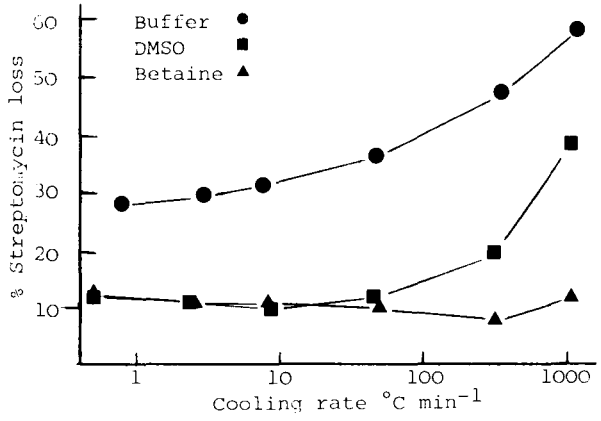
THE EVALUATION OF BETAINE AS A LIPOSOME CRYOPROTECTIVE

J. Higgins, N. Hodges, C. Olliff, A. Phillips*, Pharmacy Department, Brighton Polytechnic BN2 4GJ and *Merck Sharp & Dohme, Hoddesdon, Herts. EN11 9BU

Freezing and freeze-drying are possible means by which drug degradation and leakage from within liposomes may be retarded during their long-term storage. However, the cooling and warming processes may, themselves, result in membrane damage and drug leakage, and several agents have been employed as cryoprotectives to minimise this effect; of these, dimethylsulphoxide (DMSO) is probably the most widely used, but has the disadvantage that it may partially dissolve the phospholipid and so promote drug leakage prior to cooling (Strauss 1984). Betaine is a naturally occurring substance found to protect plant tissues from freezing stress (Coughlan & Heber 1982), and preliminary investigations demonstrated its cryoprotective effect in liposome systems (Higgins et al 1984). The purposes of this present work were to compare betaine with DMSO, and to determine the effect of liposome composition on betaine cryoprotection.

The preparation of multilamellar phosphatidylcholine (PC) liposomes and measurement of streptomycin leakage following freeze/thaw cycles has been described previously (Higgins et al 1984). Similar procedures were used to study streptomycin-containing liposomes in which 25 mole % cholesterol (chol), 10 mole % dicetylphosphate (DCP) or stearylamine (SA) were incorporated in the lipid phase. A summary of the results is given in the Figure and Table below which both show percentage streptomycin loss from liposomes following a freeze-thaw cycle in buffer or 8% w/v cryoprotective.



Liposome composition	Percentage streptomycin loss*	
	buffer alone	buffer + betaine
PC	29	11
PC + chol	50	14
PC+ DCP	23±3§	5
PC + SA	45	20±2§

*cooling rate 2.5°C min^{-1} warming rate approx 600°C min^{-1} Mean of two results except § where n = 6 for which 95% confidence limit is given.

When liposomes were suspended in 4 and 6% DMSO and betaine, plots qualitatively similar to that above were obtained, thus it is clear that under these conditions betaine is superior to DMSO. Incorporation of cholesterol in the liposome membrane increased streptomycin loss. Improved drug retention resulted from the use of negatively charged DCP, whereas SA having a similar charge to streptomycin, promoted drug leakage.

The performance of betaine under the above conditions, together with its lack of odour, established therapeutic use and low toxicity (Wilcken et al 1983) render the material worthy of further evaluation for the cryoprotection of both liposomes, and possibly mammalian cells.

Strauss, G. (1984) in Liposome Technology I (G,. Gregoriadis, Ed.) Chap. 15, CRC Press Inc., Florida
Coughlan, S.J. and Heber, U. (1982) Planta 156: 62-69
Higgins, J. et al (1984) J.Pharm.Pharmacol. 36: Supplement 24P
Wilcken, D.E.L. et al (1983) N.Eng.J.Med. 309: 448-453