unclear. Perhaps, the glycogen synthetase is more active in these areas, converting an appreciable quantity of glucose into glycogen. In the cerebellum, this contingency can be related to the low level of cyclic AMP[22].

The preceding hypothesis is also supported by the experiments with 5-hydroxytryptophan. In contrast to Blizard and Balkoski[13], who used tryptophan to prevent the MSO effect, we used 5-hydroxytryptophan, which can be easily converted to serotonin, the rate-limiting enzyme being tryptophan hydroxylase. This compound was associated with benserazide, a decarboxylase inhibitor, to prevent its peripheral degradation. In this case, MSO failed to induce a decrease in serotonin content. In the same time, the glycogen content did not increase.

Blizard and Balkoski[13] have suggested that a restriction in tryptophan availability can explain the decrease in serotonin level induced by MSO. In our hands, and as stated by Sellinger and Dietz[12], the decrease in tissue tryptophan level was insignificant and the remaining amount was very large as compared to that of serotonin in all the areas. MSO must rather exert its action on the synthesis of serotonin. In agreement with Sellinger and Dietz[12], we have observed that MSO alone had a little effect on 5-hydroxyindoleacetic acid content, some significant decrease being observed only with the high dose of 200 mg/kg in two areas. But, the treatment with 5-hydroxytryptophan led to a significant increase in the level of 5-hydroxyindoleacetic acid, suggesting a possible increase in the turnover of serotonin. This possible high turnover may be the sign of an augmented liberation of serotonin by neurons, even if the actual content of the amine did not appreciably increase. This effect may explain the decrease in glycogen content observed when using 5-hydroxytryptophan plus benserazide, since the astrocytes, which contain most polysaccharide[7], would be in a medium rich in serotonin. This idea is supported by the results in the striatum where there was no change in the levels of either 5-hydroxyindoleacetic acid or glycogen. Moreover, when MSO was associated with the two preceding drugs, 5-hydroxyindoleacetic acid levels were generally less high, and the glycogen decreases were very moderate and insignificant.

Further experiments may make it possible to describe more precisely the exact correlation between serotonin and glycogen metabolism under the effect of methionine sulfoximine.

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Retention of topical liposomal formulations on the cornea

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Summary. The ability of liposomes composed of different kinds of phospholipid materials to adhere to the surface of the cornea was studied in the rabbit. The liposomes were labelled with tracer amounts of an I^{125}-labelled phosphatidylethanolamine derivative and were instilled in 10 µl drops onto the cornea. The retention of radioactivity was monitored. The results show that liposomes containing positively charged phospholipids are better retained than an albumin control. Thus, it may be possible to develop a drug delivery liposome system which would permit long-term sustained release of ophthalmic drugs onto the cornea.
Key words. Rabbit cornea; retention; liposomes; drug delivery; ophthalmic drugs.

Topical administration of drugs to the cornea is a major route of administration to provide therapy for disorders of the external ocular surface and of inner structures of the anterior segment of the eye. The relatively short duration for an administered drop on the cornea has necessitated frequent use of topically applied drugs to achieve successful therapy for diseases such as glaucoma[1-5]. Since patient compliance in this kind of regimen is low, a major advance for ophthalmic therapy would be the development of a sustained topical ophthalmic drug delivery system. A second consequence of a drop-by-drop topical delivery system is that the peak drug concentration (directly after drop administration) may be high enough to cause significant systemic absorption and side effects[1,2,6,7].

Sustained therapy with low doses of drug has been shown to produce equal ophthalmic efficacy over time and reduced systemic side effects when compared to frequent topical use of single high dose drops[6]. Various systems (from simple gels to complex ocular inserts) are available to deliver such sustained therapy. However, all fall short of ideal for a variety of reasons. For example, a single application of a polymeric ocular insert can provide pilocarpine efficacy equivalent to approximately 18 h of frequent topical therapy with 2 % pilocarpine drops[1]. However, the delivery system is heavy; requires expert insertion into the eye; is difficult to retain in the eye; provides a sensation as a foreign particle; and has a high cost[8].

Liposomes have also been studied as an ocular drug reservoir which would be better tolerated but give similar efficacy characteristics as the ocular inserts[9,10]. The present experiments examined the ocular retention of liposomes composed of different percentage amounts of two long chain saturated phospholipids. Such a liposome formulation may provide the ideal topical ophthalmic drug delivery system if the enhanced retention can be coupled with the slow release of an encapsulated drug.

Methods and materials

New Zealand White rabbits (females, 4–5 kg, 1 year, Nitabell) were placed in a rabbit restrainer which held the unanesthetized animals in such a way that the eyes were accessible for instillation and gamma radiation counting. The animals were trained to sit quietly in the restrainer for 1 h. Trained animals were then used for the retention studies. The animals were placed in the restrainer and a lead shield placed over the nasal-lacrimal region of the nose and eye. A 4 cm diameter sodium iodide crystal detector was then placed over the eye such that the gamma radioactivity from the corneal surface was detected with a Nucleus Gamma Counter. The distance from the crystal to the eye was maintained constant by a plastic cup mounted on the front of the detector which permitted only a standard distance of 1 cm between detector and eye. The gamma activity was determined before, and at various times after instillation into the eye of 10 µl of the I^{125}-radiolabelled test material. Four animals were tested per formulation.

Calculation of retention success. The mean percentage retention at time zero gave an indication of the success with which the instilled 10-µl drop was retained in the tear fluid. The remaining percentage retention data (R) for each rabbit were fitted by least squares regression analysis (Sigmaplot software, Jandel Scientific Co.) to a monoexponential expression against time (t).

$$R = a\,e^{-bt}$$

The mean value of the exponential slope (b) was calculated for each group of animals, and compared between formulations as a loss coefficient.

Materials. The liposomal formulations were produced as previously described[10]. The lipids were labelled with an I^{125}-labelled phosphatidylethanolamine derivative, which was incorporated into the liposome bilayer according to the methods of Abra, Schreier and Szoka[11]. The following liposome formulations (with composition ratios) were tested:

DDOA:HPC (10:90; 20:80; 30:70 and 50:50);
BDSA:HPC:BHT (10:89:1; 15:84:1 and 20:79:1).

HPC	Hydrogenated phosphatidylcholine
DDOA	Dimethyldioctadecylammonium bromide
BHT	Butylated hydroxytoluene
BDSA	Benzyldimethylstearyl ammonium chloride

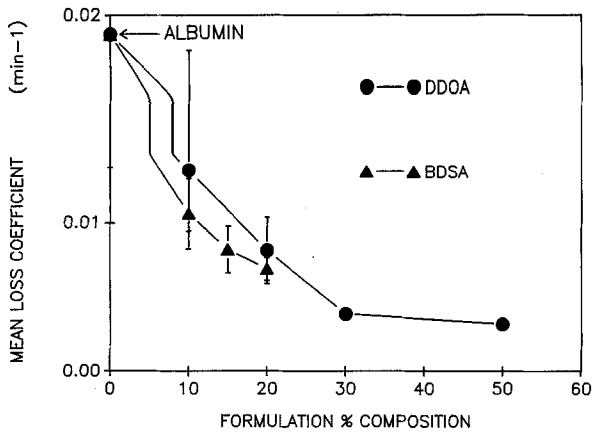
The results with these formulations were compared to the retention of I^{125}-labelled albumin:BHT (50:50).

Statistical analysis. A 5 % level of confidence was accepted as the lower limit for acceptance of significance throughout the study. The mean loss coefficient in the albumin experiments were compared to those in the liposome experiments using Student's t-test.

Results

Retention of I^{125}-labelled albumin. When 10 µl of the labelled albumin was instilled into the eye an immediate gamma count similar to the calibration standard was found (102 ± 8 %). This value rapidly declined to approximately 10 % after 60 min. The data from each individual experiment was fitted to a monoexponential curve with correlations of R against t which are all better than 0.9. The slope of the relationship varied from 0.007 to 0.031 min^{-1} (mean 0.019 ± 0.005). This mean value corresponds to a half time of 36 min.

Retention of liposome formulations. When liposomes were used, there was less variability and slower loss of the material from the eye. The figure shows the mean slopes of these monoexponential relationships. It is clear that formulations which contained DDOA (circles) or BDSA (triangles) were retained in the eye better than albumin. The decrease in the loss coefficient was related to the



The mean loss coefficients (i.e. slope of the relationship between % dose remaining vs time) with the albumin, DDOA liposomes, and BDSA liposomes are shown plotted against the relevant percentage composition of the lipid in the formulation.

relative proportion of the DDOA or BDSA in the formulation. DDOA at 10 % proportion produced no significant reduction when compared with albumin. However, progressive increase in the DDOA proportion to 20, 30 and 50 % significantly reduced the loss coefficient to 0.008 ± 0.002; 0.004 ± 0.001 and 0.003 ± 0.001 respectively. Similar results were found with increasing proportions of BDSA from 10 to 20 %.

Discussion

The liposome formulations showed consistently lower loss coefficients than found for albumin. The DDOA formulation provided maximal 'stickness' in the eye with the 30 % formulation. Above 30 % there was no further reduction in the rate of loss for the liposome from the eye, and below 30 % the rate of loss was increased towards that of albumin. These data suggest that a liposomal DDOA formulation might be chosen between 10 and 30 % which would be retained in the eye for a prolonged period. The BDSA results may be similarly compared to determine the effects of variations in composition. The loss coefficients show less variability than those found in the DDOA experiments, but are not significantly different from the DDOA at the same concentrations. In the present experiments the loss of the albumin would correlate to a T½ time of approximately 36 min. This was lengthened in a dose-dependent way by the liposome formulations, achieving nearly 4 h in the DDOA experiments and 2 h with the BDSA. The mechanism behind the adhesion of the liposomes containing DDOA or BDSA is not known at this time. It is clear that the mechanism is dependent on the amount of these lipids in the formulation and it is tempting to hypothesize that the explanation relates to the positive charge on the molecules and therefore the positive charge on the liposome. Other potential explanations include a liposome-induced impairment of ocular drainage, or the formation of loose chemical bonds between the BDSA or DDOA and the cell surface. However, at this time we have no evidence to decide between these possibilities. Whatever the mechanism, it is clear that the liposome is retained for an extended period on the cornea. The time duration would be sufficient to provide similar drug therapy as previously found with the more bulky gels and ocular insert device [1, 3]. However, the release kinetics of a suitable ophthalmic drug candidate from the liposome and the resulting ophthalmic efficacy remain to be established. In addition, the liposome materials themselves should be studied to determine if the ocular surface shows any adverse irritation reaction to the topic administration of this novel drug delivery system.

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