J. Membrane Biol. 36, 213–232 (1977)

Comparison between the Size of Granular Vesicles in Intact Cells and Vesicles Obtained by Fragmentation of Biomembranes

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Received 3 January 1977; revised 8 March 1977

Summary. Preparations of biomembranes, consisting of membrane vesicles, were analyzed with the analytical ultracentrifuge. Under certain conditions depending on the speed of rotation and the temperature, a sedimentation profile was observed that was highly characteristic for membranous material. From the sedimentation coefficients obtained, we calculated particle weights for the various well-defined membrane components. In certain types of preparations the particle weights of two adjacent components differed on average by a factor of 2. When vesicles obtained by fragmentation of biomembranes were compared with the granular vesicles present in intact cells, the accordance in diameters was striking. This may indicate that the size of vesicles is determined by purely physical factors.

Granular vesicles are found in situ in various types of cells. The size distribution of these vesicles has been determined with the electron microscope and it was shown that the standard deviation of the mean size of a certain type of vesicles was small (Bunge, Bartlett Bunge & Peterson, 1965; Costoff & McShaw, 1969; Geffen & Ostberg, 1969).

On the other hand, vesicles can be formed by fragmentation of large membranous structures, e.g. plasma membranes, nuclear membranes, and the membranes of the endoplasmic reticulum.

To our knowledge no systematic investigation concerning the size distribution of vesicles in preparations of biomembranes has been made thus far. We used analytical centrifugation in order to investigate the size distribution of vesicles of membranes in various membrane preparations. Some of the preliminary results have already been published by us elsewhere (Boom, Bont, Hofs & De Vries, 1976). In the present paper some practical and theoretical aspects of the experiments are dealt with

in more detail. In addition, a comparison was made between the size distribution of vesicles present in situ and that of vesicles obtained by fragmentation of biomembranes.

Materials and Methods

Preparation of Nuclear Membranes

The isolation procedure of nuclear membranes has been described previously (Boom *et al.*, 1976).

Preparation of Membranes of the Endoplasmic Reticulum

Both rough and smooth membranes of the endoplasmic reticulum were isolated from rat liver cells as described previously (Bloemendal, Bont & Benedetti, 1967).

Preparation of Plasma Membranes

Plasma membranes were isolated from calf thymocytes as described by Van Blitterswijk, Emmelot and Feltkamp (1973).

Analytical Centrifugation

The preparations were analyzed at 260 nm in a Spinco Model E analytical ultracentrifuge equipped with UV absorption optics and an automatic scanning system developed in this laboratory (Van Es & Bont, 1966).

The absorbance of all preparations was between 1 and 2, corresponding to a concentration of about 0.1 mg/ml.

Preparation of Liposomes

Liposomes were prepared from lecithin and 4 mol % phosphatidic acid according to De Gier, Mandersloot and Van Deenen (1968).

Results

Sedimentation Analysis of Nuclear Membranes from Rat Liver

The sedimentation velocity was studied in a Spinco model E analytical ultracentrifuge equipped with UV absorption optics coupled with an automatic scanning system as described previously (Van Es & Bont, 1966). The results of a typical sedimentation experiment are given in Fig. 1. It is evident that the membranes contained well-defined fractions as revealed by the steplike sedimentation pattern. In Table 1 the results of nine experiments are summarized. An apparent particle weight was obtained from the sedimentation coefficients in the following way. We

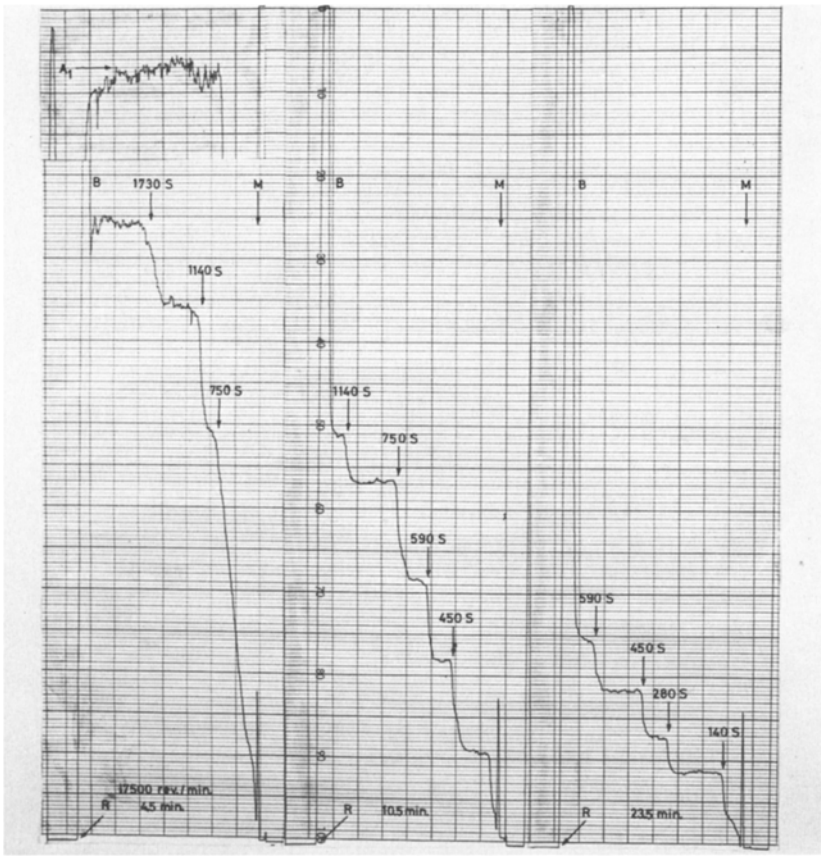

Fig. 1. Sedimentation characteristics of nuclear membranes. The absorbance was traced 4.5, 10.5 and 23.5 min, respectively, after reaching maximum speed ("17,500 rev/min). R = reference point; B = bottom; M = meniscus; A_1 = absorbance at start of experiment (superposed)

Table 1. Mean sedimentation coefficients (w, 20 °C) and standard deviation of nine subfractions distinguishable in nuclear membranes, calculated from the results of nine experiments (Maximum speed = 16,200 or 17,500 rev/min)

Subfraction no.								
1	2	3	4	5	6	7	8	9
Mean sedimentation coefficient								
3500	2420	1870	1280	865	575	430	285	150
Standard deviation								
–	±4.2%	±2.5%	±5.1%	±4.3%	±3.5%	±2.6%	±2.4%	±16%

The nos. 4 and 5 tend to split up in two components usually just beyond the resolution power of the instrument. No. 1 is only incidentally visible. The experiments were performed with nine different freshly prepared batches.

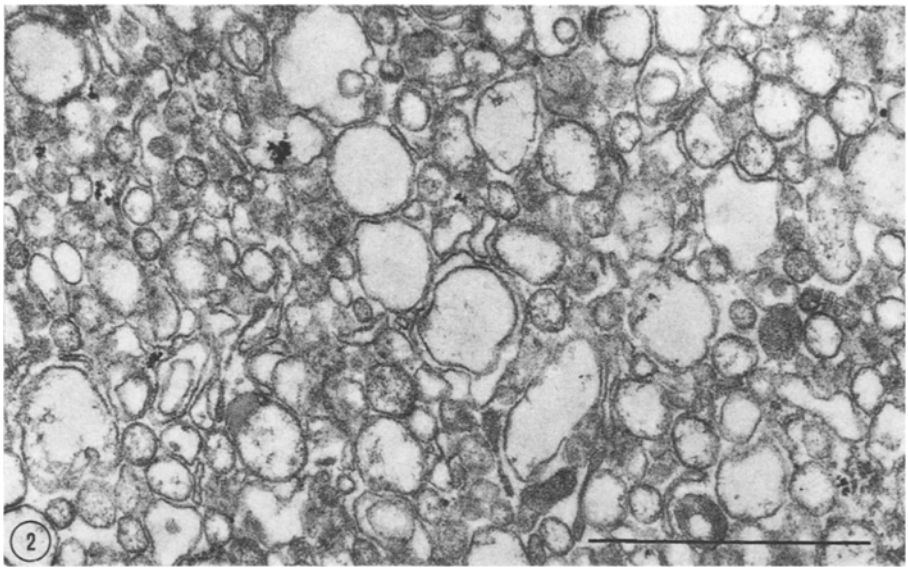

Fig. 2. Electron micrograph of a preparation of nuclear membranes. Membranes of the nucleus were isolated as described under Materials and Methods; thin section stained with uranyl acetate. The preparation consists of membranous profiles. The bar represents 1 μm

assumed that the membrane fragments were composed of vesicles. This was confirmed by electron microscopic analysis (*see* Fig. 2). Secondly, we assumed that the membrane vesicles sediment as hollow spherical particles. The sedimentation coefficient S of particles is given by the equation:

$$S = \frac{m\left(1-\frac{\rho}{d}\right)}{f}.$$

In this equation m = mass, f = frictional ratio, d = density of the membranes and ρ = density of the medium. The mass m is given by $m = Vd$ where V = volume of the particle and d = density. The volume of membranous material in a spherical particle is given by

$$V = 4\pi \int_{R_i}^{R_o} R^2 dR,$$

where R_i = inner radius and R_o = outer radius. Since the density of biomembranes is a function of R, the equation for the mass is given by

$$m = 4\pi \int_{R_i}^{R_o} R^2 d(R)\, dR,$$

where $d(R)$ is the density as a function of R. We assumed that biomembranes have a thickness Δr of about 100 Å (Emmelot, Bos, Van Hoeven & Van Blitterswijk, 1974) and since in a certain preparation the average density is the same (d) for all membrane vesicles, we can approximate the integral by the equation

$$m = 4\pi \left[\frac{R_o + R_i}{2}\right]^2 \Delta r d \cdot k$$

where $k = 10^{-24}$, when all dimensions are given in Å (10^{-8} cm).

Since we assumed $\Delta r = R_o - R_i = 100$ Å, we can write $m = 4\pi\ (R_o - 50)^2\, d\ 100\, k$. The frictional ratio f for spherical particles is given by $f = 6\pi\eta R_o$. The sedimentation coefficient is now given by the equation

$$S = \frac{4(R_o - 50)^2\, d\, 100 \left(1 - \frac{\rho}{d}\right) P}{6\pi\eta R_o} = \frac{2 \cdot (R_o - 50)^2}{3\eta R_o} 100 (d - \rho) P.$$

The viscosity η (0.01 poise), and the density ρ (1 g/ml) for the medium are those of water. The density d of membranes was taken as 1.17 g/ml. When all dimensions are expressed in Ångstrom (10^{-8} cm) and S in Svedberg units (10^{-13} sec), the factor $P = 0.1$. Now the equation becomes

$$S = \frac{(3.4)(R_o - 50)^2}{3R_o}.$$

From this equation R_o can be solved for every S value. The ratios of the masses of two adjacent components (*see* Fig. 1 and Table 1) were calculated in the following way. In Table 2 the number of the component (column 1), the sedimentation coefficient (column 2), the R_o values (column 3) and $(R_o - 50)^2$ values (column 4) are given. The masses of the particles are proportional to the latter values and the ratios of two adjacent components were therefore easily calculated and are given in column 5. The values of $m_{(n+1)}/m_n$ deviates from 2 for the values m_2/m_1, m_4/m_3, and m_8/m_7.

The mean value of the ratios, when m_2/m_1 is discarded, is

$$\overline{\frac{m_{n+1}}{m_n}} = 2.0 \pm 0.2.$$

In addition, when we consider the value $\sqrt[8]{m_9/m_1} = 2.07$ it is evident that on average two adjacent components differ by a factor of 2 in particle weight.

Table 2. Calculation of radius and mass ratios from sedimentation coefficients

No. of component	Sedimentation coefficient (Svedberg units)	R_o (Ångstrom)	$(R_o-50)^2\times10^{-4}$	m_{n+1}/m_n
1	150	221	2.93	
2	285	344	8.66	2.96
3	430	474	17.99	2.08
4	575	603	30.60	1.70
5	865	860	65.66	2.15
6	1280	1227	138.62	2.11
7	1870	1748	288.51	2.08
8	2420	2234	477.06	1.65
9	3500	3187	984.36	2.06

For the sedimentation coefficients *see* Table 1 and for the calculation of the outer radius of the particles and for further details *see text.*

From this result it can be derived that on average

$$\frac{m_{n+2}}{m_n}=\left[\frac{R_{n+2}-50}{R_n-50}\right]^2=4 \quad \text{or} \quad \left[\frac{R_{n+2}-50}{R_n-50}\right]=2.$$

Actually we calculated 2.0 ± 0.2 for the mean value of this ratio. Since $R\gg50$, the ratio can be approximated by $R_{n+2}/R_n=2$. This relation can easily be verified from the data in Table 2.

Separation of Membrane Vesicles on Sucrose Density Gradients

In order to get more conclusive evidence for the interpretation of our results, we separated the membrane vesicles according to size. To this end the preparation was layered on top of a sucrose gradient and after centrifugation for 90 min, the various fractions were isolated (*see* Fig. 3). With the isolated fractions we performed the following experiments. First they were analyzed in the analytical centrifuge and subsequently studied in the electron microscope. The results are summarized in Table 3. It was concluded that the higher the sedimentation velocity in the sucrose gradient, the higher the S value in the analytical ultracentrifuge (U.C.). In addition, from the data obtained with the electron microscope, we concluded that the more rapidly sedimenting components are not aggregates of small vesicles, but the higher the S values the larger the size of the vesicles (Figs. 4–6).

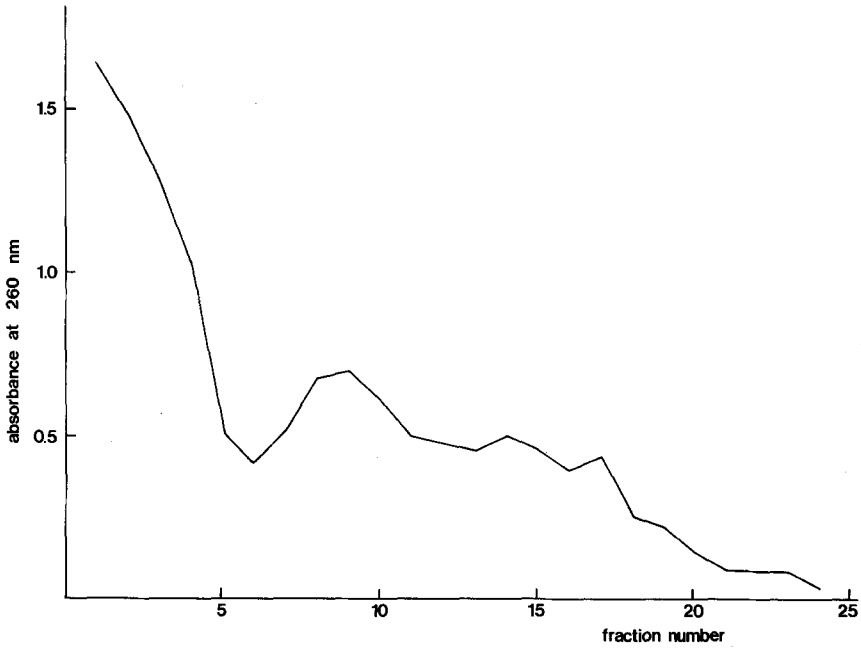

Fig. 3. Sedimentation profile of nuclear membranes in a linear sucrose gradient (5–40% w/v). Various fractions of the gradient were analyzed in the analytical ultracentrifuge (*see* Table 3) and in the electron microscope. The higher the fraction number of the gradient, the higher the *S* value in the analytical ultracentrifuge and the larger the vesicles

Table 3. *S* values of membranes fractionated by sucrose gradient centrifugation[a]

Fraction no. of sucrose gradient[b]	Interval of *S* values
9	140–400
14	800–1300

[a] The fractions of the gradient were diluted with medium to a sucrose concentration of 0.2 M.

[b] For the sedimentation profile in the sucrose gradient *see* Fig. 3.

Sedimentation Analysis of Cytoplasmic Membranes

We were interested in knowing whether these findings were restricted to membranes isolated from a nuclear fraction or whether they were an aspect of biomembranes in general. One could argue that nuclear membranes contain DNA, be it only as a small fraction of the total mass of the membranous material. This nonmembranous nuclear material could possibly be responsible for the peculiar fragmentation of the nuclear

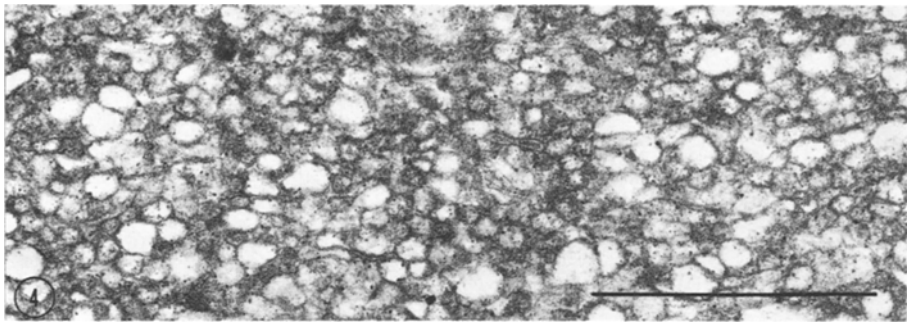

Fig. 4. Electron micrograph of a fraction isolated from nuclear membranes. The preparation shown in Fig. 2 was fractionated on a sucrose density gradient (*see* Fig. 3). A top fraction (fraction 2) of the gradient was examined in the electron microscope in the same way as the unfractionated preparation (*see* Fig. 2). The bar represents 1 μm

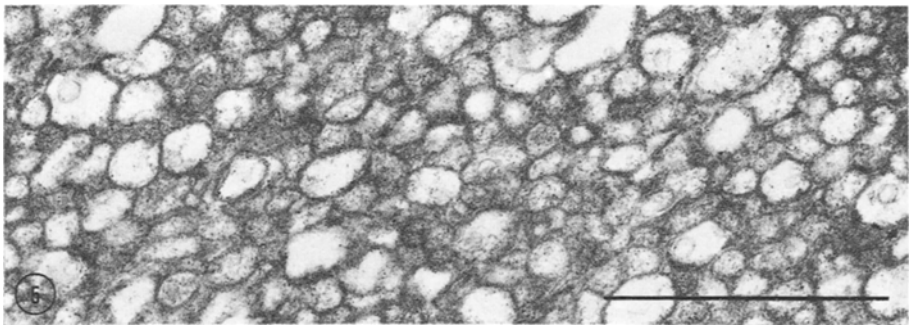

Fig. 5. Electron micrograph of a fraction isolated from nuclear membranes. Fraction 12 of the gradient (*see* Fig. 3) was examined. For further details *see* Fig. 4. The bar represents 1 μm

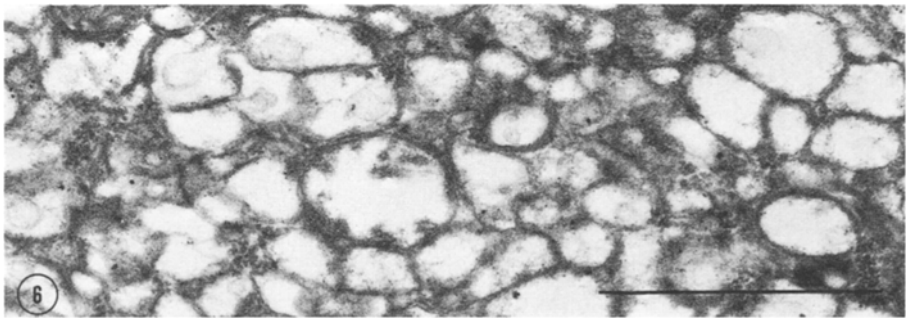

Fig. 6. Electron micrograph of a fraction isolated from nuclear membranes. A bottom fraction of the gradient (fraction 19) was examined. For further details *see* Fig. 4. The bar represents 1 μm

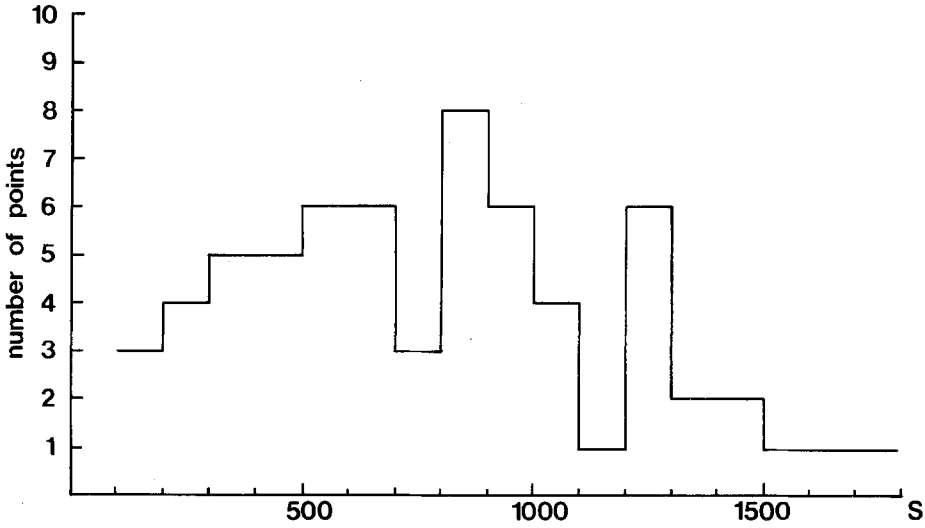

Fig. 7. Histogram of the sedimentation coefficients of membranes from the endoplasmic reticulum. For the construction of this histogram 64 *S* values, obtained from 13 experiments were used. For further details *see text*

membranes. Therefore we decided to investigate the membranes of the endoplasmic reticulum. It is well known that the membrane fraction of the endoplasmic reticulum consists of two groups of membranes, viz rough membranes (membranes dotted with ribosomes) and smooth membranes (membranes without ribosomes) (Palade, 1955).

In a previous publication (Boom *et al.*, 1976) the sedimentation profiles of smooth membranes and nuclear membranes were shown. The conclusion was drawn that the number of components can differ from one preparation to another, irrespective of their source. In addition, vesicles with the same sedimentation coefficients were found in both preparations. Rough membranes of the endoplasmic reticulum also showed well-defined fractions in the analytical ultracentrifuge, but the spread in *S* values was much higher than those given in Table 1 for nuclear membranes. We could not calculate the mean of the sedimentation coefficients and their standard deviation but instead the frequency for the occurrence of a certain *S* value was plotted against the *S* value (Fig. 7). The number of *S* values used for the construction of the histogram is rather low (64) but it seems as if vesicles with *S* values of about 600, 850 and 1250 were preferentially formed. For these three *S* values the same formula and the same assumptions were made, as used for the nuclear membranes, and again it was found that particle weights of the components with 600, 850 and 1250 *S*, respectively, differed with a

factor of 2. At *S* values smaller than 500 no peaks in the distribution of *S* values were observed. This could be due to the fact that polyribosomes also give rise to well-defined sedimentation patterns that were superimposed on the sedimentation patterns of the vesicles. Furthermore, polyribosomes attached to the vesicles, made a relatively large contribution to the particle weight of the sedimenting vesicles, especially at lower *S* values. Therefore, even though definite sedimenting components were observed in rough membranes, no regularities in the *S* values could be detected, especially at relatively low *S* values. The experiments used for the construction of Fig. 7 were performed with membranes of the endoplasmic reticulum from rat liver. The endoplasmic reticulum from chicken liver showed similar steplike sedimentation patterns.

Sedimentation Analysis of Plasma Membranes

Since both nuclear membranes and cytoplasmic membranes contained nonmembranous material like, respectively, DNA and RNA we decided to investigate the outer membrane of the cell; i.e., a suspension of plasma membranes. Since preparations of plasma membranes isolated from tissue like liver contain large sheets of membranes, interconnected by the various types of junctional complexes (Emmelot *et al.*, 1974), we investigated plasma membranes isolated from calf thymocytes. The latter type of preparations consisted of membrane vesicles. Also in these preparations definite fractions were observed (Fig. 8).

For the four most rapidly sedimenting components the following sedimentation coefficients were calculated: 1719, 2546, 4307 and 5715 *S*. These *S* values correspond with a radius of 1615, 2345, 3899 and 5143 Å, respectively, and with mass ratios for two adjacent components of 1.7, 2.8 and 2.1. The *S* values of the first two components differ by less than 10% from the *S* values of components 7 and 8, respectively, in Table 2, obtained for nuclear membranes.

In general, all preparations of membranes irrespective of their source (provided that they contained membrane vesicles) showed a discontinuous distribution of membrane particles.

Sedimentation Analysis of Artificial Membranes (Liposomes)

From our sedimentation studies we concluded that biomembranes existed as particles with a well-defined size with the exclusion of other sizes. These conclusions were supported by gradient centrifugation, since

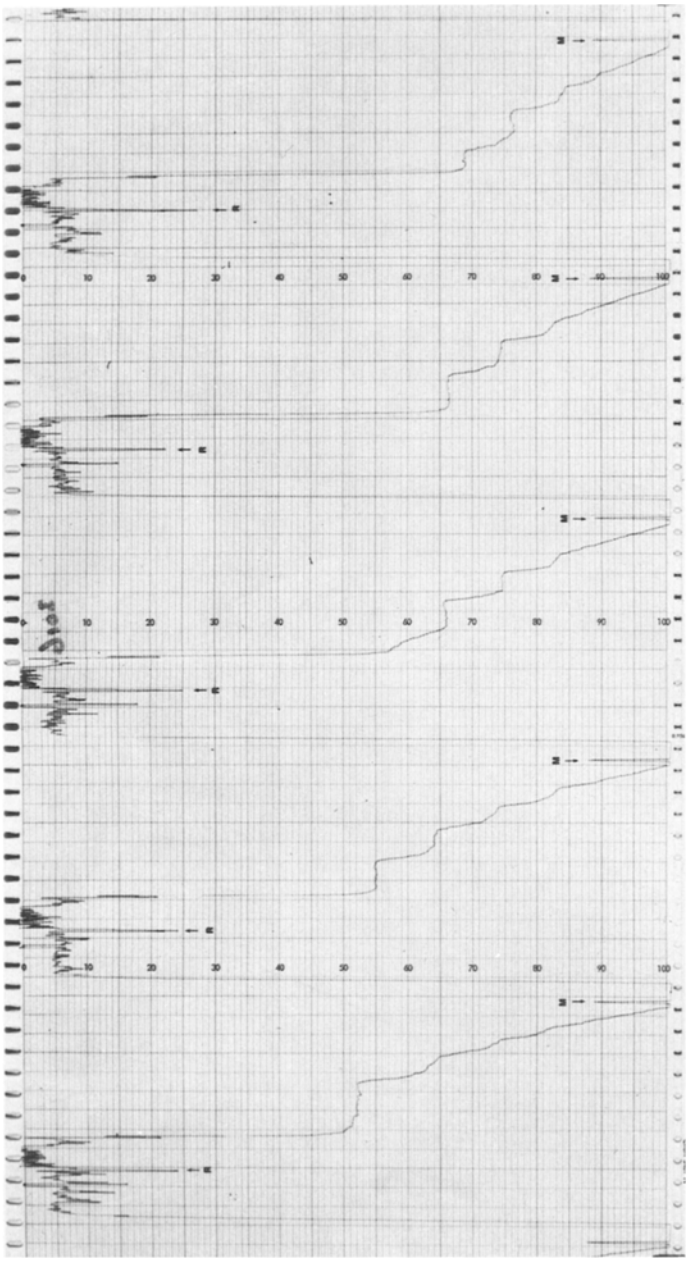

Fig. 8. Sedimentation analysis of plasma membranes in the analytical centrifuge. Plasma membranes were isolated as described by Van Blitterswijk *et al.* (1973), and analyzed in the analytical U.C. at 20 °C. R = reference point at 7.3 cm. M = meniscus. The first tracing (at the left) was made 3 min after reaching maximum speed (= 7000 rev/min). Interval between two tracings 90 sec

we were able to separate the membranes into various size classes. Electron microscopy, in addition, confirmed our findings.

Nevertheless, in order to exclude every possible form of misinterpretation of our experimental data we looked for materials that could serve as control.

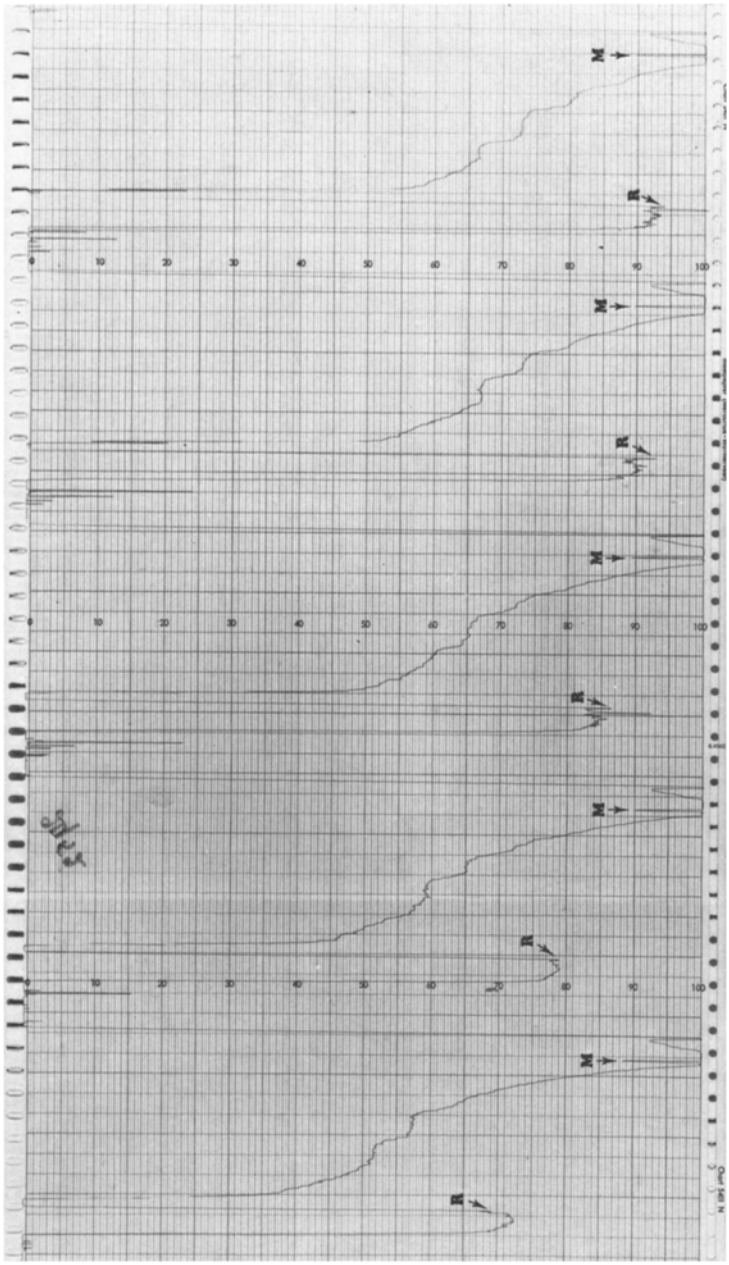

Fig. 9. Sedimentation analysis of liposomes in the analytical U.C. Liposomes were prepared as described under Materials and Methods and analyzed in the analytical U.C. at 20 °C. R = reference point at 7.3 cm. M = meniscus. The first tracing (at the left) was made 9 min after reaching maximum speed (= 8000 rev/min). Interval between two tracings was 90 sec

In order to test whether indeed this phenomenon was restricted to biomembranes we made artificial membranes (liposomes) and studied their sedimentation behavior in the analytical U.C. In Fig. 9 it is shown that also a suspension of artificial membranes showed a sedimentation

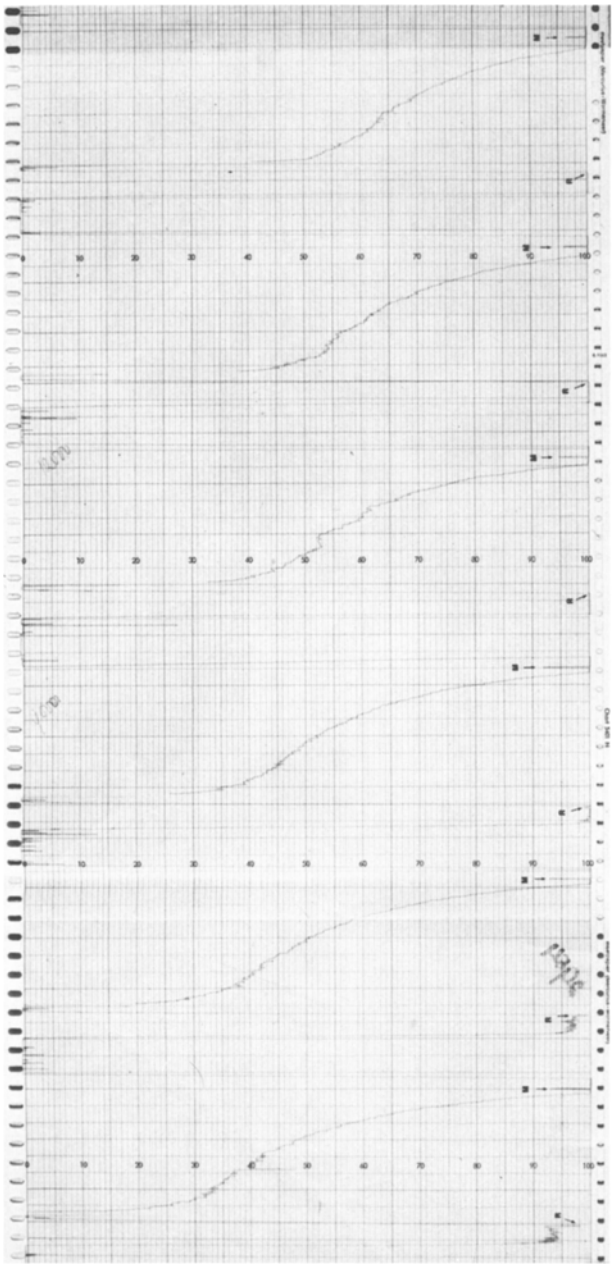

Fig. 10. Sedimentation profile of liposomes in the analytical U.C. Liposomes were prepared as described under Materials and Methods and analyzed in the analytical U.C. These liposomes were kept at 4 °C for one night and analyzed at 20 °C in the analytical U.C. R = reference point at 7.3 cm. M = meniscus. The first tracing (at the left) was made 7 min after reaching maximum speed (= 9000 rev/min). Interval between two tracings was 90 sec

profile characteristic for the presence of well-defined components. We concluded that the formation of well-defined classes of vesicles in biomembranes must be attributed to the phospholipid moiety. In Figs. 10 and 11 two other experiments with liposomes are shown. When a

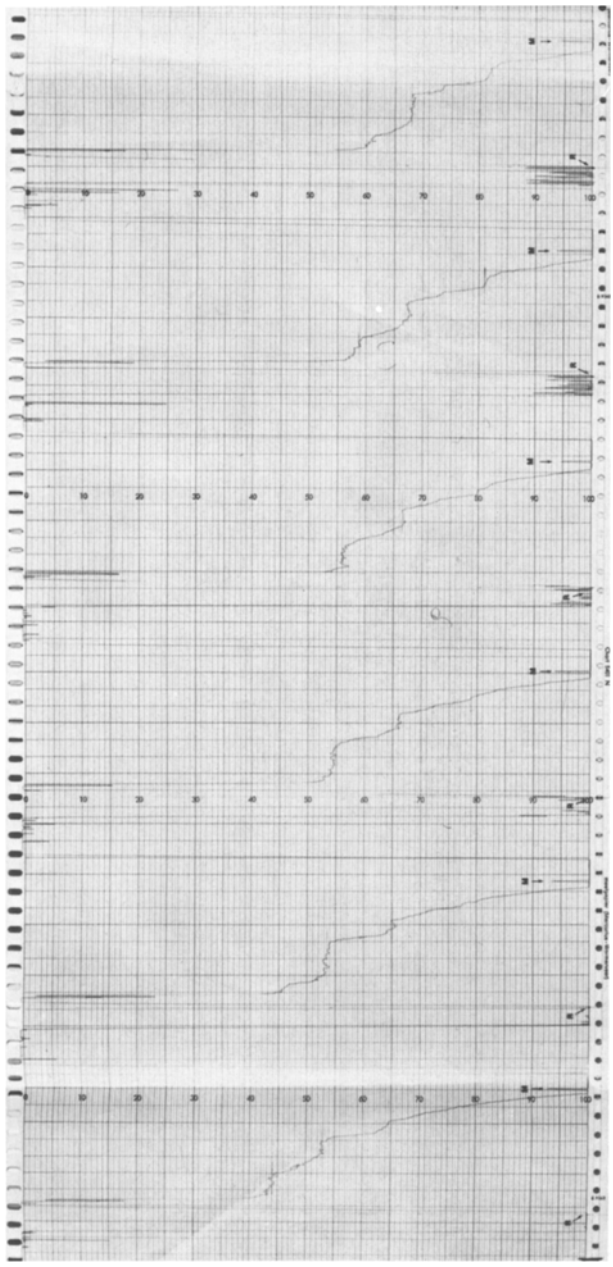

Fig. 11. Sedimentation analysis of liposomes in the analytical U.C. For this experiment an aliquot from the same preparation as used in Fig. 10 was analyzed. The preparation was kept at 4 °C for one night and was analyzed at 20 °C after standing for 3 hr at room temperature. All other experimental details were identical to those described in the legend to Fig. 10

preparation of liposomes, kept overnight at 4 °C, was subsequently analyzed at 20 °C in the analytical U.C., a continuous distribution of sizes was observed instead of a few well-defined classes of particles (*see* Fig. 10). When, however, the same preparation as shown in Fig. 10 was

Table 4. Sedimentation coefficients and radii of liposomes

No. of components	S values of Fig. 9	S values of Fig. 11	Average radius
1	956	962	1595
2	1267	–	
3	1684	1708	2121
4	2230	–	
5	3320	3114	2921
6	5135	4423	

The sedimentation coefficients of the components observed in Figs. 9 and 11 were calculated and with the aid of the equation $R=51.5\sqrt{S}$ (*see text*) the radius R was obtained. The R values for components 1, 3 and 5, respectively, are the average of the two series.

analyzed after a few hours standing at room temperature again a steplike sedimentation pattern was obtained (*see* Fig. 11). The experiments shown in Figs. 10 and 11 were performed with the same batch under identical conditions, i.e. at the same speed of rotation, at the same temperature and in the same time interval after reaching maximum speed.

The sedimentation coefficients for liposomes were determined and the results obtained from Figs. 9 and 11 were summarized in Table 4. If we assume the liposomes to be solid spheres, their radius R is related to the sedimentation coefficient by the equation

$$R^2=\frac{9}{2}\frac{\eta}{(d-1)}S.$$

This equation becomes

$$R=51.5\sqrt{S}.$$

R in Ångstrom (10^{-8} cm), $\eta=0.01$ poise; d, the density of liposomes $=1.017$ g/ml, S in Svedberg units (10^{-13} sec).

The obvious difference in sedimentation behavior as expressed for liposomes in Figs. 10 and 11 was often obtained for biomembranes, when suspensions of biomembranes were analyzed at higher speeds than that required for the results shown in the Figures.

Discussion

Membrane preparations that exist in the form of vesicles were examined in the analytical ultracentrifuge equipped with absorption

optics. When analyzing the tracings, we have to keep in mind that part of the absorbance of membranous material at 260 nm was due to light-scattering, since the absorbance diminished after the addition of a detergent, e.g. SDS, to the suspension. By the addition of detergent membrane vesicles are broken down into particles too small to give light scattering at 260 nm (Bont, Emmelot & Vaz Dias, 1969).

When complex structures like biomembranes are fragmented, one would expect a random distribution of sizes with a mean size dependent on the mode of preparation of the membranous material. With subtle isolation methods the mean size should correspond to very large particles; a rough isolation procedure should result in relatively small particles. The fact that fragments of nuclear membranes only existed in a few classes, that were characterized by well-defined sedimentation coefficients, posed the question whether this was a more general phenomenon of biomembranes. Fragmented biomembranes often exist in the form of vesicles, but especially preparations of plasma membranes isolated from tissue like liver do not consist of isolated vesicles but mainly of large sheets of membranes, interconnected by the various types of junctional complexes (Emmelot *et al.*, 1974). In the latter case we could not detect the steplike pattern, since the bulk material sedimented so rapidly, even at the lowest possible speed, that analysis was impossible. All preparations composed of vesicles showed the characteristic sedimentation behavior but sometimes only at a specific temperature interval. Each type of preparation had its own maximum speed above which the characteristic sedimentation profile was not observed. This maximum speed was inversely related to the S values. An explanation for this observation might be that the components sediment so rapidly that the movement of the sedimenting boundaries interferes with their registration by the scanner. Secondly, due to their relatively high sedimentation velocity, the vesicles might be deformed from a spherical shape to a more elongated form. Since at other temperatures and/or higher rotor speeds one would have failed to observe this phenomenon, it is impossible to state with certainty, that a preparation of membranes that does not show the characteristic sedimentation profile, is not composed of well-defined membrane fragments. When working at another rotor speed and/or temperature one might possibly find the characteristic sedimentation pattern. At the moment it cannot be excluded that under certain conditions indeed all suspensions of membranes show only a few classes of well-defined membrane particles. The occurrence of artifacts seems unlikely for the following reasons.

1. Each type of preparation shows his own characteristic sedimentation profile with typical sedimentation coefficients (*cf.* Table 1).

2. The membrane vesicles can be separated on sucrose gradients. The heavier fractions contain only components with high *S* values, the light fractions only low *S* values, and the intermediate fractions almost exclusively the intermediate *S* values. In addition the *S* values could be correlated with the results obtained with the electron microscope: the higher the *S* value the larger the particles.

It was therefore concluded that the sedimentation profile was not due to sedimentation artifacts. Each sedimenting component exists of a vesicle with definite mass. The more rapidly sedimenting components are not aggregates of smaller ones since electron-microscopic studies revealed that the heavier components did not contain aggregates of small vesicles. Now one could argue that biomembranes have a mosaic structure and when the membranes are desintegrated, rupture leaves certain fragments of the mosaic intact. A component with a definite sedimentation coefficient should then correspond to a certain part of the mosaic. Though the possibility that the fractions represent fragments of a mosaic structure is not ruled out, it would be a rather peculiar coincidence that the masses of these structures differ on average with a factor of 2. In addition, artificial membranes (liposomes) also exhibited a discontinuous size distribution and this again favors the assumption that the different vesicles do not represent various identical structures of a mosaic. There is, however, one major difference between biomembranes and liposomes that has to be taken into account. Vesicles of biomembranes can be considered as hollow spheres. Liposomes, on the other hand, are composed of concentric lamellae (Bangham & Horne, 1964), and can best be considered as solid spheres.

One could argue that every new shell that was added to the "onion" gave rise to a new component in the analytical centrifuge. In liposomes with charged lipid bilayers, these bilayers are separated by considerable water compartments. According to Bangham and Horne (1964) the mean width of the lipid layer is estimated to be 44.2 Å and of the water compartment 25.6 Å. Therefore, the total difference in radius between two liposomes differing with only one shell equals 69.8 Å, i.e. the sum of the two layers. Since the difference in radius is always much higher, one has to conclude that liposomes are also preferentially formed with certain sizes.

The control experiments with liposomes strengthened our conclusion that vesicles of biomembranes exist in only a few size classes. For a

Table 5. Diameters of membrane vesicles in preparations of fragmented membranes compared with granular vesicles in the intact cell

Source of vesicles	Number of component in Table 2									
	1	2	3		4	5	6	7	8	9
Fragmented membranes	442	688	948		1206	1720	2454	3496	4468	6374
Synaptic vesicles (Geffen & Ostberg, 1969)	443	688								
Synaptic vesicles (Bunge *et al.*, 1965)			950		1250					
Rat pituitary granular vesicles[a] (Costoff & McShaw, 1969)			TSH 850	ACTH 923	1415 FSH	LH 1475	STH 2410	LTH 3395		

[a] Values are the mean of the two values given for intact cells and isolated vesicles, respectively (*see* Costoff & McShaw, 1969).

definite conclusion whether or not the fractions observed in biomembranes represent fragments of a mosaic structure, detailed experiments on the composition of isolated membrane vesicles will be necessary.

The phenomenon was not restricted to the membranes from the rat, since plasma membranes from the calf and membranes of the endoplasmic reticulum from the chicken (experiments not shown) exhibited the same phenomenon.

The findings presented so far, prompted us to investigate whether in intact membranous components in a living cell, e.g. granular vesicles, a purely physical phenomenon is operative for the determination of their size. In order to investigate this aspect, we first multiplied the radius obtained in Table 2 with a factor of 2. The diameters obtained in this way were compared with the values for granular vesicles given in the literature. In Table 5 the results of this comparison are summarized. Not only that the various granular vesicles present in living cells are homogeneous in size (Bunge *et al.*, 1965; Costoff & McShaw, 1969; Geffen & Ostberg, 1969), but the absolute values of the diameters correspond with the diameters calculated by us in Table 2. From the striking similarity between our in vitro experiments and the in vivo situation, we concluded that also in the living cell a purely physical mechanism determines the size of vesicles. With the exception of FSH and LH from rat pituitary (Costoff & McShaw, 1969) all other granular vesicles corresponded

rather well with one of the nine components observed by us in fragmented membranes. Owing to the standard deviations in both measurements (centrifugation and electron microscopy, respectively) and owing to approximations made for the calculation of the radius from the sedimentation coefficients, the sometimes excellent agreement between certain components of these two series of data must be partly due to chance.

It is well known that neurotransmitters are released in quanta and that the amount of material in a quantum is determined by the size of the vesicle in which it is stored (Hall, 1970). In this context our results may indicate that not only for neurotransmitters but also for hormones the vesicle hypothesis might be applicable.

At present we cannot explain why the diameters of membrane vesicles form a geometric series. The results presented here, however, seem to indicate that the size of membrane vesicles is determined by purely physical factors.

We are grateful to Professor J.Th.G. Overbeek (Department of Physical Chemistry, University of Utrecht) for his advice and critical reading of the manuscript and to Professor S.A. Wouthuyzen (Department of Theoretical Physics, University of Amsterdam) and Dr. R.P.H. Rettschnick (Department of Physical Chemistry, University of Amsterdam) for fruitful discussions. Our thanks are also extended to Dr. C.A. Feltkamp for making the electron micrographs.

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