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Short Communication

The in vitro incorporation and release of hydroxocobalamin by liposomes

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Many therapeutic agents, for example, anti-tumour drugs, enzymes and hormones, have been incorporated in liposomes and liposomal carriers have been investigated as potential drug delivery systems (Gregoriadis, 1976a and b; Tyrrell et al., 1976; Ryman et al., 1978; Ryman, 1979; Ryman and Tyrrell, 1979). The localization of liposomes in liver and spleen (Gregoriadis and Ryman, 1972a and b; Gregoriadis, 1973; Gregoriadis and Neerunjun, 1975) would appear to be of particular advantage for potent drugs acting specifically in these organs; the targetting might permit a reduction of the administered dose and with microgram requirements the amount of lipid carrier would be small. The anti-pernicious anaemia drug, hydroxocobalamin, which is given intramuscularly on account of its irregular and poor absorption from the gastrointestinal tract, and which is stored in the liver (Martindale, 1977), might advantageously be administered in liposomes. It is necessary firstly to determine to what extent it is incorporated and released in vitro.

We have used hydroxocobalamin as its hydrochloride and the synthetic lipids L-α-dipalmitoyl phosphatidylcholine (DPPC) and L-α-dimyristoyl phosphatidylcholine (DMPC), and for conferring charge on the liposomes, dicetyl phosphate and stearylamine (all from Sigma Chemicals). 10^{-4} moles of lipid, including 10 mol % of charged material where present, were dissolved in 25 ml chloroform and the solution evaporated to a dry thin film in a round-bottom flask on a 'Rotavapor' under vacuum at 48°C. 25 ml 0.01 M phosphate buffer, pH 7.0, containing 50 mg hydroxocobalamin was added to the flask with mild agitation forming hand-shaken multilamellar liposomes. This suspension was left for 96 h at 4–6°C, which ensured that the lipid was fully hydrated. The liposomes were separated by centrifugation at 136,000 *g* for 30 min and re-dispersed in 25 ml 0.01 M phosphate buffer. The centrifuging was repeated and the liposomes resuspended in 25 ml fresh buffer. The suspension was placed, in a stoppered glass tube, in a shaking water bath at 37°C. At zero time and subsequently at 1, 2 and then at 24 h intervals aliquots were centrifuged as before and the supernatants assayed at λ_{max} 354 nm. A calibration graph of hydroxocobalamin in 0.01 M phosphate buffer, pH 7.0, obeyed Beer–Lambert's law at 354 nm over the range 0–100 μg/ml. The entrapment per 2×10^{-5} moles lipid is given in Table 1 and the release of hydroxocobalamin is shown in Fig. 1. Similar results were obtained in duplicate experiments ($\bar{x} \pm <10\%$). The entrapment was determined by centrifuging 5 ml of sample at zero time, removing the supernatant and sonicating with 5 ml fresh buffer for 15 min in an ice bath. The liposome pellet obtained was now colourless and repeated sonication showed that no entrapped drug was left in the liposomes.

TABLE 1

HYDROXOCOBALAMIN [a] ENTRAPMENT BY 2×10^{-5} MOLES LIPID

Lipid	10 mol % charged species	Entrapment	
		μg	mg/mg%
Dipalmitoyl phosphatidylcholine (DPPC)	Nil	0	0
DPPC	Stearylamine	167.5	1.22
DPPC	Dicetyl phosphate	179.0	1.25
Dimyristoyl phosphatidylcholine (DMPC)	Nil	0	0
DMPC	Stearylamine	114.3	0.9
DMPC	Dicetyl phosphate	180.75	1.36
DPPC and DMPC (45 mol % of each)	Dicetyl phosphate	173.3	1.36

[a] 50 mg added initially to 10^{-4} moles lipid.

Negatively charged liposomes prepared with either DPPC or DMPC showed maximal entrapment to about the same extent, but the rate of release of hydroxocobalamin was much greater from the negatively charged DMPC liposomes. About 88% was released in 168 h in contrast with about 20% from the negatively charged DPPC liposomes. Release was more rapid with the DMPC than the DPPC liposomes presumably due to its lower phase transition temperature, 23.9°C as compared with 41°C, and thus greater bilayer

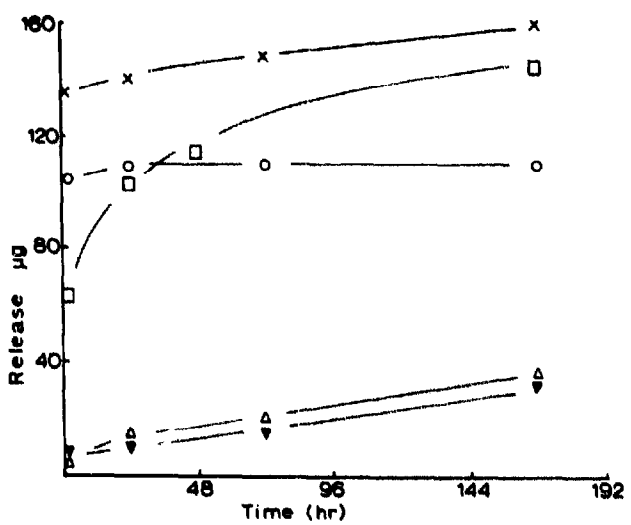

Fig. 1. Release of hydroxocobalamin from liposomes (37°C, pH 7.0) (μg per 2×10^{-5} M lipid). ×, dimyristoyl phosphatidylcholine (90 mol %) + dicetyl phosphate (10 mol %); ○, dimyristoyl phosphatidylcholine (90 mol %) + stearylamine (10 mol %); △, dipalmitoyl phosphatidylcholine (90 mol %) + dicetyl phosphate (10 mol %); ▼, dipalmitoyl phosphatidylcholine (90 mol %) + stearylamine (10 mol %); and □, dimyristoyl phosphatidylcholine (45 mol %) + dipalmitoyl phosphatidylcholine (45 mol %) + dicetyl phosphate (10 mol %).

fluidity and hence permeability at 37°C (Tyrrell et al., 1976; Knight and Shaw, 1979; Ryman and Tyrrell, 1979). When liposomes were prepared from a mixture of DMPC and DPPC (45 mol % of each) and dicetyl phosphate (10 mol %) the entrapment (mg/mg%) was unchanged but the release rate was decreased as compared with the DMPC and dicetyl phosphate liposomes (Table 1 and Fig. 1). It would thus appear to be possible to modify the release rate, as required, by altering the ratio of the phospholipids DMPC and DPPC. In contrast to charged liposomes, uncharged liposomes had negligible drug entrapment. The presence of a charged species electrostatically increases the spacing between the phospholipid bilayers causing an increase in the volume of the aqueous compartments and thus in the amount of drug entrapped (Bangham et al., 1967).

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