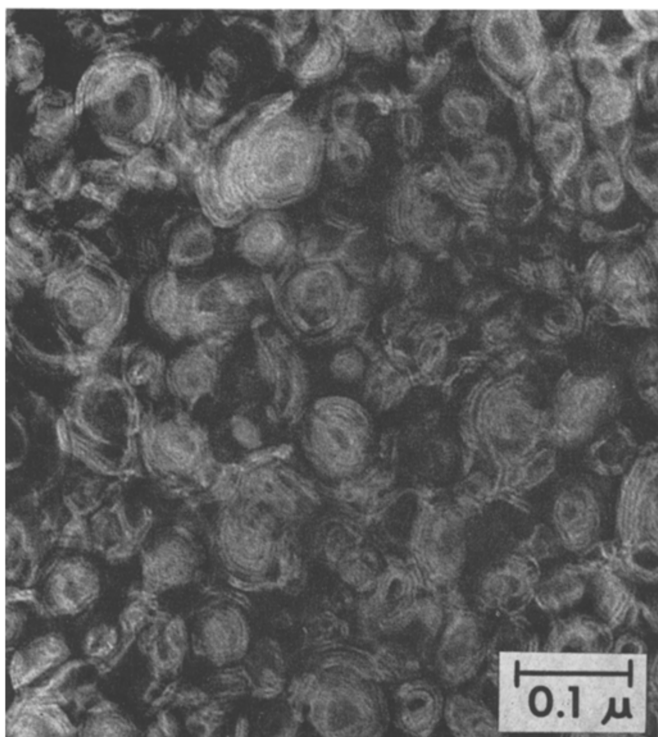


Fig. 11. Negatively stained preparation of unfixed vesicle suspension (× 143,000)

using hydrodynamic methods. Preliminary experiments in our laboratory have shown that mitochondrial lipids will readily form similar vesicles *(unpublished observations)*.

Before quantitative permeability studies can be undertaken, the size distribution of the vesicles must be known. Column chromatography resulted in a fraction of vesicles possessing a trapped volume distribution more suitable to permeability studies than the unchromatographed preparation. It also permits a limited choice of average vesicle size to determine bilayer properties as a function of vesicle radius.

The size distribution obtained by electron microscopy was checked by light-scattering measurements. The approximately inverse fourth-power dependence of turbidity on the wavelength of incident light indicates that the vesicles are small enough to use the Rayleigh-Debye approximation (Kerker, 1969) over a wide range of the relative refractive index. In fact, the relative refractive index of the vesicles must be close to unity because the volume fraction of the vesicle occupied by buffer is large. The light-scattering data thus afford an independent determination of the average radius of gyration to be compared with the value calculated from the electron micrographs. As seen in column C of Table 1, the two values are in fair agreement.

If we intend to calculate the vesicle weight and trapped volume from the size distribution obtained by electron microscopy, we must know the density of the lipid. Owing to the high permeability of lipid membranes to water (Reeves & Dowben, 1970), it can be assumed that, at equilibrium in a density-gradient centrifugation experiment, the density of the trapped volume in the vesicles is equal to that of the external medium. Hence this measured density can be taken as that of the solvated lipid membrane. Since the extent of solvation and the specific volume of the lipid-bound buffer are not known, the isopycnic density measured at 7 °C can be regarded only as an approximation of the density of dry lipid. This value of 1.065 g/cc is rather high for lipids. A value of 1.015 to 1.016 g/cc has been reported for solid lecithin (Elworthy, 1959). However, it is of interest that the partial specific volume, $\bar{V}$, of lecithin at 5 °C is 0.948 cc/g (Reiss-Husson, 1967). By assuming that the hydrated density is equivalent to the reciprocal of $\bar{V}$ (Gagen, 1966), a value of $\bar{V}=0.939$ cc/g is obtained for Asolectin. Huang (1969) reported a value of 0.9885 cc/g at 20 °C for lecithin, determined with a magnetic densitometer. We determined pycnometrically a value of 0.932 cc/g for unfractionated Asolectin vesicles at 23 °C.

The buoyant density of 1.065 g/cc and the vesicle bilayer thickness of 43 Å are consistent with a value of 13.7 for the ratio of the average lipid

molecular weight to the average area per lipid molecule. Similar values cai be obtained from studies of other liquid-crystalline phospholipid-wate systems (Luzzati, 1968). As seen in columns F and G, the vesicle weight anc trapped volume data are, within experimental error, in agreement with th size distribution determined by electron microscopy.

The light-scattering values for the radius of gyration and vesicle weigh and the values for trapped volume obtained from isotope studies are in dependent of the shape of the vesicles. These values are in fairly gooc agreement with those calculated from the size distribution measured ii electron micrographs which are based on the assumption that the vesicle are spherical. This shows that the size distribution as determined by elec tron microscopy is a reasonably accurate one and may be used for the stud of lipid bilayer permeability in this system. The high surface-to-volum ratio of the system allows determination of values for solutes of low per meability. For rapidly permeating substances, stopped-flow techniques coul be used. Moreover, proteins can be bound to the lipid bilayer withou changing its characteristic geometry and this should be valuable for a stud of structural aspects in lipid bilayer-protein interaction as well as its in fluence on permeability. The vesicle preparation described here thus offer a system that complements other model systems for membranes, especiall the black lipid films.

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