On the size-dependent disintegration of small unilamellar phosphatidylcholine vesicles in rat plasma

Evidence of complete loss of vesicle structure

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The destruction of small unilamellar egg phosphatidylcholine vesicles in rat plasma was monitored by measuring release of encapsulated ^{125}I -poly(vinylpyrrolidone) or carboxyfluorescein and by determining transfer of radiolabelled phosphatidylcholine to plasma lipoproteins by means of gel filtration. The susceptibility of the vesicles to the destructive action of plasma increased with decreasing vesicle size, as observed by incubating plasma with individual fractions constituting the small-vesicle peak on Sepharose CL-2B. This results in selective destruction of small vesicles when heterogeneous vesicle populations are incubated with plasma. Samples of homogeneous vesicle populations were incubated with a wide range of plasma concentrations, which resulted in extents of solute and phospholipid release ranging from 10 to 90%. When the extents of solute release were plotted against the extents of lipid release a linear, virtually 1:1, relationship was found, for both carboxyfluorescein and poly(vinylpyrrolidone) as the solute. This suggests that the release of solutes from small unilamellar phosphatidylcholine vesicles as a result of their interaction with plasma (lipo)proteins involves the total destruction of a fraction of the vesicles, the magnitude of which is determined by the vesicle:plasma ratio. Our results argue against a previously presented view suggesting that the interaction between such vesicles and plasma results in the formation of pores through which encapsulated solutes diffuse at M_r -dependent rates [Kirby & Gregoriadis (1981) *Biochem. J.* 199, 251–254]. The discrepancies between the two studies in observations as well as in interpretation are discussed.

The interaction of plasma with liposomes is of particular interest to those who envisage liposomes as a potential drug carrier system for systemic application. Several research groups have reported on this subject since the potentially destructive action of plasma on liposomes was first described to result from interaction with high-density lipoprotein by Krupp *et al.* (1976) and by ourselves (Scherphof *et al.*, 1978). The incorporation of cholesterol in the liposomal bilayer greatly improved the stability of the liposomes in plasma (Finkelstein & Weissman, 1979; Kirby *et al.*, 1980; Allen & Cleland, 1980; Damen *et al.*, 1981) but, nonetheless, the interest in liposome–plasma interactions remained. The increased plasma susceptibility of liposomes at the gel-to-liquid-crystalline phase transition (Scherphof *et al.*, 1979) led Yatvin

and colleagues to design a temperature-controlled drug release system (Yatvin *et al.*, 1978; Weinstein *et al.*, 1979). The incorporation of cholesterol into the liposomes, although effectively inhibiting solute release, could not entirely overcome phospholipid label release which was shown to be the result of an exchange mechanism operative between the liposomes and the high-density lipoprotein (Damen *et al.*, 1981). In attempts to elucidate further the mechanisms of interaction between liposomes and plasma lipoproteins we discovered (Damen *et al.*, 1980) and partially purified (Damen *et al.*, 1982) a protein factor from the non-lipoprotein fraction of plasma which acted as a stimulatory agent for both the exchange and net transfer of phospholipid between liposomes and high-density lipoprotein. A similar protein was recently par-

tially purified from plasma and characterized as a phospholipid exchange protein (Tall *et al.*, 1983). Recently we demonstrated that the exchange of phosphatidylcholine between cholesterol-rich unilamellar vesicles and high-density lipoprotein only involves the phospholipid in the outer monolayer of the liposomal membrane (Scherphof *et al.*, 1983). As regards the mechanism of plasma-induced solute release from cholesterol-free liposomes we have favoured the view that during physical contact between liposome and high-density lipoprotein the lipoprotein takes up the liposomal phospholipid, leading to an increase in lipoprotein size, termination of the existence of the liposome and, consequently, the complete release of aqueous liposomal contents (Scherphof *et al.*, 1980, 1984b). Kirby & Gregoriadis (1981) claimed to have evidence that the interaction of high-density lipoprotein with small unilamellar phosphatidylcholine vesicles causes the formation of pores in the liposomal membrane of a size allowing selective release of encapsulated solutes. In the present paper we report evidence in support of our initial point of view and provide alternative explanations for the results obtained by Kirby & Gregoriadis (1981).

Experimental

Egg phosphatidylcholine was obtained from Sigma. Carboxyfluorescein was purchased from Eastman-Kodak (Rochester, NY, U.S.A.) and purified on Sephadex LH-20 (Ralston *et al.*, 1981). Poly(vinylpyrrolidone), *M*_n 25 000–30 000, was purchased from Merck. ¹²⁵I-labelled poly(vinylpyrrolidone) and [¹⁴C]methyl iodide were from The Radiochemical Centre, Amersham. Phosphatidyl-[methyl-¹⁴C]choline was prepared by substituting one of the choline methyl groups in egg phosphatidylcholine with a radioactive methyl group as described by Stoffel (1975).

Heparinized plasma was obtained from starved female Wistar rats of 200–250 g and dialysed overnight against several changes of 0.15 M NaCl/10 mM-Tris/HCl, pH 7.8. Sepharoses and Sephadex were from Pharmacia, and Bio-Gel was from Bio-Rad.

Preparation of liposomes

A solution of 30–50 μmol of [¹⁴C]phosphatidylcholine and 0.06–0.1 μmol of α-tocopherol in 1–2 ml of benzene was lyophilized and the dry phospholipid powder was dispersed in 1 ml of 0.15 M NaCl buffered with 10 mM-Tris/HCl, pH 7.4 (NaCl/Tris) or in 100 mM-carboxyfluorescein. When, in addition, ¹²⁵I-poly(vinylpyrrolidone) was to be encapsulated, 45 mg of unlabelled polymer mixed with approx. 30 μCi of the labelled com-

pound (5 mg), was dissolved in the carboxyfluorescein-containing NaCl/Tris buffer. The lipid dispersion was sonicated in a sealed glass tube under nitrogen for 1–2 h at 4–10°C in a bath-type sonifier (Applied Sciences). Non-encapsulated solute was removed by gel filtration on Sephadex G-100. The pooled and almost optically clear void volume fractions were applied to a Sepharose CL-2B column (28 cm × 1.0 cm), eluted with NaCl/Tris, to obtain a preparation of small unilamellar phosphatidylcholine vesicles, free from multilamellar liposomes. If required, (pooled) column fractions were concentrated by ultrafiltration through Amicon PM-10 filters.

Incubations

Aliquots of individual or combined Sepharose column fractions, containing 1 μmol of phospholipid unless otherwise indicated, were mixed with various amounts of dialysed plasma and the final volume was made up to 1.0–2.0 ml with NaCl/Tris. This mixture was incubated for 30–60 min at 37°C and subsequently chromatographed on a Bio-Gel A-1.5m column (40 cm × 1 cm). The column fractions were assayed for ¹⁴C and, when appropriate, ¹²⁵I radioactivity, with a liquid-scintillation counter and a gamma counter, respectively. When carboxyfluorescein was encapsulated the fractions were also assayed for fluorescence after addition of 1% Triton X-100 (final concentration). Fluorescence was measured on a Perkin-Elmer MPF 43 spectrofluorimeter set at excitation and emission wavelengths of 490 and 520 nm, respectively. Release of radioactive or fluorescent solute and of radioactive phospholipid was estimated by comparing fluorescence intensities or radioactivities of the combined void-volume fractions (liposomes) with those in the included volume. Recoveries from the Bio-Gel columns were always better than 90%.

Results

In pilot experiments we observed that with a variety of liposome:plasma ratios the major release of both phospholipid and solute from the liposomes takes place almost instantaneously. Samples that were subjected to gel filtration immediately after mixing liposomes and plasma at 37°C yielded virtually the same extents of solute release and phospholipid transfer as samples that were incubated for up to 1 h. In order to avoid possible differences introduced by slight variations in the time required for the sample to penetrate into the column bed, we chose, nonetheless, routinely an incubation time of 30 min, knowing that then an equilibrium or steady-state situation had been attained.

The observations presented in Fig. 1 demon-

Fig. 1. Bulk uptake of liposomal phosphatidylcholine by plasma high-density lipoprotein

Three different samples ($\times$, $0.4 \mu\text{mol}$; $\bullet$, $1.5 \mu\text{mol}$; $\circ$, $6.0 \mu\text{mol}$ of liposomal lipid) of small unilamellar vesicles consisting of ^{14}C -labelled phosphatidylcholine were incubated for 1 h at 37°C in a final volume of 1.3 ml consisting of 0.9 ml of rat plasma and 0.4 ml of NaCl/Tris buffer, pH 7.4. The incubation mixtures were chromatographed on an Ultrogel AcA-34 column to separate the remaining liposomes from (high density) lipoproteins and the fractions were assayed for radioactivity.

Fig. 2. Heterogeneity of small unilamellar phosphatidylcholine vesicles

^{14}C Phosphatidylcholine liposomes containing 100 mM-carboxyfluorescein were prepared as described in the Experimental section. The sonicate was chromatographed on a Sepharose CL-2B column and the fractions were analysed for radioactivity content ($\times$) and fluorescence ($\bullet$) after addition of Triton X-100 (1% final concentration). $\circ$, Encapsulated volume (litre/mol of lipid).

strate the net loss of liposomal lipid that occurs when liposomes are incubated in plasma. Increasing amounts of liposomes, consisting of ^{14}C phosphatidylcholine, were incubated with a constant amount of plasma (0.9 ml). When only a small amount of liposomal lipid is present ($0.4 \mu\text{mol}$), as much as 90% of the radioactive phosphatidylcholine is transferred to the high-density lipoprotein under these conditions and the transferred phospholipid is eluted in the fractions in which the native high-density lipoprotein is also eluted (20–24). When the amounts of liposomal phospholipid are increased, the proportion of phospholipid that is transferred decreases but the absolute quantities transferred increase substantially. This apparently leads to a considerable increase in lipoprotein particle size as judged by the shift in elution volume of the second peak towards the void volume. Obviously, the lipoprotein particle acquires additional phospholipid at the expense of the liposomes.

One of the problems encountered in the work with liposomes is their size heterogeneity. Even the so-called small unilamellar vesicles obtained by gel

filtration following ultrasonication are not perfectly homogeneous. This is demonstrated in Fig. 2, which shows the elution profile of a carboxyfluorescein-containing ^{14}C phosphatidylcholine sonicate on a Sepharose CL-2B column. Nearly all radioactivity is in the small-liposome peak. The right-hand portion of this peak is reasonably homogeneous, the encapsulated volume varying from 0.21 litre/mol of lipid in fraction 15 to 0.19 litre/mol in fraction 19. The fractions in the left-hand portion of the peak, however, have encapsulated volumes varying from 0.65 litre/mol in fraction 10 to 0.24 litre/mol in fraction 14. This represents an almost two-fold difference in diameter. The fractions eluting more towards the void volume show steeply increasing encapsulated volumes, up to approx. 3 litre/mol in the void-volume fractions which contain a small proportion of multilamellar vesicles.

Since we previously found phospholipase susceptibility of small unilamellar vesicles to be dependent on the radius of curvature of the vesicles (Wilschut *et al.*, 1978) a similar size dependency was expected for the interaction with lipoprotein. Fig. 3 shows this to be a fact. When we incubated a

Fig. 3. Lipid and solute release from small unilamellar liposomes in plasma: dependence on liposome size

Phospholipid-labelled vesicles containing carboxyfluorescein were prepared as described in the Experimental section and chromatographed on Sepharose CL-2B. Samples (0.5 μ mol of phospholipid) obtained from separate fractions of the eluate constituting the second peak (fractions 10–17 in Fig. 2) were individually incubated for 60 min at 37°C with 0.8 ml of rat plasma supplemented with 0.4 ml of NaCl/Tris, pH 7.8. After the incubation the mixtures were chromatographed on Bio-Gel A-1.5 m and the fractions were assayed for radioactivity and fluorescence after addition of Triton X-100. The encapsulated volume of the liposomes was calculated from the ratio of fluorescence and radioactivity in the Sepharose fractions before plasma incubation. The percentages of carboxyfluorescein (○) and radioactivity (●) release were calculated as described in the Experimental section.

fixed sample of phospholipid of a number of individual fractions from the small-vesicle peak on Sepharose with a constant amount of plasma, the extent of phospholipid transfer from the liposomes to the high-density lipoprotein gradually declined with increasing liposome size, the latter being expressed as encapsulated volume per quantity of phospholipid. The gradual increase in liposomal stability with increasing liposome size is also reflected in solute retention. Release of encapsulated carboxyfluorescein during plasma incubations followed the same pattern as the release of phospholipid, as is also seen in Fig. 3. The similarity of lipid and solute release suggested to us that the mechanism of solute release involved the complete disintegration of the vesicle. In order to substantiate further this view we performed a large number of experiments in which liposomal lipid and plasma were incubated in widely varying ratios. High lipid-to-plasma ratios would presumably lead to relatively little liposome disintegration, while incubations with a large excess of plasma would result in massive disintegration. Both solute and phospholipid release were determined and plotted against each other (Fig. 4). The 32 experiments thus performed with carboxyfluorescein as a solute

Fig. 4. Correlation between solute and lipid release from small unilamellar liposomes in plasma

Liposomes labelled with [¹⁴C]phosphatidylcholine and containing carboxyfluorescein or [¹²⁵I]-poly(vinylpyrrolidone) as aqueous solutes were prepared as described in the Experimental section and chromatographed on Sepharose CL-2B. Samples (0.5 μ mol of phospholipid) of the pooled fractions constituting the descending portion of the second peak, with an encapsulated volume of 0.17–0.20 litre/mol, were incubated for 30 min at 37°C with 0.05–2.0 ml of rat plasma supplemented with 2.45–0.5 ml of NaCl/Tris, pH 7.8. After incubation, 2 ml portions of the incubation mixtures were chromatographed on a Bio-Gel A-1.5 m column and the fractions were assayed for radioactivities and fluorescence to determine the proportions of phospholipid and solute released from the liposomes. ●, Carboxyfluorescein; x, poly(vinylpyrrolidone).

reveal a linear relationship between loss of lipid and loss of solute with a slope of 47.5°. The 15 experiments with poly(vinylpyrrolidone) as a solute provided essentially the same result, only in this case the slope of the straight line was 44°.

Kirby & Gregoriadis (1981) reported that when liposomes containing only buffer were incubated with plasma in the presence of 200 mM-carboxyfluorescein, they could, by gel filtration, isolate liposomes from the incubation mixture that contained the fluorophore in concentrations that were somewhat higher than when the incubations were done in the absence of plasma. This observation was taken as evidence in support of the view that the vesicles, despite heavy losses of phospholipid, retain their overall structure in plasma. The phospholipid loss would, in the authors' opinion, give rise to the formation of pores. We did similar experiments and found essentially the same results (Table 1). We prepared liposomes exactly accord-

Table 1. *Effect of plasma on the permeation of carboxyfluorescein into preformed liposomes*

Small unilamellar liposomes were prepared as described by Kirby & Gregoriadis (1981) from [^{14}C]phosphatidylcholine. In six separate incubations, portions of 0.5 μmol of phospholipid were incubated for 30 min at 37°C in 1.2 ml of incubation medium composed of 0.4 ml of 300 mM-carboxyfluorescein and 0.8 ml of plasma or NaCl/Tris. A 1 ml sample of the incubated mixture was chromatographed on Sepharose CL-6B. Fractions were assayed for radioactivity and for fluorescence before and after addition of Triton X-100 (1% final concentration). Total liposome fluorescence was calculated by summation of the fluorescence (arbitrary units) of the separate liposome-containing fractions, before and after addition of Triton. Fluorescence latency was calculated as described by Kirby & Gregoriadis (1981).

Incubation no.	Medium	Pooled liposomes			Peak fraction			Radioactivity in pooled liposomes	
		Fluorescence		Latency (%)	Fluorescence		Latency (%)		
		- Triton	+ Triton		- Triton	+ Triton		(d.p.m.)	(% of control)
1	Buffer	3201	10455	69.4	402	2871	86.0	14170	100
2	Plasma	1058	3201	83.2	295	2391	87.7	4690	33.1
3	Buffer	2758	9680	71.5	609	4290	85.8	13510	100
4	Plasma	952	2758	76.9	330	1771	81.4	5350	33.9
5	Buffer	3019	7629	60.4	720	2805	74.4	14050	100
6	Plasma	1178	3940	70.1	528	2130	75.3	5564	39.6

ing to Kirby & Gregoriadis (1981), incubated them in 100 mM-carboxyfluorescein in presence or absence of 65% (v/v) rat plasma and subjected the incubation mixture to gel filtration on Sepharose CL-6B. Liposomes are eluted in or close to the void volume, well separated from high-density lipoprotein and free fluorophore. When examining the latency values for the pooled liposomes it becomes clear that for all three plasma samples, both for the control incubation in buffer and the incubation in plasma, a high degree of fluorescence latency is found, indicating that the fluorophore had entered the liposomes in high concentration. In all three cases the latency for the liposomes retrieved from the plasma incubation had a higher value than the control liposomes, also in line with the observations by Kirby & Gregoriadis (1981). The difference between control and plasma-incubated liposomes vanishes, however, when individual peak fractions from the Sepharose column are assayed. Whereas the average values for the pooled liposomes differed almost 10% in favour of the plasma-treated liposomes (67.1 versus 76.7%), the peak fraction of the plasma-treated liposomes even had a slightly lower average latency than that of the buffer-treated liposomes (81.4 versus 82.1%). We tend to explain the difference in latency on the basis of the higher plasma susceptibility of the relatively small liposomes (cf. Fig. 2). In the plasma incubations the smaller liposomes disappear more readily and thus the remaining liposomes have a larger average diameter than those in the original preparation. This has a favourable influence on the value found for the latency of the carboxyfluorescein fluorescence, since intermolecular quenching is slightly reduced in extremely small

compartments (Van Renswoude *et al.*, 1979). The higher latencies found in the plasma incubations do, therefore, not necessarily imply that in plasma the liposomes are more permeable to carboxyfluorescein than they are in buffer.

Another aspect of the results in Table 1 that should be emphasized is that only a fraction of the liposomes added to the plasma incubations actually survive. This becomes clear upon examination of the fluorescence intensities. Only one-third to one-half of the fluorescence intensity in the buffer incubations is recovered in the liposomes of the plasma incubations.

These values are compatible with the radioactivity measurements presented in the last two columns of the Table; only 30–40% of the total phospholipid radioactivity is recovered in the liposomes after incubation in plasma, the remainder having become associated with the high-density lipoprotein.

Discussion

In contrast to the exchange of phospholipid that is observed between cholesterol-rich liposomes and plasma high-density lipoprotein (Damen *et al.*, 1981) the transfer of phospholipid from pure phosphatidylcholine liposomes to plasma high-density lipoprotein represents a net loss of phospholipid from the liposomes and a concomitant gain of phospholipid by the lipoprotein (Kirby *et al.*, 1980; Kirby & Gregoriadis, 1981; Damen *et al.*, 1981). The increase in particle size of the lipoprotein during incubation with excess liposomes, as shown in Fig. 1, is compatible with this view.

The question is, however, what happens to the

liposomes after they have suffered such a serious loss of phospholipid? Gregoriadis and co-workers have favoured the formation of holes or pores in the liposomes and thus the maintenance of the liposome as a structural entity, possibly supported by the incorporation of plasma proteins plugging the holes (Kirby & Gregoriadis, 1981; Gregoriadis *et al.*, 1983). We, on the other hand, have suggested (Scherphof *et al.*, 1981) that pure phosphatidylcholine vesicles merely dissolve in plasma, i.e. they cease to exist as identifiable entities while all their phospholipid is accommodated by lipoprotein particles. The results presented in Fig. 4 of this paper provide evidence in support of this view. Firstly, we observed a nearly 1:1 relationship between solute and lipid release over the entire range from about 10 to about 90% release. In our opinion this fits best with the assumption that when a vesicle is successfully attacked by one or more lipoprotein particles all of its phospholipid is accommodated by the lipoprotein and all of its contents are released. If upon such an attack the vesicle lost only a fraction of its phospholipid, it would be difficult to understand why also precisely the same fraction of the encapsulated solute would escape before the pores formed were efficiently plugged by plasma proteins. Secondly, we observed the same phenomenon for the high- M_r solute poly(vinylpyrrolidone); this suggests that solute release is independent of particle size and this argues against pore formation. Although we found a slight difference between the slopes of the carboxyfluorescein and the poly(vinylpyrrolidone) lines, suggestive of a somewhat more extensive release for the fluorophore, this difference is not nearly as dramatic as that reported by Kirby & Gregoriadis (1981). The discrepancy between their results and ours is possibly due to a difference in poly(vinylpyrrolidone) concentrations used in the two studies. Assuming an encapsulated volume for small unilamellar phosphatidylcholine vesicles of 0.25 litre/mol of lipid and 4000 molecules/vesicle (Wilschut, 1982), it can be calculated that in the preparations used by Kirby & Gregoriadis (1981) less than 1 out of a 100 vesicles contained a molecule of the polymer, the remaining 99 being 'empty'. Since poly(vinylpyrrolidone), despite its apparent water solubility, is readily extracted with chloroform from an aqueous solution (G. L. Scherphof & H. Ellens, unpublished work) it is conceivable that, when present in extremely low molecular quantities, it tends to remain associated with high- M_r (lipoprotein) complexes eluting in the void volume of an Ultrogel AcA 34 column. The small difference between the two solutes, as observed by us, may also be caused by these lipophilic properties of the polymer. In addition, it is quite conceivable, in view of the difference in M_r , that there is a difference in the rates of

release of carboxyfluorescein and poly(vinylpyrrolidone) from vesicles which survived the destructive action of plasma. Such release may be stimulated by plasma factors labilizing the liposomal membrane structure; with large unilamellar phosphatidylcholine vesicles, we observed considerable plasma-induced carboxyfluorescein leakage without the occurrence of significant phospholipid release (Scherphof *et al.*, 1984a).

On thermodynamic considerations the existence of small phospholipid vesicles which have lost more than half, even up to 90%, of their phospholipid would seem unlikely. Since 20 nm is approximately the minimal size of a single-bilayer liposome (Huang, 1969), liposomes of that size cannot, conceivably, survive upon losing even a small fraction of their lipid. Larger liposomes on the other hand might shed significant proportions of their phospholipid and still remain in existence simply by adjusting their volume.

Our observations as presented in Fig. 4 are, however, difficult to reconcile with such a possibility, since loss of surface would not be expected to coincide precisely with an equivalent loss of volume, i.e. contents. It could be argued that loss of phospholipid surface is compensated for by incorporation of proteins; binding of plasma proteins to liposomes has indeed been observed (see Scherphof *et al.*, 1981, for a review). Again, however, it is difficult to envisage how this could explain that release of phospholipid and contents coincide so well, as shown in Fig. 4. Therefore, we favour the view that once a liposome has lost some of its phospholipid to a lipoprotein particle it will cease to exist. The remainder will either recombine with one or more remnants of other lipoprotein-attacked vesicles to form a new vesicle or each remnant will continue to lose lipid until all of it has been accommodated by lipoprotein particles. In either case one would expect all solute to escape from such vesicles; the former event would, however, lead to lower lipid release relative to solute release, which is contradictory to our observations. We tend to think, therefore, that the latter course of events applies, or at least dominates. Since at higher liposome:plasma ratios lipid and solute release remain limited, there is apparently a limited capacity of the lipoprotein to destroy liposomes. This is conveniently explained by the observation that the lipoprotein itself has a finite capacity to accommodate the lipid surplus (Scherphof *et al.*, 1984a). We would have to postulate that, as the lipoprotein becomes more and more saturated with liposomal phospholipid, its potency to attack new liposomes decreases. Attempts to demonstrate that this is true have been frustrated by the observation that the newly acquired phospholipid in or on the lipoprotein is readily susceptible to exchange with the

phospholipid of fresh liposomes. The capacity to cause release of liposomal contents was, however, found to be reduced under these conditions (G. Scherphof & H. Morselt, unpublished work).

Our results on the entrance of carboxy-fluorescein into 'empty' vesicles essentially confirm those of Kirby & Gregoriadis (1981). In the presence as well as in the absence of plasma, partially quenched fluorescence is observed in the vesicles recovered after incubation. The slightly stronger quenching in plasma-incubated vesicles is, however, interpreted by us to result at least in part from the selective disintegration by plasma of the smallest vesicles (with the lowest quenching). With homogeneous vesicle preparations there is no longer any difference between control and plasma incubations. It is noteworthy that, under conditions where the carboxyfluorescein is outside, the fluorophore apparently permeates readily through the liposomal membranes, while vesicles with the same concentration of encapsulated carboxy-fluorescein almost fully retain their contents for weeks. The consideration that in the former condition there is such a large excess of fluorophore that only a very minor fraction of it suffices to load the empty vesicles probably does not fully explain this difference.

Finally, we wish to emphasize that our results and interpretations only apply to cholesterol-free phosphatidylcholine vesicles. Cholesterol-rich (more than 30 mol%) vesicles react with plasma in a completely different way; solute release is largely suppressed and phospholipid released from the liposomes is substituted by phospholipid from the lipoprotein(s) (Damen *et al.*, 1981). This implies maintenance of the structural integrity of the vesicles, although a limited increase in permeability may occur as a result of interaction with plasma (lipo)proteins. With cholesterol contents lower than 30%, net phospholipid loss may result in a decrease of vesicle size, since the vesicles will initially be larger than cholesterol-free vesicles due to the presence of the cholesterol (Forge *et al.*, 1978).

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