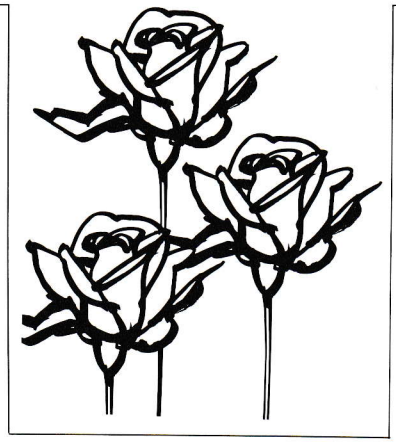

Liposomes

In Dermatological Preparations

Part II

By Hans Lautenschläger,
Nattermann Phospholipid GmbH, Köln, Federal Rep. of Germany

In Part I, some theoretical background on the structure of liposomes and their probable mechanisms of action on and in the skin were reviewed. Part II will discuss in more detail the utilization of liposomes in dermatological and cosmetic formulations. Appropriate uses are suggested, and some practical limits pointed out.

Dosages

The potential mechanisms of action described in Part I provide a background against which we may consider dose levels of phospholipids or liposomes in skin care preparations.

1. The dose level should not be too low if the depth of penetration is a fundamental concern. Some cosmetic liposomal gel preparations, which claim to have deep effects, are probably dosed too low for this to be possible.

2. The number and amounts of additives in a formulation should be kept as low as possible, so the effect is not reduced by interactions with other components of a formulation. This runs contrary to normal consumer habits, so appropriate factual information and advertising measures should be utilized to educate users.

For example, liposomes of highly unsaturated phospholipids in their "original formulations" with the viscosity of water could be applied onto the skin as sprays. In contrast to water, 10% dispersions are quickly absorbed and leave a slight cooling effect. The usual skin care measures (day cream, etc.) then can follow in a second step.

3. Care must be taken to ensure that deep-penetrating liposomal formulations are as free as possible from additives that have not been subjected to adequate toxicological testing, because the phospholipids in liposomes exert a pentration-enhancing effect.

4. If good distribution of an active agent is required, which is to be limited to the horny layer, then sphingolipids are probably suitable lipid raw materials.[28] This also applies to niosomes that contain saturated alkyl groups. Niosomes, like phospholipid-containing liposomes, are characterized by excellent distribution within the horny layer, as can be demonstrated with the aid of fluorescence microscopy.[6,29]

5. It should be expected that the cosmetic effect may differ considerably from individual to individual. Considering the mechanism of action discussed, a normal or oily skin will, for example, react quite differently from a dry, lipid-deficient skin. Here, from the manufacturer's point of view, it is important to recommend a qualitatively and quantitatively suitable product to individual consumers and to include an appropriate information leaflet.

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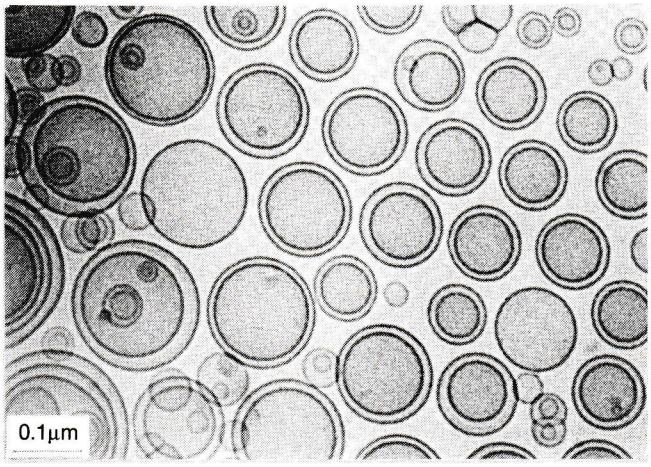

Photo 1. Cryofixed liposomes: 1% dispersion

Loaded Liposomes

"Loaded" liposomes differ from "empty" liposomes by containing cosmetically or dermatologically active agents. When used for topical application, the effects of loaded liposomes are those of both the phospholipids and their "load."

As has already been mentioned, the storage of actives occurs at either of two sites: in the aqueous interior of the liposome (water-soluble substances) and in the membrane (lipophilic and amphiphilic substances). Experience has revealed that loading the membranes is perhaps more interesting. Vitamin E, retinoids, steroids, other lipophilic and amphiphilic agents, and vegetable oils remain in the liposomes, largely uninfluenced by admixture of the liposomes with other components of the formulation, such as water.

In the case of water-soluble substances inside the liposomes, losses particularly of low molecular weight substances by leakage must be expected when water is added. However, this leakage can be counteracted by ensuring that the outside phase contains similar concentrations to those enclosed in the liposomes. This is, for instance, the practice in the case of the often-used water-soluble natural moisturizing factors (NMF).

It should be remembered in this context that even a 10% dispersion of liposomes represents a very tight packing of the liposome spheres, so that the outside phase amounts to only a third of the volume. Photo 1 shows an electron micrograph of a 1% liposome dispersion. Thus, separation from the water-soluble substances present in the outside phase is usually unnecessary, and also too expensive in most cosmetic and dermatological applications.

Effect of Temperature

The phase-transition temperature (gel-to-fluid phase transition) is an important criterion in the choice of a basic liposomal formulation. A low phase-transition temperature, such as occurs with unsaturated phospholipids, for example, also allows loading with temperature-sensitive actives such as retinoids, proteins, and enzymes.

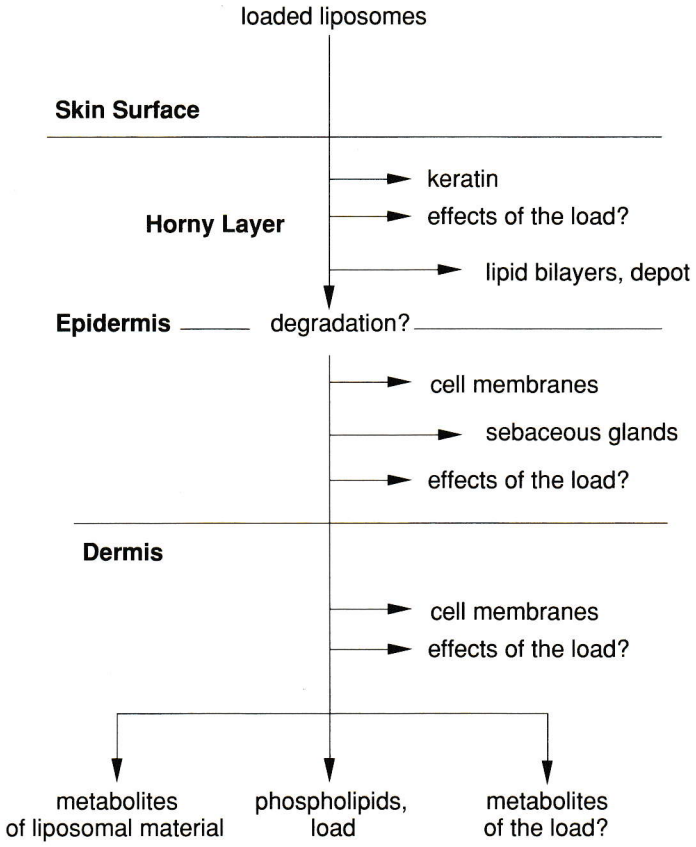

Figure 1. Potential effects of loaded liposomes in the skin (simplified schematic)

A high phase-transition temperature is required for hydrogenated or synthetic phospholipids. However, this high temperature limits the actives which may be included. In addition, actives stored in liposomes with high phase-transition temperatures are generally set free more slowly than those in liposomes with a lower phase-transition temperature.

Loaded liposomes, in addition to exerting their intrinsic effects, must transport their loads to the site where they are to exert their cosmetic or dermatological effects (Figure 1). If the horny layer is the target, then the distribution behavior in this layer has priority. If transport is to take place into a deeper layer of the skin, then the penetration-enhancing properties of the liposomes or of their major components the phospholipids have top priority.

It is presumed that the lipid bilayers of the horny layer are fluidized by phospholipids. In the case of steroids, retinoids, and similar actives which can exert their effects only after appropriate absorption, this is the most important prerequisite action, along with a certain depot effect of the horny layer. How far loaded liposomes actually penetrate into the living tissue is still unclear.

The penetration-enhancing effect of phospholipids has been confirmed in a study made in the Institute für Medizinische Balneologie und Klimatologie of the University of Munich.[30,31] In this study, a liposomal concentrate was loaded with monoclonal an-

tibodies (molecular weight: 20 to 60 kD) and applied to porcine skin in vivo. After 40 minutes, it was possible to demonstrate the presence of the antibody complex in both the dermis and the epidermis by specific coloration (APAAP method). The antibody alone cannot penetrate the skin.

Dermatological Applications

Currently, the most important groups of substances being investigated for the local treatment of dermatological disorders with the aid of liposomal systems are probably antimycotics, and topical antibiotics in general, corticosteroids and retinoids. Antiseptics should perhaps also be included here.

The first antimycotic, containing the active ingredient econazole, has been marketed since mid-1988 in Switzerland under the name Pevaryl-Lipogel.[32] The liposomal formulation and the same dose level of a four-fold higher concentration of active ingredient was measured in the horny layer in comparison with the normal cream formulation. The healing rates were significantly higher with the liposomal application.[33]

Similar results have been obtained for the liposomal application of hydrocortisone. The concentrations of active ingredient in the epidermis were 4 to 8 times higher and in the dermis 9 to 14 times higher than when the active ingredient was applied as a water-in-oil emulsion.[34] The administration of triamcinolone has led to comparable results.[35]

It is hoped that in the case of the retinoids—in particular vitamin A acid and its derivatives—liposomal preparations will allow a reduction in dose levels and, hence, an appreciable reduction in side effects. Indeed, with liposomal application of tretinoin, two-fold to three-fold higher concentrations of active ingredient are found in the epidermis and dermis than with normal cream preparations. Conversely, the levels found in the plasma and urine are higher on treatment with the cream.[36]

The interest in this group of substances is reflected by the large number of patent applications. The particular fields of application here are the treatment of acne and the so-called anti-aging preparations.

Empty liposomes may have a future as bath oils with dermatological properties. The fields of wound healing and, in particular, treatment of sun damage (sunburn) also must be mentioned.

Cosmetic Applications

The functional aims of cosmetics naturally are different from those of purely dermatological preparations. But it must be pointed out that the boundaries are very fluid, and the law requires a high degree of prophylaxis especially in the case of skin-care cosmetics.[41] This prophylaxis is very much in the

forefront in liposomal cosmetics, because the positive "side effects" of the liposome already discussed come to the aid of the cosmetic user. The following types of preparation are worthy of mention:

1. Skin-care preparations with empty or moisturizer-loaded liposomes, which reduce the transdermal water losses and, hence, are suitable for the treatment of dry skin. Further potential effects are skin smoothing and supplying linoleic acid to the sebaceous glands.
2. Liposomes loaded with other skin-care agents.
3. Sun-protection formulations with UV absorbers. Liposomal formulations would have the advantage here that the active ingredient would be distributed optimally in the horny layer, and also would acquire a certain "water resistance."
4. Liposomally-encapsulated radical scavengers and related substances of the vitamin E, superoxide dismutase (SOD) or flavonoid type. In this context, beta-carotene as $^1O_2$-quencher should be mentioned.
5. Liposomal formulations of tanning agents such as tyrosine,[42] etc.
6. Fitness frictions of the Franzbranntwein character, i.e. with inclusion of essential oils and possible omissions of alcohol from these formulations.
7. Aftershaves with added skin-care properties.
8. Very mild cleansing lotions that simultaneously provide a skin-care base. Liposomal formulations can, as is well-known, readily disperse oils and other fatty materials, but are, in contrast to other surface-active compounds, not aggressive.
9. Care rinses for the scalp and hair. The improved combing properties of the hair and the conditioning effect on the scalp are a result of the association of phospholipids and keratin.
10. The composition of oils for the treatment of maternal stretch marks, usually made up of natural triglycerides, lecithin and oil-soluble vitamins, reveals a possible application of oil and vitamin-containing liposomes. The depot capacity of highly unsaturated liposomes (up to 30% w/w lipid dry substance) means that a 10% dispersion of liposomes can contain up to 3% of an encapsulated oil. The percentage would naturally be less for hydrogenated liposomes.
11. Bath oils. The main impediment to using liposomes in bath oils is the higher cost of manufacture.
12. Lotions for use after bath and sauna.

Limits to Liposomal Dispersions

Formulation of liposomal dispersions is limited by compatibility with other components of the formulation. In this respect, important ingredients of formulations are tensides, ethanol, propylene glycol and cream ingredients.

Surface-active agents have a great effect on liposomes. There is, for example, the well-known phenomenon of large multilamellar (multi-shelled) liposomes being transformed via large unilamellar (single-shelled) liposomes to small unilamellar liposomes and finally to mixed micelles as a function of the concentration of surfactant.[43,47] For this reason, it is impossible to formulate liposomal liquid soaps or shampoos. However, the transformation of liposomes to mixed micelles is reversible. The dialysis method of preparing liposomes depends on this.

Surprisingly, some liposomes are relatively stable towards amphiphiles such as ethanol and propylene glycol. This is important for dermatologicals because liposomal dispersions constitute an excellent nutrient medium and are prone to the same bacterial contamination problems as oil-in-water emulsions. It is possible to add ethanol at concentrations of 10 to 20% and propylene glycol at the usual concentration of 5 to 10% as preservation additives. Without these additives it is almost impossible to preserve high concentration (5-10%) liposomal dispersions using acceptable levels of preservatives.

The problem of compatibility with surfactants crops up again and again, whenever liposomal dermatologicals are formulated as creams. It is often forgotten that the admixture of a stable emulsion with a liposomal dispersion that is in equilibrium necessarily brings about a nonequilibrium situation.

Exchange processes take place that often are, in the end, detrimental to the liposomes. These processes become particularly apparent during stress tests at

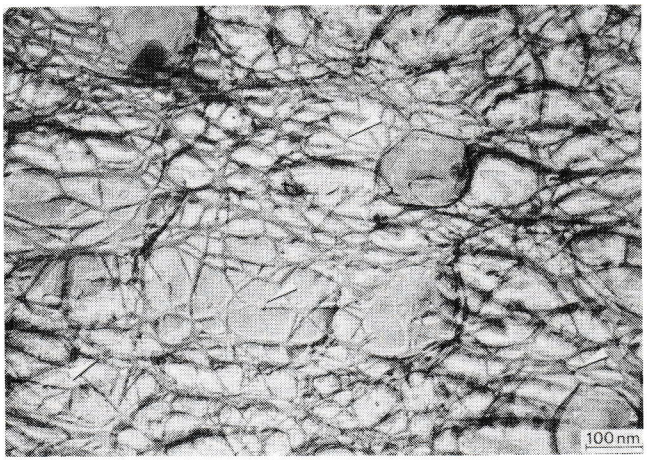

Photo 2. Electron micrograph of a liposomal skin-care gel after freeze-etching preparation[44]

elevated temperatures. Gel formulations are created much more readily, as may be demonstrated by various analytical methods, especially the electron microscope.[44] The electron micrograph (Photo 2) illustrates liposomes in a gel matrix of xanthan gum and aloe vera.

Often the question arises whether liposomes can be enriched with oils after their preparation. This has been done with niosomes and, for example, macadamia oil.[29] Also, water-insoluble lipid phases (including essential oils) disperse spontaneously on brief mixing with phospholipid-containing liposome concentrates.

At first sight, this process would seem to be very suitable for the loading of already produced liposomes with oils, but closer examination reveals some disadvantages. At the first contact of liposomes with the oil phase it must be expected that the liposomes will be destroyed. They initially form emulsion-like states in the oil-in-water interface regions which then form all possible intermediate states with other liposomes. In other words, the process is very difficult to reproduce and leads to inhomogeneous particles.

It should be borne in mind also that amphiphilic substances with their polar functional groups are only enriched in the outer layer of the liposomes and can lead to instability of the liposomes. In contrast, liposomes with a high leakage rate for low molecular, polar, water-soluble substances naturally can be loaded with such substances from the external phase. For this reason, it is always appropriate to allow for the same concentration internally and externally. Specific subsequent loading techniques, such as with the intermediate aid of detergents,[45] will not be dealt with here, because they are too complex for the formulation of topical preparations.

Adding Ingredients

This leads directly to another aspect of the formulation of liposomal dermatologicals—it is always necessary, after production of the liposomes, to add formulation ingredients and additives that have little or no effect on the action but are essential for later application and acceptance. These include preservatives, perfumes and aromatic oils, consistency regulators and active ingredients that are not to be encapsulated.

Here too, as in the case of oil-in-water emulsions, an equilibrium can be formed in which there can be a higher concentration of these substances at the surface of the liposomes. The problem of preservation, already discussed, is one of the consequences.

These interactions also can be demonstrated macroscopically with liposome concentrates and high molecular weight polyvalent substances, for instance proteins such as water-soluble collagen that associate with phospholipids. If such substances are incorporated from the start in oligo- or multilamellar vesicles, so that their binding sites are more or less saturated within the vesicle, then they yield dispersions with a viscosity similar to that of water. But if these substances are added to the completed liposomal dispersion, then, even though the chemical conditions are the same, the final product is so viscous that it no longer flows (Figure 2).

This is a very important aspect of the availability of liposomes in dermatological and cosmetic preparations. It must be expected that, if high molecular weight polyvalent substances are added after liposome formation, they will have deleterious ef-

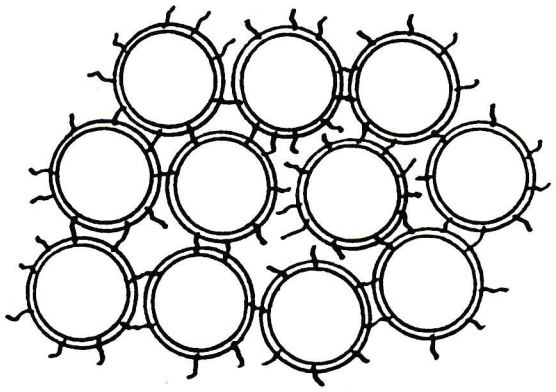

Figure 2. Crosslinking of liposomes by polyvalent components of the formulation (simplified)

Photo 3. Electron micrograph of a liposomal gel with 2% liposomes (calculated as a dry substance) and 5% dispersed wheat-germ oil after freeze etching

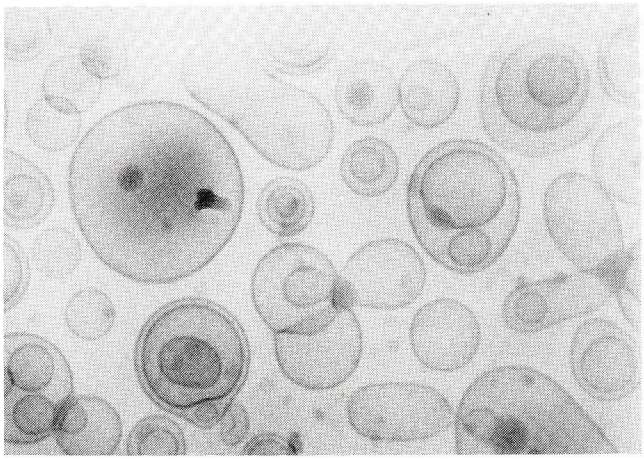

Photo 4. Cryofixed propeller-liposomes, 1% dispersion[47] (calculated as a dry substance)

fects on the penetration and transport properties of the liposomes.

Lipid-Rich Systems

Liposomal systems delivering lipids to the skin may be utilized in the following ways:

- 2-phase treatment,
- Lipids in classical liposomes,
- Liposome-emulsion systems,
- Lipid-containing gels,
- Propeller-liposomes, and
- Semiliposomal systems.

Compatibility of liposomes with surfactants, a problem already mentioned, is important when manufacturers desire to present consumers with the usual cream, but now enriched with liposomes. The consumer is accustomed to the feeling of a cream and has certain distrust of a colorless or even transparent liquid gel.

For this reason, some manufacturers of cosmetics suggest a two-phase treatment for their customers: application of a liposomal formulation followed by the usual day or night cream. This is certainly a good recommendation, particularly if care is taken that additives which may limit bioavailability of the liposomes are used sparingly, if at all in the liposomal preparation.

On the other hand, lipid-rich liposomal systems are of particular interest for cosmetics because of the good distribution (in the horny layer) of the "active" phospholipid ingredient.

The capacity of classical liposomes for lipids of the triglyceride type is not sufficient for skin care. With very few exceptions, the concentrations of liposomes do not exceed 1%; they are frequently lower. The dilution of a 10% liposome dispersion (calculated as a dry substance) which is loaded with 3% oil (with respect to the concentrate) to a 1% dispersion (calculated as a dry substance) in the end product, yields an oil concentration of only 0.3%.

Oil-in-water creams with very low proportions of emulsifiers have been described in the literature. For example, a liposome dispersion can be stirred into a cream base made up of 10% paraffin oil, 0.5% polysorbate, 0.5% sorbitan mono-oleate and 85% water without the liposomal system being destroyed.[46] The author has also stress-tested (37°C; −10/+20°C) various formulations of this type.

Instead of using a small amount of emulsifier, similar results can be obtained with a suitable gel former, such as certain polyacrylates (e.g. Carbomer 940, 1342). In these formulations, which have a cream-like consistency for oil concentrations of up to 10%, it is possible to avoid additional emulsifiers completely.

Such preparations are rapidly taken up by the skin, do not leave an oily glistening on the skin surface and make the skin soft and supple. The acceptance by the skin is extraordinarily good. Liposomes can be seen in an electron micrograph alongside the oily regions of such a formulation (Photo 3).

A further possibility for achieving lipid-rich liposomal systems involves specific mixtures of naturally occurring vegetable phospholipids that are able to stabilize oil at a proportion by weight of 1:1, yielding a controllable particle size of 100 to 400 nm on average. However, such vesicles no longer take up

the typical spherical shape, but are more reminiscent of a propeller, in which a tiny droplet of oil is to be found in the center (Photo 4).[47]

Whether this type of liposome is an ideal base for lipid skin care is still open to question. They are likely, however, to be ideal carriers for lipid-soluble cosmetic and pharmaceutical agents. In the ideal case, these formulations have the advantage of not requiring the use of emulsifying or gel-forming additives, so mobility of the vesicle is not restricted.

Another interesting liposomal lipid delivering system is a "semiliposomal" product. This product consists of classical liposomes, propeller-liposomes and emulsified oils in a varied mixture. The mixture, which is very non-homogeneous from the electron microscopic point of view, is prepared using a mixture of pure phosphatidylcholine (about 50%) and a native oil (about 50%). The compound is homogenized into water, and the formulation is stabilized by a small amount of polyacrylate.

Because the membrane-forming agent is identical with the emulsifier of the emulsion particles, exchange processes cause no change in composition. The manufactured products show great stability at higher temperatures (40 °C). A look at the manufacturing process shows that a semiliposomal final state can also be reached from a non-liposomal starting material as well as a starting mixture of emulsion and liposomal dispersion.

compound → semiliposomal system ← emulsion + liposomes
(starting material) (final state) (starting mixture)

Chemical Stability

The molecular structure of niosomes is characterized by ether linkages. Therefore, it is very inert towards hydrolytic influences and largely independent of the pH of the formulation. By contrast, phospholipid-containing liposomes have, on account of the ester linkages present, considerable hydrolytic degradation in the region of pH less than 5 and pH greater than 8. This is not unexpected, because the phospholipid molecule is a phase-transfer catalyst.

Experience has shown that the problem is not a large one since dermatologicals usually have a pH between six and seven but it must, nevertheless, be borne in mind. The rate of hydrolysis depends on the type and concentration of other components of the formulation. This point must be tested individually.

The sensitivity of natural phospholipids to oxidative influences is often used as an argument against their use. The experience obtained with native oils, which also oxidize easily but which are finding more and more use at the moment shows that in the cosmetic field, the active ingredient idea is spreading more and more.[48] The rapid degradation typical of a natural product (after the container has been opened) is accepted as "natural" by consumers.

Then again, formulations can be stabilized by "vitaminization" with vitamin E, vitamin C and vitamin A and their derivatives, either alone or in combinations. These vitamins are themselves valuable cosmetic agents, and can be used as active dermatological ingredients,[49] working synergistically with the phospholipids.[50]

Of course synthetic antioxidants such as BHA and BHT also can be used. An alternative or additional strategy, particularly for cosmetics, is to fill them into dispensers or tubes and, if necessary, to include an expiration date. But niosomes and liposomes with saturated fatty acids formulated together with, say, cholesterol should also be protected by antioxidants, because peroxides can build up in these formulations.

Safety of Liposomal Raw Materials

Here, liposomal raw materials manufactured exclusively from natural phospholipids are in a particularly favorable situation because most experience has been obtained with them. Some of these materials are used in large quantities in the food, diet food, cosmetics and pharmaceutical industries in nonliposomal form, administered orally, topically and intravenously. No side effects are known, as explicitly stated in the monograph "Sojalecithin."[51]

On the contrary, the polyunsaturated fatty acids and the choline present in bonded form in the phospholipid molecule are necessary for human life—they are essential. Hydrogenated and synthetic phospholipids also are included in this classification, although the effect of the essential fatty acids is naturally absent here.

Other liposomal raw materials that are now known are unlikely to achieve the broad use of the phospholipids because of their chemical structures. This says nothing about their safety, but rather something about the fact that, when developing pharmaceutical products, account must be taken not only of the kinetics of an ingredient with which liposomes are loaded but also of the kinetics of an "additive" liposome and, if necessary, of other additives (stabilizers). It is of interest that niosomes, loaded with doxorubicin for instance, can be administered intravenously in animal experiments.[52]

Summary

In spite of leaving many questions open, I have attempted to provide a simplified overview of a very rapidly growing field in dermatology and cosmetics, and to put it into some sort of context. I would like to summarize some of the most important results concerning the field of liposomal dermatologicals and cosmetics.

1. Liposomes are a multi-faceted topic, which cannot be covered via a routine formulation. But this is the cause of the fascination of this topic for a formulator. He or she is consistently confronted with new questions again and again, and experiences unexpected surprises. For this reason it is not possible to consistently recommend one system or another, because each formulation has its limiting conditions.

2. Liposomes are formulations and active ingredients in one.

3. The quality of the effect depends on the dose.

4. The liposomal potential is dependent on other ingredients in a formulation.

5. Liposomes have both a dermatological and a cosmetic justification.

6. The primary topic in the dialogue between raw material manufacturers and manufacturers of preparations must be the working out of criteria for the evaluation of liposome-containing dermatologicals and the setting of technological standards.

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Address correspondence to Dr. Hans Lautenschläger, Neusser Gasse 50, D-5024 Pulheim 3, Fed. Rep. of Germany.

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