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The Effect of Organotin Compounds on the Permeability of Model Biological Membranes

William R. Cullen,* F. Geoffrey Herring and Basil U. Nwata
Environmental Chemistry Group, Department of Chemistry, University of British Columbia, Vancouver, B.C., Canada V6T 1Z1

The efflux of dimethylarsinic acid (DMA) from liposomes formed from egg phosphatidylcholine (EPC) is increased when tributyltin chloride (TBT) is added to the extraliposomal compartment; however the addition of monobutyltin trichloride (MBT) slows down the efflux. When the liposomes are prepared from EPC and organotin compounds, different mechanisms for DMA efflux seem to operate: TBT–EPC liposomes show a mixture of facilitated and passive diffusion; MBT–EPC liposomes show only passive diffusion. The facilitated diffusion of DMA^- seems to be stopped by the addition of TBT to the extraliposomal compartment.

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INTRODUCTION

This study is concerned with the effect of some organotin compounds, namely tributyltin chloride and monobutyltin trichloride, on model biological membranes, liposomes, formed by the hydration of egg phosphatidylcholine (EPC) or a mixture of organotin compound and EPC, in Tris buffer. The experiments were originally designed to study the permeation of these organotin compounds through liposomes; however, the liposomes do not form in the presence of ^{1}H NMR-detectable concentrations of organotin compounds. Therefore, the approach adopted was to use a molecular probe, dimethylarsinic acid (DMA), which is capable of easy permeation through the liposomes, to monitor the effects of low concentrations of organotin compounds on the permeability of these model biological membranes.

The permeation of dimethylarsinic acid (DMA) through EPC liposomes has been studied by Cullen and co-workers.[1–4] DMA has the following properties that make it suitable as a probe molecule for permeation studies.

(1) DMA permeates across EPC liposomes by passive diffusion.
(2) DMA has good aqueous solubility which enables high concentrations to be trapped in the small aqueous volumes of the liposomes. This in turn makes it easy to observe the NMR signals of DMA in the liposomes.
(3) The rate of efflux of DMA fom EPC liposomes is slow enough to permit its study by using NMR spectrometry.
(4) The methyl hydrogen atoms of DMA give rise to a simple NMR spectrum, a singlet, which can be shifted by using spectroscopic shift reagents.

Passive diffusion occurs when a concentration gradient exists across a membrane. Molecules move through the membrane because of thermal molecular motion.[5] The direction of transport is determined by the concentration gradient, and diffusion is in the direction of lower solute concentration, until the concentration on each side of the membrane is equalized. Passive diffusion obeys Fick's first law.

In general, the rate of diffusion is determined by the concentration difference across the membrane, by the molecular size of the permeant, by the viscosity and width of the membrane, and by

Correspondence to: William R. Cullen.
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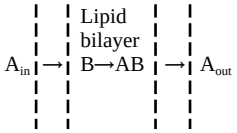
temperature. In passive diffusion, it is assumed that lipophilic solutes penetrate the membrane by dissolving in the hydrophobic layer and then diffusing across the bilayer, while hydrophilic solutes pass through aqueous pores in the membrane. A detailed description of passive diffusion has been given by Heinz.[6]

In facilitated diffusion, the transport of the permeant is aided by the presence of another molecule capable of acting as a carrier or capable of forming channels in the membrane. The direction of transport is along the concentration gradient and Fick's first law is not obeyed.[7] The mechanism of solute transport by facilitated diffusion is described in terms of two models.[5, 6, 8]

In the first model, the permeant moves across the membrane via channels which are transient pores formed in the membranes by ionophoric substances. Channels can show specificity for different permeants.

The second model postulates that a carrier molecule binds specifically to the permeant molecule on one side of the membrane barrier, then transports it through the barrier, releasing it at the other side. The carrier molecule is able to move freely within the bilayer without leaving it. A schematic diagram of the various steps involved in facilitated diffusion is shown in Scheme 1.

The pioneering work of Selwyn *et al.*[9, 10] showed that organotin compounds (trimethyltin, triethyltin, tripropyltin, tributyltin and triphenyltin species) partition into the membranes of mitochondria, liposomes, erythrocytes and chloroplasts, and mediate chloride–hydroxide transport across the membrane. A few other related organotin-induced properties of membranes have been described.[11–14] In the environment, the toxicity of other pollutants may be enhanced if organotin cations preferentially mediate their diffusion across biomembranes. Hence, the present study was planned to investigate further the effect of organotin compounds on the permeability of biomembranes. The selected probe permeant, dimethylarsinic acid, is also an environmentally occurring toxic compound.



Scheme 1 Schematic diagram of facilitated diffusion (efflux) mediated by a carrier: A is the permeant, B is the carrier molecule, AB is the carrier–permeant complex.

EXPERIMENTAL

Chemicals

Tributyltin chloride was purchased from Ventron (Alfa Inorganics): monobutyltin trichloride and deuterated 3-(trimethylsilyl)propionic acid sodium salt (2,2,3,3-d_4, TSP; Aldrich); dimethylarsinic acid (DMA; Fisher); tris(hydroxymethyl)aminomethane hydrochloride (Tris buffer; Sigma) and α-D(+)-glucose (Sigma); egg phosphatidylcholine (EPC; Avanti Polar Lipids, Birmingham, AL, USA). Solutions of Tris buffer were prepared by dissolving appropriate amounts in deionized water and adjusting the pH to 7.4 with sodium hydroxide. All solvents used for lipid extraction were Spectrograde. A stock solution of DMA (25 mg ml^{-1}) was prepared in Tris buffer (300 mM), and its pH was adjusted to 7.4 with sodium hydroxide solution. The organotin compounds were freshly dissolved in Tris buffer (40 mM), and their pH was adjusted to 7.4 with sodium hydroxide solution if necessary.

Preparation of large unilamellar vesicles (LUVs) from egg phosphatidylcholine (EPC) and the encapsulation of dimethylarsinic acid

A stock solution of EPC was prepared by dissolving EPC (1 g) in chloroform (10 ml). This stock solution was stored in the freezer until needed. The stock solution (2 ml) was pipetted into a test-tube, the solvent was evaporated off by using a gentle flow of nitrogen gas, and then the resulting paste was dried for 3 h on a vacuum line. Multilamellar vesicles (MLVs) were then prepared by adding 300 mM Tris buffer (1 ml) containing dimethylarsinic acid (25 mg ml^{-1}) at a pH of 7.4. The suspension was vortex-mixed for 5 min, and then subjected to five freeze–thaw cycles, according to the method of Meyer *et al.*[15] The sample was dipped in liquid nitrogen for about 2 min and thawed in a water bath (30 °C). The freeze–thawed vesicles were then forced, by pressure from a nitrogen tank (200–500 psi; 1380–3450 kPa), to pass through two stacked

200 nm pore-sized polycarbonate filters (Costar Corporation, Cambridge, MA, USA) in an extruder (Lipex Biomembranes Inc., Vancouver, Canada) to afford large unilamellar vesicles (LUVs). The LUVs were divided into two portions of about 0.4 ml each, to allow duplication of each DMA efflux experiment. For the DMA efflux experiments, the LUVs (0.4 ml) were applied onto a Sephadex G-50 gel permeation column (1.5 cm i.d. $\times$ 4 cm), pre-equilibrated in Tris buffer (40 mM, pH .7.4). Elution was achieved by the use of further 40 mM Tris buffer (pH 7.4). Upon application of the LUVs onto the gel permeation column, timing was initiated. Only about the first milliliter of the eluted LUVs was collected. An aliquot of the eluted LUVs (400 μl) was pipetted into the NMR tube (5 mm; Norell Inc.) which already contained the following: α-D(+)-glucose (28 mg), manganese sulfate (40 μl of 30 mM), TSP (25 μl of 40 mM), and Tris buffer (135 μL, 40 mM, pH 7.4). The amount of glucose added was calculated to approximately balance the osmotic pressure acting on the liposomes.

The time course for the efflux of DMA from the EPC LUVs was followed by acquiring NMR spectra at appropriate time intervals, until equilibrium was reached.

Experiments were performed on the liposomes in which DMA had been encapsulated, to determine

(1) efflux of encapsulated DMA from the liposome in the absence of any organotin compound; and
(2) efflux of encapsulated DMA from the liposome, with organotin compound (tributyltin chloride or monobutyltin trichloride) added to the extraliposomal compartment.

Preparation of butyltin–EPC LUVs and the encapsulation of DMA

The stock EPC solution (2 ml) in chloroform was pipetted into a test-tube together with aliquots of tributyltin chloride or monobutyltin trichloride (0.5, 1.5 or 5 μg ml^{-1}) in chloroform (1 ml), and vortex-mixed. The chloroform was evaporated off, and the butyltin–EPC mixture was dried on a vacuum line for 3 h. DMA (1 ml of 25 mg ml^{-1} solution) in Tris buffer (300 mM, pH 7.4) was added to the dried butyltin–EPC mixture and vortex-mixed for about 5 min, to achieve the encapsulation of DMA in the butyltin–EPC MLVs that were formed. The butyltin–EPC MLVs were forced to pass through two stacked 200 nm pore-sized polycarbonate filters in the extruder, as described above, to produce LUVs.

Butyltin–EPC liposomes of the following composition were prepared.

(a) 0.5 μg tributyltin chloride/0.2 g EPC (TBT–EPC A).
(b) 1.5 μg tributyltin chloride/0.2 g EPC (TBT–EPC B).
(c) 5.0 μg tributyltin chloride/0.2 g EPC (TBT–EPC C).
(d) 0.5 μg monobutyltin trichloride/0.2 g EPC (MBT–EPC A).
(e) 1.5 μg monobutyltin trichloride/0.2 g EPC (MBT–EPC B).

The butyltin–EPC LUVs were divided into two portions of about 0.4 ml each, to permit the duplication of each DMA efflux experiment. A portion of the butyltin–EPC liposomes (0.4 ml) was added to a Sephadex G-50 gel permeation column (1.5 cm i.d. $\times$ 3.0 cm) and eluted as described above. The first fraction (about 0.7 ml) of the eluted butyltin–EPC was collected. An aliquot of the eluted butyltin–EPC liposomes (400 μl) was quickly pipetted into the NMR tube, which already contained glucose (28 mg), aqueous manganese sulfate solution (40 μl of 30 mM solution), TSP (25 μl of 40 mM solution) and Tris buffer (135 μl of 40 mM solution, pH 7.4). When the presence of the same butyltin chloride used to form the liposome was desired in the extraliposomal compartment, 135 μl of a 16.7 μM solution in Tris buffer of the tin compound was added.

Experiments were performed on the butyltin–EPC liposomes to determine

(1) efflux of encapsulated MDA from the butyltin–EPC liposomes with no butyltin chloride added into the extraliposomal compartment; and
(2) efflux of DMA from the butyltin–EPC liposomes with tributyltin chloride or monobutyltin chloride added to the extraliposomal compartment.

NMR spectroscopy

A Bruker AM400 NMR spectrometer was operated in the water suppression mode. For each experiment involving the efflux of encapsulated DMA, 25–30 data points, collected over

11–17 h, were processed. Each data point represents an average of 48 scans. The accumulated free induction decays (FIDs) were Fourier-transformed with a line broadening of 10 Hz to produce the NMR spectra. In Fig. 1, peak A (sharp singlet) is assigned to the DMA inside the liposome. Peak B (broad singlet) is assigned to DMA that has diffused out of the liposomes: the peak has been broadened and shifted by the manganese sulfate, the spectroscopic shift/broadening reagent added to the NMR tube. All peaks were referenced to TSP (peak C). Either of the peaks could be used to monitor DMA efflux from the liposomes. However, it was convenient to monitor the decrease of the peak due to DMA remaining in the liposome, because the peak area was easier to obtain by integration. The peak due to the Tris buffer was used as an internal standard to nullify the effect of fluctuations in the instrument's operating parameters. The peak area ratios, DMA inside the liposome:Tris buffer, were calculated and plotted as a function of time to describe the efflux behavior of the DMA molecules. Each data point is a combined signal from the methyl resonance of the two species of DMA, namely DMAH and DMA, present in solution.[1]

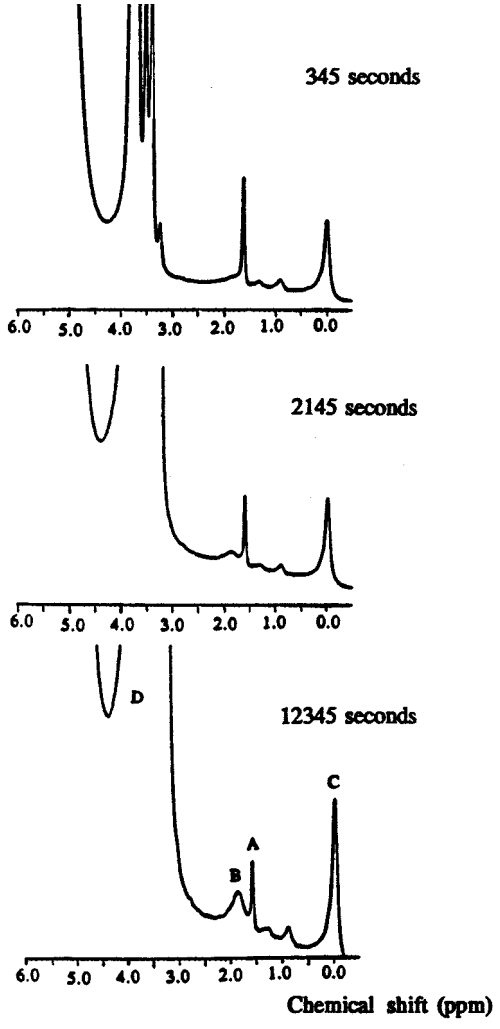


Figure 1 ^{1}H NMR spectra of DMA diffusing from EPC liposomes. Peaks A and B are due to DMA inside and outside the liposomes respectively. Peak D is due to Tris buffer. All peaks are referenced to TSP (peak C).

Treatment of data

The experimental data for each efflux experiment were analyzed for passive or facilitated diffusion by using Eqn [1] or [2] respectively. These equations were developed[16] from the work of Langmuir,[17] Widdas[18] and Hall and Baker.[19] A more precise treatment has been elaborated by Males *et al*.[20] but Eqns [1] and [2] are sufficient for the present purposes.

$$I^t_{in} = I^{eq}_{in} + (I^0_{in} - I^{eq}_{in}) \exp - \{(1+f)kt\} \quad [1]$$

$$I^t_{in} = I^{eq}_{in} + (I^0_{in} - I^{eq}_{in}) \exp - \left\{(1+f)\frac{\beta}{\phi}kt\right\} \quad [2]$$

A plot of $\ln(I^t_{in}/I^{eq}_{in})$ versus time gives a straight line with slope $-(1+f)k$ for passive diffusion, or $-(1+f)(\beta/\phi)k$ for facilitated diffusion, where f is the ratio of the internal volume to the external volume. At equilibrium $f = V_{in}/V_{out} = I^{eq}_{in}/I^{eq}_{out}$, where I^{eq}_{in} and I^{eq}_{out} are the peak integrals (area) due to DMA inside and outside the liposomes at equilibrium, respectively; β/ϕ is the ratio of the formation constant to the dissociation constant for the carrier–permeant complex. The total volume of reagents in the NMR tube for each experiment was 0.6 ml; therefore, $V_{in} + V_{out} = 0.6$ ml. Equation [1] was fitted to the experimental data points by an iterative procedure until convergence was obtained.[16] This was done by using the commercially available mathematical software Sigmaplot 5.0 (Jandel Scientific). The value of $(1+f)k$ is obtained from the plot of $\ln(I^t_{in}/I^{eq}_{in})$ vs t on the approach to equilibrium. The value of f is obtained from I^{eq}_{in}/I^{eq}_{out}; hence k can be calculated. The previously determined values of I^{eq}_{in}, f and k were kept constant while I^0_{in} was permitted to vary by about ± 0.005 units about the calcu-

lated value. A good fit of Eqn [1] to the experimental data points indicates efflux by passive diffusion, provided the magnitude of the efflux rate constants is in the range expected for passive diffusion. This provision is necessary because in some situations Eqn [1] can also fit data for facilitated diffusion.

If the experimental data points did not fit Eqn [1] they were analyzed for facilitated diffusion by using Eqn [2].

The NMR signal obtained is composed of methyl resonances from both DMAH and DMA^-, and the calculated values for the efflux rate constant k, and the permeation coefficient P, were corrected for the permeating species according to Eqns [3] and [4].

$$k = \alpha k' \quad [3]$$

$$P = \alpha P' \quad [4]$$

where α is the fraction of DMAH in solution, and is given by the relationship $\alpha = [H^+]/(K_a + [H^+])$ for a monoprotonic weak acid. For DMAH, α is 0.0593 at pH 7.4.[1] K_a is the dissociation constant; k' and P' are the corrected values for the permeating species DMAH. The fraction of DMA^- in solution is $1 - \alpha$, and has a value of 0.9407.

Determination of permeability coefficients

The permeability coefficients were calculated according to Eqn [5]:

$$P = \frac{kV_{in}/\text{mmol lipid}}{A} \quad [5]$$

where k (s^{-1}) is the efflux rate constant, V is the trap volume of the liposome per μmol of phospholipid, and A is area per μmol phospholipid, and has been calculated to be 1.81×10^3 cm^2 per μmol phospholipid.[1, 3, 21]

The lipid concentration (μmol phospholipid) was determined by phosphorus assay.[22, 23]

RESULTS AND DISCUSSION

Liposomes do not form in the presence of butyltin compounds at concentrations high enough to be detectable by using conventional ^1H NMR spectroscopy. Consequently the efflux of encapsulated butyltin compounds from liposomes cannot be conveniently studied by NMR spectroscopy. Therefore experiments were conducted to observe the efflux of encapsulated dimethylarsinic acid (DMA) from the liposomes in the presence and absence of the organotin compounds added to the extraliposomal compartment. At pH 7.4, DMA exists in solution as DMAH and DMA^-, and the ^1H NMR spectra reflect the combined proton resonances of the two species. Thus the figures presented in this work describe the efflux of DMA, while the tables of data are corrected for DMAH or DMA^- efflux. Under conditions of passive diffusion, the permeation of DMA^- is very slow[1, 4] and DMAH is the major species permeating out of the liposomes. Data for DMA^- are given only where it permeates by facilitated diffusion.

The time course for the efflux of DMA from EPC liposomes in the presence of tributyltin chloride in the extraliposomal compartment is shown in Fig. 2. The efflux behavior conforms with a first-order passive diffusion process and the data can be fitted by using Eqn [1]. Similar efflux behavior is shown when monobutyltin trichloride is added to the extraliposomal compartment (Fig. 3).

Calculated efflux data for DMAH from EPC liposomes in the absence and presence of organotin compounds are given in Table 1. In the presence of tributyltin chloride (33.2 μM) the permeation half-life of DMAH efflux becomes about 2.5 times smaller than in its absence, indicating an increased rate of permeation (data not shown). This is reflected in the permeability coefficient which is also about three times greater in the presence of tributyltin chloride. The efflux rate constant k'_{DMAH} also increases by more than two-fold. This increased permeability suggests that some properties of the EPC liposomes have been changed by the tributyltin chloride. According to Heywood *et al.*[12] the tributyltin cation can cause membrane disruption and rupture (lysis) of EPC liposomes. Such rupture or pore formation in the liposomal bilayer would result in the observed increased permeability to permeants.

The increased permeability of the EPC liposomes to DMA in the presence of tributyltin chloride could also arise if the tributyltin cation acted as a carrier and mediated the transport of DMA^- by facilitated diffusion. The ability of the tributyltin action to facilitate the diffusion of Cl^- and OH^- has been mentioned above.[9, 10, 14]

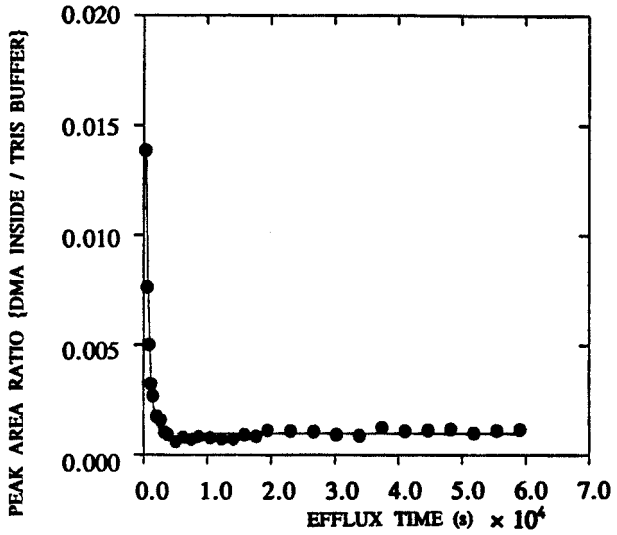


Figure 2 Time course for DMA efflux from EPC liposomes with tributyltin chloride added to the extra liposomal compartment. Data are fitted by using Eqn [1].

Unfortunately, Eqns [1] for passive diffusion and [2] for facilitated diffusion, as used in this study, are not capable of distinguishing between situtions where there is a 100% passive or facilitated diffusion.[16]

The efflux of DMAH is appreciably slower when $BuSnCl_3$ is in the external compartment (Table 1). The permeation half-life of DMAH is about three times larger, and the permeability coefficient is decreased by a factor of about 4.3. A probable mechanism for this retarded efflux is permeation of the tin compound into the lipid bilayer causing a decrease in the membrane fluidity. Compounds capable of decreasing membrane fluidity, such as cholesterol[24] and α-tocopherol,[25] are known to decrease permeability. A decrease in the membrane fluidity caused by dibutyltin dichloride has been observed on phosphatidylinositol 4-monophosphate and phosphatidylinositol 4,5-diphosphate

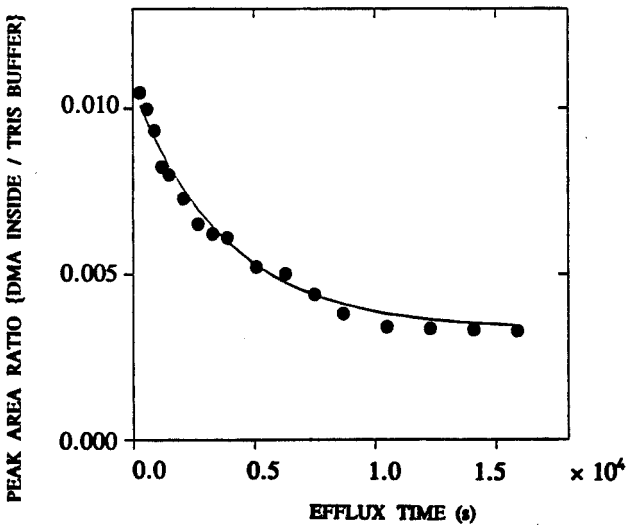


Figure 3 Time course for DMA efflux from EPC liposomes with monobutyltin trichloride added to the extraliposomal compartment. Data are fitted by using Eqn [1].

Table 1 Effect of external organotin compounds on the efflux of DMAH from EPC liposomes

External Sn compd	k'_{DMAH}	P'
None	$(1.1\pm0.04)\times10^{-2}$ [a]	$(1.7\pm0.2)\times10^{-8}$
Bu_3SnCl (33.2 μM)	$(2.8\pm0.1)\times10^{-2}$	$(4.9\pm1.0)\times10^{-8}$
$BuSnCl_3$ (33.2 μM)	$(3.7\pm0.1)\times10^{-3}$	$(4.0\pm1.2)\times10^{-9}$
Bu_3SnCl (8.3 μM)	$(0.8\pm0.1)\times10^{-2}$	$(1.3\pm0.1)\times10^{-8}$
Bu_3SnCl (16.7 μM)	$(1.5\pm0.02)\times10^{-2}$	$(1.6\pm0.1)\times10^{-8}$

[a] The value of $(0.97\pm0.15)\times10^{-2}$ was measured for the efflux in the presence of Hepes buffer.[3] Tris buffer was used in the present study.

vesicles.[26, 27] Unfortunately, there are no reports of the interaction of monobutyltin trichloride with liposomal membranes. However, from the data presented in Table 1, it seems that the monobutyltin species, unlike the tributyltin species, can neither induce membrane disruption nor act as carrier: hence, the observed retarded permeation of DMAH.

Tributyltin species degrade in the environment by progressive loss of butyl groups. These products are generally progressively less toxic, perhaps because debutylation leads to products less capable of causing membrane disruption.

The effect of tributyltin chloride concentration on the efflux of DMAH across EPC liposomes was studied and the results are also shown in Table 1. As the concentration of tributyltin chloride in the extraliposomal compartment is increased from 0 to 8.3 μM, there is an initial decrease in the efflux rate constant and the permeability coefficient, followed by an increase as the tributyltin concentration is raised from 8.3 to 33.2 μM. This increase indicates either that the liposomal membrane becomes more permeable to DMAH or that the tributyltin cation is facilitating the efflux of DMA^-. A plot of efflux rate constant versus tributyltin chloride concentration shows a linear relationship described by the equation $y=8.01\times10^{-4}x+1.46\times10^{-3}$; the regression coefficient is 0.9990. The data point corresponding to zero concentration of tributyltin chloride does not fall on the regression line, probably because tributyltin chloride substantially modifies the properties of the liposomes and the data effectively reflect two different types of liposomes. Nonlinearity could also result if different modes of transport of DMA exist for the liposomes that are in contact with tributyltin chloride and those not in contact with it, or if chloride/hydroxide exchange leads to a pH change in the liposomes.

The effect of increasing the concentration of external monobutyltin trichloride on the permeation of DMA across EPC liposomes is to decrease the permeability substantially upon addition, and there is no recovery on adding more tin compound (data not given).

Efflux of DMA from butyltin chloride–EPC liposomes

The efflux of DMA from liposomes prepared from mixtures of tributyltin chloride (TBT) and egg phosphatidylcholine (EPC) was studied to establish the permeability properties of these model membranes. In the first of these experiments no tributyltin chloride was added to the extraliposomal compartment. The liposomes were prepared as described in the Experimental section and are designated as TBT–EPC A, B or C liposomes, depending on the initial reagent composition. It should be noted that the actual tin content of the liposomes is unknown.

As an example, data for the efflux of DMA from TBT–EP C liposomes (5.0 μg TBTCl/0.2 g EPC) are plotted in Fig. 4 (normally such a plot is linear). These data can be fitted by a combination of Eqns [1] and [2] (Eqn [6]; M is the percentage contribution of passive diffusion to efflux, while N is the percentage contribution of facilitated diffusion to efflux as shown in Fig. 5. Thus the efflux of DMA from TBT–EPC C seems to proceed via a mixture of passive diffusion and facilitated diffusion. Facilitated diffusion is the major mode of DMA^- transport at the early stages of the efflux, while passive diffusion of DMAH dominates the later stages. At intermediate times, a mixture of facilitated diffusion and passsive diffusion is observed. Relevant data are listed in Table 2.

$$I'_{in}=M(I^{eq}_{in}+(I^0_{in}-I^{eq}_{in})\exp-\{(1+f)kt\})$$
$$+N\left(I^{eq}_{in}+(I^0_{in}-I^{eq}_{in})\exp-\left\{(1+f)\frac{\beta}{\phi}kt\right\}\right) \quad [6]$$

The efflux of DMA from TBT–EPC A (5.0 μg TBTCl 0.2 g EPC) and TBT–EPC B (1.5 μg TBTCl 0.2 g EPC) can also be accounted for by mixture of facilitated diffusion and passive diffusion (Table 2). It seem that as the concentration of tributyltin chloride in the liposome is

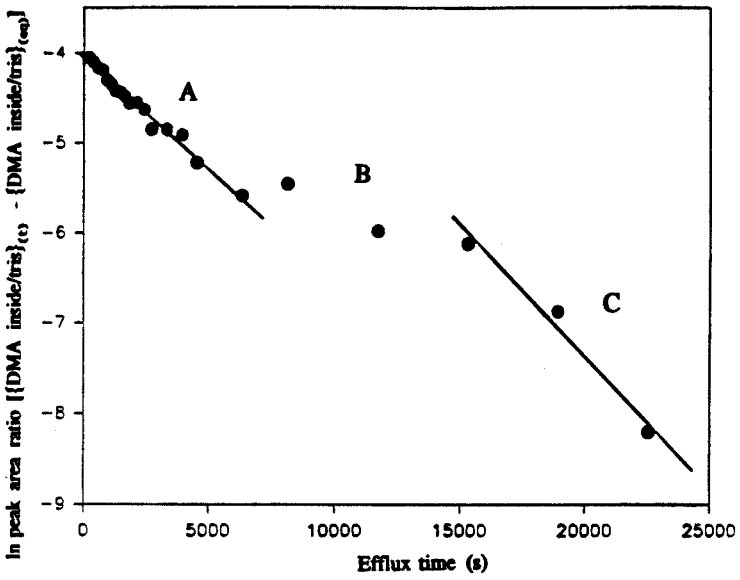


Figure 4 Log plot of DMA efflux from TBT–EPC C liposomes. A = region of mainly facilitated diffusion. C = region of mainly passive diffusion. B = region of mixed passive and facilitated diffusion.

increased, facilitated diffusion decreases while passive diffusion increases.

When tributyltin chloride (16.7 μM) is added to the extraliposomal compartment of TBT–EPC B liposomes, the DMA efflux data show normal behavior (Fig. 6) and the facilitated efflux pathway seems to be blocked, possibly because the external organotin compound suppresses the release of the TBT–DMA complex from the interface. The rate constant for the efflux of DMAH from TBT–EPC B in the presence of externally added tributyltin chloride is $(1.8 \pm 0.1)\times 10^{-2}$ s^{-1} ($P' = (2.8 \pm 0.5)\times 10^{-8}$ cm s^{-1}), while in the absence of externally added tributyltin chloride, it is 2.2×10^{-3} s^{-1} (Table 2).

Monobutyltin trichloride–EPC liposomes,

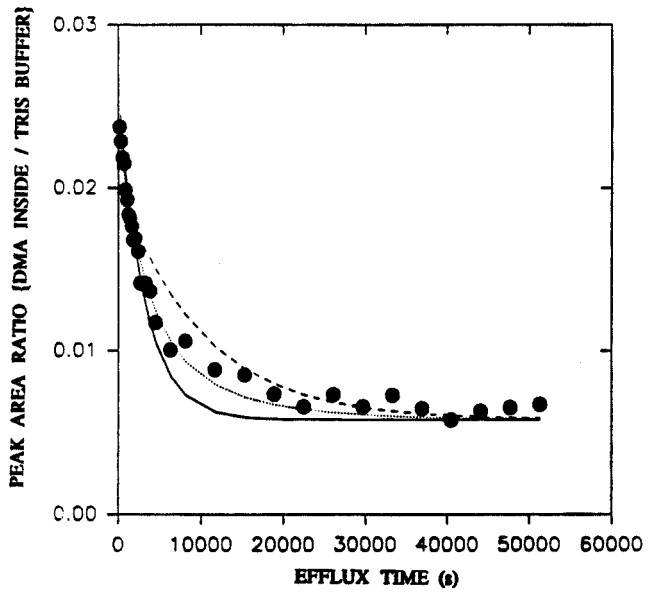


Figure 5 Time course for DMA efflux from TBT–EPC C liposomes. The data are the same as those shown in Fig. 4, fitted by using (– – –) Eqn [1], (——) Eqn [2] and (. . . .) Eqn [6].

Table 2. Diffusion parameters for the efflux of DMA from tributyltin–EPC liposomes interpreted as a mixture of passive and facilitated diffusion[a]

Liposome	$k'_{(DMA)}$ (s^{-1})	$k'_{(DMAH)}$ (s^{-1})	β/ϕ[b]	Facilitated diffusion (%)	Passive diffusion (%)
TBT–EPC A	2.0×10^{-4}	3.1×10^{-3}	2.2	66	34
TBT–EPC B	1.4×10^{-4}	2.2×10^{-3}	1.8	58	42
TBT–EPC C	1.1×10^{-4}	1.8×10^{-3}	2.2	54	46

[a] Tributyltin chloride was not added to the extraliposomal compartment.
[b] Mean value of $\beta/\phi = 2.1 \pm 0.2$.

designated MBT–EPC, were also used to study the permeation of DMA. Data for the efflux of DMA from MBT–EPC B (1.5 μg $MBTCl_3$/0.2 g EPC) with no monobutyltin trichloride in the extraliposomal aqueous compartment indicate that only one mode of diffusion is involved. The efflux parameters for DMAH, assuming passive diffusion, are shown in Table 3. In the presence of externally added monobutyltin trichloride (16.7 μM), again only one mode of DMA efflux is found (Table 3).

The permeability data for the MBT–EPC liposomes, either in the presence or absence of externally added monobutyltin trichloride, are essentially the same, and are similar to the values obtained for 'EPC only' liposomes. Therefore, monobutyltin trichloride does not have the ability to act as a carrier for DMA^-. It should be noted, however, that membrane permeability is greatly retarded if monobutyltin trichloride is added externally to the extraliposomal compartment.

In order to study the effect of the butyltin chloride content of the liposome on the permeability properties of TBT–EPC and MBT–EPC liposomes, experiments were conducted with tributyltin chloride (16.7 μM) added to the extraliposomal compartment of TBT–EPC liposomes, because under these conditions passive diffusion of DMA is the dominant pathway. Monobutyltin trichloride (16.7 μM) was also spiked into the extraliposomal compartment of MBT–EPC liposomes to maintain similar experimental conditions with MBT–EPC liposomes.

The data from these experiments are given in Table 4. As the tributyltin chloride concentration of the liposome is increased on going from TBT–EPC A to TBT–EPC B, the permeability of the

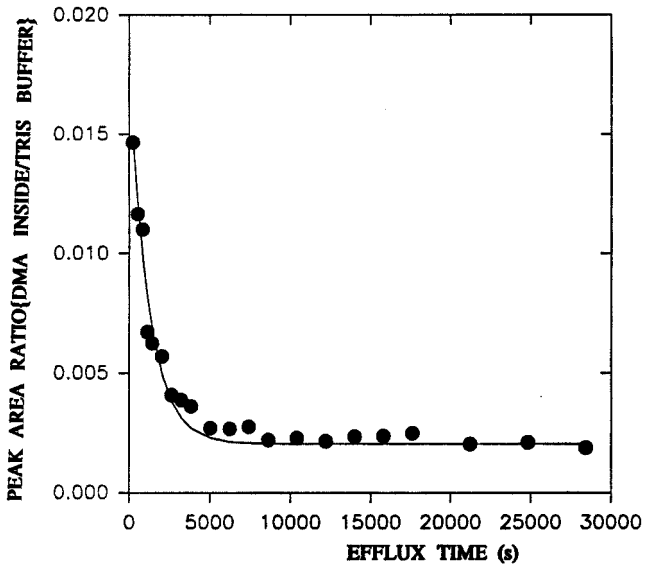


Figure 6 Time course for DMA efflux from TBT–EPC B liposomes with tributyltin chloride (16.7 μM) present in the extraliposomal compartment.

Table 3. Permeability data for efflux of DMAH from MBT–EPC B liposomes

Concn of $BuSnCl_3$ in extraliposomal compartment (μM)	Parameter	Value
0	k' (s^{-1})	$(8.3\pm0.4)\times10^{-3}$
0	P' (cm s^{-1})	$(1.7\pm0.1)\times10^{-8}$
16.7	P' (cm s^{-1})	$(1.7\pm0.1)\times10^{-8}$

liposomes to DMAH also increases by a factor of about 1.6 in accordance with a model invoking an increase in membrane disruption. For efflux from the MBT–EPC liposomes (with monobutyltin chloride present in the extraliposomal volume) the permeability coefficients and rate constants show very little variation with an increase in the monobutyltin trichloride composition of liposomes.

Clearly there is much to be learned from membrane permeation studies. The interaction of substances with membranes and their motion through membranes (or lack thereof) may contribute just as much to their toxicity to living organisms as, say, their binding to sulfhydryl groups on proteins inside the cell. The reader is directed to related studies on mercury species by Boudou and his co-workers, for example Refs 28, 29.

CONCLUSIONS

The present study shows that tributyltin chloride and monobutyltin chloride exert different and opposite effects on the model cell membranes. Tributyltin chloride makes the model membranes more permeable while monobutyltin trichloride makes them less leaky. This observation may account for the lower toxicity of monobutyltin trichloride.

The study also indicates that tributyltin cations are able to function as mobile carriers for dimethylarsinate, DMA^-, while monobutyltin cations lack this ability. It is probable that tributyltin cation can act as a carrier for a wide range of anions.

Table 4. Effect of butyltin chloride concentration of TBT–EPC and MBT–EPC liposomes on permeability[a]

Liposome	P' (cm s^{-1})	k' (s^{-1})
TBT–EPC A	$(1.7\pm0.2)\times10^{-8}$	$(1.1\pm0.1)\times10^{-2}$
TBT–EPC B	$(2.8\pm0.5)\times10^{-8}$	$(1.8\pm0.1)\times10^{-2}$
MBT–EPC A	$(4.9\pm0.8)\times10^{-8}$	$(3.6\pm0.1)\times10^{-2}$
MBT–EPC B	$(4.4\pm0.3)\times10^{-8}$	$(3.2\pm0.2)\times10^{-2}$

[a] TBT (16.7 μM) was added to the external liposomal compartment of the TBT liposomes and MBT (16.7 μM) to the MBT liposomes.

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