Ion-Pairing Interactions between ^{99M}Tc-Based Myocardial Imaging Agents and Oleic Acid

Charles M. Heard[1,2] and Jonathan Hadgraft[1]

Received June 19, 1995; accepted October 17, 1995

Purpose. The purpose was to test the hypothesis that ion-paired facilitated transport is of importance in successful myocardial uptake of cationic imaging complexes. In vitro ion-pairing interactions between oleic acid and seven cationic technetium-99m complexes based on the ligands 1,2-bis[bis(2-ethoxyethyl) phosphino ethane] (tetrofosmin), 1,2-bis(dimethyl phosphino ethane) (DMPE) and 1,2-bis(diethyl phosphino ethane) (DEPE) has been studied. The complexes studied were: $[^{99m}Tc\ O_2\ (tetrofosmin)_2]^+$ (commercially available as myocardial perfusion imaging kit, Myoview®), $[^{99m}Tc\ O_2\ (DMPE)_2]^+$, $[^{99m}Tc\ O_2\ (DEPE)_2]^+$, $[^{99m}Tc\ Cl_2\ (DMPE)_2]^+$, $[^{99m}Tc\ Cl_2\ (DEPE)_2]^+$, $[^{99m}Tc\ (DMPE)_3]^+$ and $[^{99m}Tc\ (DEPE)_3]^+$.

Methods. Ion-pairing interactions were monitored using a rotating diffusion cell containing a solid supported liquid membrane and by formation of lipid monolayers.

Results. Depletion of complex from the donor phase into an isopropyl myristate model membrane was generally in proportion to distribution coefficient and transfer to the receptor compartment was in all cases very small. However, by the inclusion of 5%w/v oleic acid, which is used in myocardial metabolism, partitioning was enhanced by amounts which varied depending on the tendency to form complex/oleate ion-pairs. Transfer to the receptor compartment was increased for most complexes when given sufficient time to diffuse through the membrane. The complex $[^{99m}Tc\ O_2\ (tetrofosmin)_2]^+$ appeared to form particularly stable ion-pairs with oleic acid. Monolayer formation also indicated ion-pairing interactions.

Conclusions. The results suggested that whether or not a complex is taken up by the myocyte may depend on its ability to 'hitch a ride' by ion-pairing with the myocytes energy source—a molecule of long chain fatty acid.

KEY WORDS: myocardial perfusion imaging agents; DMPE; DEPE; tetrofosmin; Myoview®; ion-pairing; monolayers; model membranes.

INTRODUCTION

Scintigraphic visualisation of the heart is a valuable tool in the diagnosis of ischaemia and the location of infarcted myocardial tissue. Until recently, the best available method for achieving this was by administering the radionuclide of thallium, $^{201}Tl^+$. For a number of reasons[1] it had been considered desirable to replace $^{201}Tl^+$ with the nuclide ^{99m}Tc, but unlike thallium ions which are taken up by myocyte cells, technetium ions are not cardioselective and a long-standing goal of radiopharmaceutical research had therefore been to improve targeting by complexation with appropriate ligands. Early work concentrated on positively charged ionic complexes as it is well established that cations can accumulate in the myocardium. Limited success was achieved using the ligand 1,2-bis(dimethyl phosphino)ethane (DMPE) which, by varying reaction conditions and the species present, is capable of producing a variety of stable cation core types,[2] which in turn demonstrate a range of imaging efficacy. However, the similar ligand 1,2-bis(diethyl phosphino)ethane (DEPE) demonstrated little cardiospecificity[3] when complexed to ^{99m}Tc. Of the many other structural analogues of DMPE to have been evaluated, ligands based on alkyl ether represented significant improvements.[4] In particular, 1,2-bis[bis(2-ethoxyethyl) phosphino]ethane has, in the form of the complex $[^{99m}Tc\ O_2\ (tetrofosmin)_2]^+$, demonstrated imaging properties at least as good as $^{201}Tl^{+5}$ and is now commercially available in kit form (Myoview®).

However, the mechanism for successful perfusion of the myocardium remains to be elucidated. As these compounds bear a single positive charge, an early hypothesis was that they were behaving as potassium analogues in the sodium-potassium-ATPase system. However, evidence was subsequently found to suggest that, where this was the case for $^{201}Tl^+$, it was not so for technetium complexes such as $[^{99m}Tc\ Cl_2\ (DMPE)_2]^{+6}$, where lipophilicity was claimed to be a major factor. Kinetic uptake has been studied using cell cultures and isolated hearts.[7] There have been few other reported mechanistic investigations into the uptake of such complexes into myocardial tissue using model systems.

Where a specific active transport mechanism is not in operation, the rate-limiting step in the uptake of a pharmaceutical by a biological membrane is generally considered to be interfacial transfer. The normal myocardium utilises predominantly long-chain fatty acids as a source of energy, including oleic acid which accounts for 60% of the total used.[8] Oleic acid has also been used successfully as an ion-pairing agent in facilitated transfer across lipoidal membranes[9] and is also a recognised skin penetration enhancer, where its mode of action is believed to be linked to its cis-structure and disruption of lipid bilayers when applied to skin surface.[10] Together, these facts suggested that an ion-pairing interaction between a cationic imaging complex and a negatively charged carboxylate group of long chain fatty acid, which would be lipophilic overall, could provide a facilitated transport mechanism for the entry of such complexes into myocardial tissue.

This work was therefore aimed at testing the hypothesis that successful myocardial uptake of imaging agents depends upon ion-pair formation. Although concerned with a specific field of research this phenomenon could be of significance in the transport of other charged species through other biological membranes.

MATERIALS AND METHODS

Materials

Seven complexes (Table 1) based on the ligands 1,2-bis[bis(2-ethoxyethyl) phosphino ethane] (tetrofosmin), 1,2-bis(dimethyl phosphino ethane) (DMPE) and 1,2-bis(diethyl phosphino ethane) (DEPE) were investigated. Ligand structures and ^{99M}Tc complexes representing core oxidation states of +I,

[1] Welsh School of Pharmacy, UWC, Cardiff, United Kingdom CF1 3XF.
[2] To whom correspondence should be addressed.

Abbreviations: DMPE, 1,2-bis(dimethyl phosphino ethane); DEPE, 1,2-bis(diethyl phosphino ethane); HD, hexadecanol; IPM, isopropyl myristate; OA, oleic acid; RCP, radiochemical purity; tetrofosmin, 1,2-bis[bis(2-ethoxyethyl)phosphino ethane].

Table I. IPM/pH7.4 Buffer Distribution Coefficient (P) at Room Temperature and 37°C. [a]Also, first order rate constants (k) for depletion of complex from donor phase using IPM or IPM/5% oleic acid membrane

Complex	$P_{ambient}$	sd × 10^{-2}	$P_{37°C}$	sd × 10^{-2}	k_{IPM} × 10^{-3} min^{-1}	sd × 10^{-3}	$k_{IPM/oleic\ acid}$ × 10^{-3} min^{-1}	sd × 10^{-3}
$[^{99m}Tc\ O_2\ (DMPE)_2]^+$	0.0079	0.39	0.0068	0.13	0.02	–	0.75	0.07
$[^{99m}Tc\ O_2\ (DEPE)_2]^+$	0.0033	0.18	0.0069	0.09	0.03	–	0.27	–
$[^{99m}Tc\ Cl_2\ (DMPE)_2]^+$	0.132	3.0	0.11	1.1	0.14	–	2.35	0.71
$[^{99m}Tc\ Cl_2\ (DEPE)_2]^+$	1.86	32.0	1.63	9.8	0.37	0.06	3.85	0.57
$[^{99m}Tc\ (DMPE)_3]^+$	0.158	4.7	0.14	2.2	0.15	0.03	3.86	0.15
$[^{99m}Tc\ (DEPE)_3]^+$	3.52	84.0	3.13	27.0	1.40	0.18	1.54	0.09
$[^{99m}Tc\ O_2$ (tetrofosmin)$_2]^+$	0.054	2.8	0.078	1.9	0.01	–	3.52	0.14

+III and +V are illustrated in Figures 1a and 1b respectively. ^{99M}Tc, in the form of sodium pertechnetate eluate was obtained from an Amertec generator (Amersham International, Little Chalfont, Bucks, UK). DMPE, DEPE and Myoview®, were gifts from Amersham International. Hexadecanol, oleic acid and ethylenebis(oxyethylenenitrilo)tetraacetic acid and were obtained from Aldrich. All other reagents were AnalaR grade or equivalent. Sodium chloride solution (0.9%) was deoxygenated by nitrogen purge prior to use. Syntheses were carried out in sealed rubber-stoppered glass vials, evacuated of oxygen prior to use. Reactants and reagents were added to vials using a combination of hypodermic and microliter syringes and preparations were used without further purification. Each complex was prepared freshly as required. Syntheses were carried out

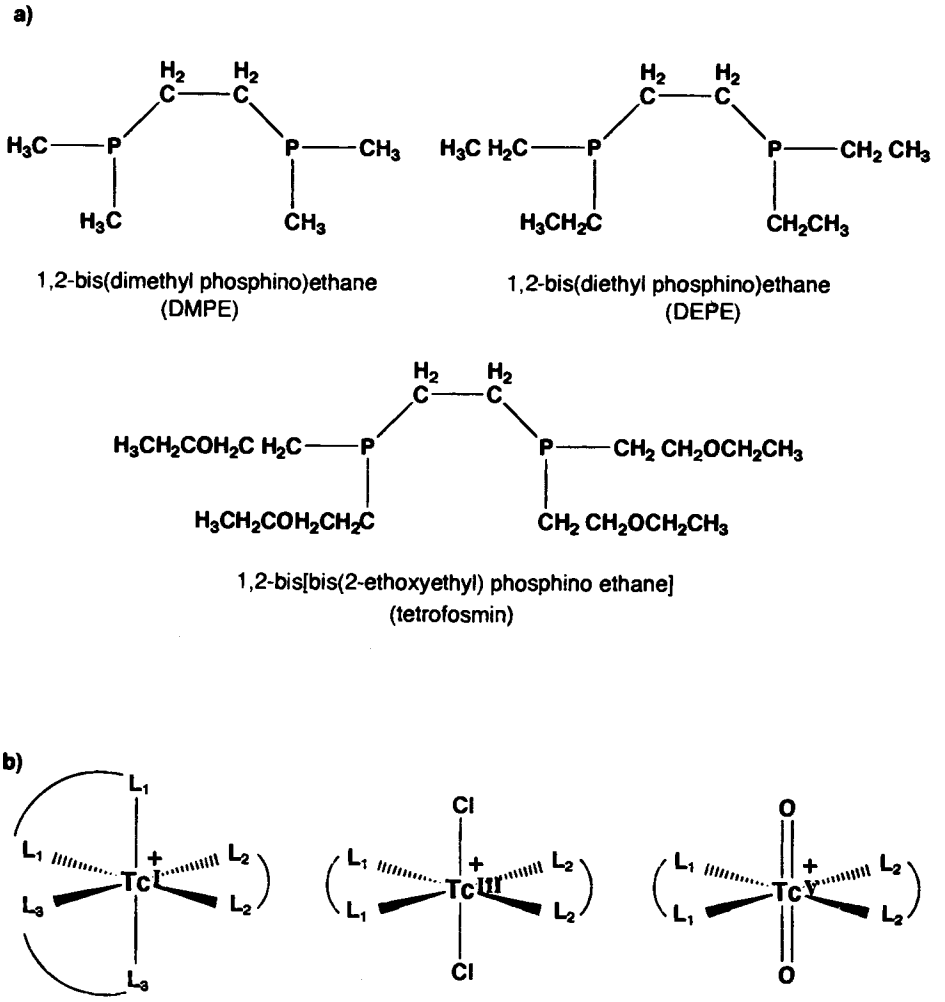

Fig. 1. Chemical structures of: a) bidentate ligands: 1,2-bis(dimethyl phosphino)ethane, 1,2-bis(diethyl phosphino)ethane and 1,2-bis[bis(2-ethoxyethyl) phosphino ethane]; b) technetium complexes in the +I, +III and +V oxidation states.

in parallel, using equally divided pertechnetate eluates (typically 2GBq, approximately 10ng of technetium).

Preparation of Tc^{I} Ligand Complexes

Ethanol (1ml), de-ionised water (1ml) and 50μl of 10M sodium hydroxide solution were added to a sealed stoppered vial. To this was added an excess (25μl, ca. 3.3 mMol) of ligand. Pertechnetate was added and the vial shaken. A vacuum was then created inside the vial by evacuating 20ml of headspace to minimise the risk of explosion. The vial was then transferred to a lead container and heated in an oil bath at 120°C for 2 hours. The preparation was allowed to cool for 15 minutes before opening.

Preparation of Tc^{III} Ligand Complexes

Sodium chloride (10mg) was added to a test tube along with 15mg ethylenebis(oxyethylene nitrilo)tetraacetic acid and 2ml of saline solution. The tube was shaken and the dissolved contents added to a sealed vial. Pertechnetate solution was added. In a second tube 5mg of ferric chloride hexahydrate was dissolved in 1ml of ethanol. To this was added 20μl of ligand and the solution shaken. The contents of this tube were added to the sealed vial which was then treated as described above.

Preparation of Tc^{V} Ligand Complexes

10μl of ligand were added to 5ml saline solution in a sealed vial. Pertechnetate was added and the vial shaken. The mixture was left to react for 1 hour at ambient temperature.

Determination of Radiochemical Purity (RCP)

The RCP of each product was determined for each preparation, using both paper and thin layer chromatography methods.[11] All analyses demonstrated RCP of >95%.

Transmembrane Transfer Studies

Myocyte diffusion was modelled using the rotating diffusion cell, which has been used previously to model transmembrane transfer and ion-paired facilitated transport of a wide range of compounds.[12,9] In this system the donor compartment can be considered analogous to the bloodstream or extra-cellular medium and the receptor compartment analogous to the intracellular medium. To represent the lipoidal nature of the myocyte membrane isopropyl myristate (IPM) was employed as it consists of a blend of hydrophobic and hydrophilic moieties representative of a biological membrane. 5% w/v solutions of oleic acid in IPM solutions were also used to represent myocyte membranes having sequestered oleic acid from the bloodstream. Cellulose nitrate membranes (0.2μm, Whatman, Maidstone, UK), were rendered hydrophobic with dichlorodimethylsilane then saturated with the relevant lipid phase. The cell was operated at its highest rotational speed, ensuring minimal stagnant diffusion layer. Receptor (130 mL) and donor (40 mL) phases were buffered at pH 7.4[13] using tris(hydroxymethyl) aminomethane/HCl. All experiments were conducted at 37°C. With the cell set in motion (5.0 Hz), 1mL aliquots of aqueous technetium complex was added to the donor compartment and a 1mL sample taken after 5 seconds mixing, representing the 'initial' bulk donor phase count. Subsequent 1mL samples were taken from both the donor and receptor compartments over a period of two hours. Samples were counted at the end of each run on a LKB Multigamma counter.

Partitioning

The distribution coefficient, P, was measured at room temperature and 37°C in an IPM/pH 7.4 tris(hydroxymethyl)aminomethane/HCl—buffered system. Aliquots of complex solution were added to tubes containing 3ml of each phase. Tubes were then vigourously vortex-mixed for 10 seconds and the phases separated by centrifugation at 1000 × G for 15 minutes. 1ml samples were then taken from the upper IPM phase and counted. P was calculated by difference.

Interaction with a Lipid Monolayer

The ability of an amphiphilic molecule to form a monolayer when applied to an aqueous sub-phase has long been known. Lateral compression of a monolayer can, in the form of pressure/area isotherms, yield information relating to intermolecular interactions as the molecules approach each other. In particular, molecular species present in the subphase before compression can influence monolayer formation as a result of interactions with headgroups.[14] Pressure/area isotherms were constructed using a Langmuir-Blodgett trough (NIMA Technology, Coventry, UK) in the absence and presence of $[Tc\ O_2\ (tetrofosmin)_2]^+$, $[Tc\ O_2\ (DMPE)_2]^+$, $[Tc\ O_2\ (DEPE)_2]^+$[11] in the subphase. Complexes were added to 'ultra-pure' water at concentrations of 0, 15.4, 46.3 and 77.1 μMol/700mL and carefully applied to the trough surface. Monolayers were constructed from hexadecanol (HD), a simple non-ionic amphiphilic lipid and 10% solutions of oleic in HD to determine ion-pairing interactions. Solutions of lipid (8.2×10^{-8} M) were prepared in chloroform; 100μl of which was carefully applied to the subphase surface (700ml). Following a further 15 minutes equilibration compression commenced at a rate of $50cm^2min^{-1}$. In control experiments, $[Tc\ O_2\ (tetrofosmin)_2]^+$, was used following purification by an ion-exchange method.[11]

RESULTS

Transmembrane Transfer

Figure 2 shows the depletion of each complex from the donor phase (partitioning into membrane) over a period of 120 min, as a percentage of the initial radioactivity (mean of two determinations). The complexes $[^{99m}Tc\ O_2\ (DMPE)_2]^+$ and $[^{99m}Tc\ O_2\ (DEPE)_2]^+$ behaved similarly in that they both failed to partition appreciably into the IPM membrane. However, partitioning was observed in the presence of a membrane containing 5% oleic acid, albeit by relatively small amounts. Both $[^{99m}Tc\ Cl_2\ (DMPE)_2]^+$ and $[^{99m}Tc\ Cl_2\ (DEPE)_2]^+$ demonstrated a small amount of partitioning into the IPM membrane, but unlike the two +V complexes, the partitioning of both +III complexes was very much enhanced by the presence of oleic acid in the membrane. The complex $[^{99m}Tc\ (DMPE)_3]^+$ behaved very similarly to the two +III complexes. However, the complex $[^{99m}Tc\ (DEPE)_3]^+$ demonstrated partitioning to a considerable extent into the IPM membrane which was not increased by the

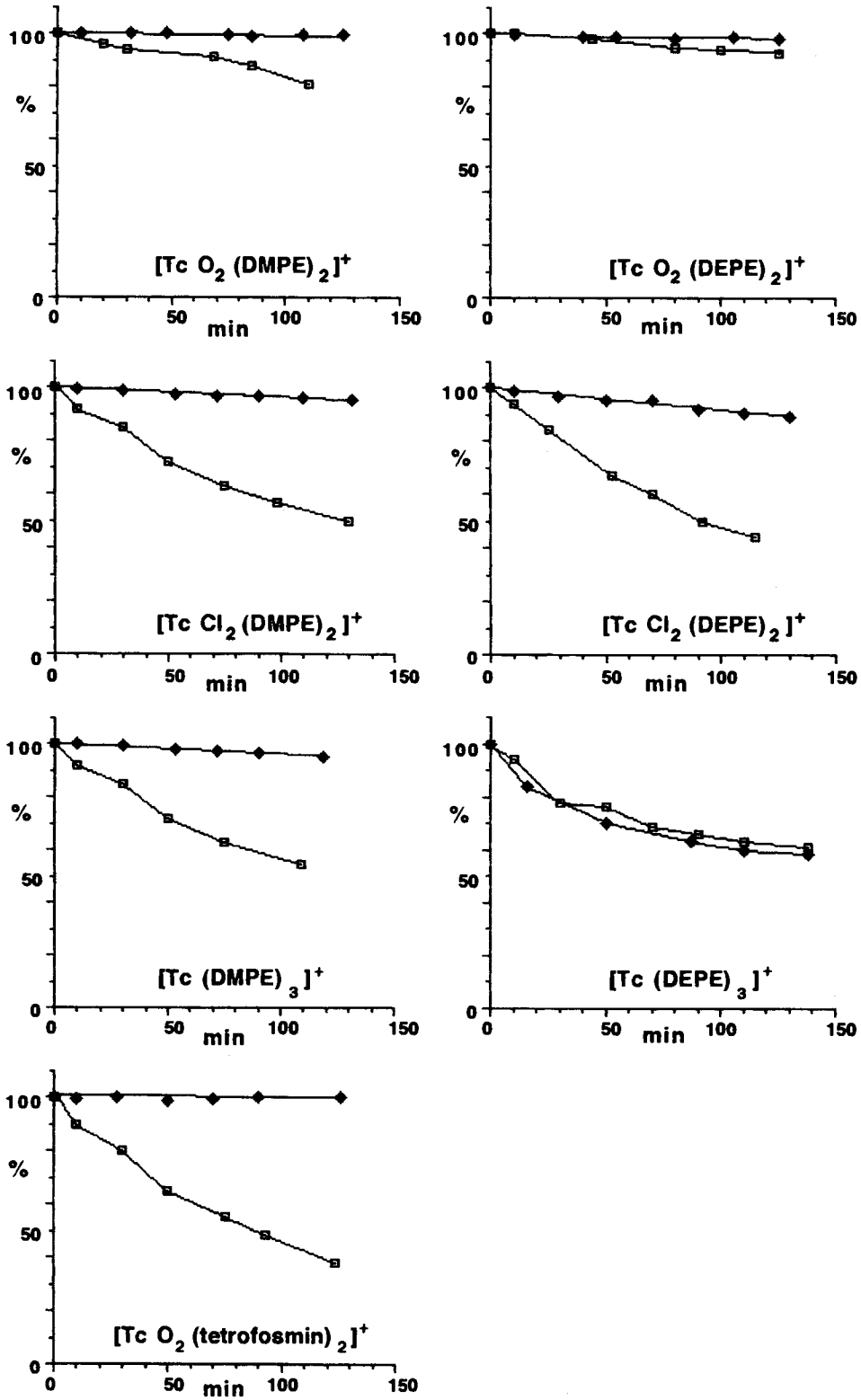

Fig. 2. Percent depletion of complex from donor phase or partitioning into membrane: diamond, IPM; square, 5% oleic acid in IPM. Average of 2 determinations.

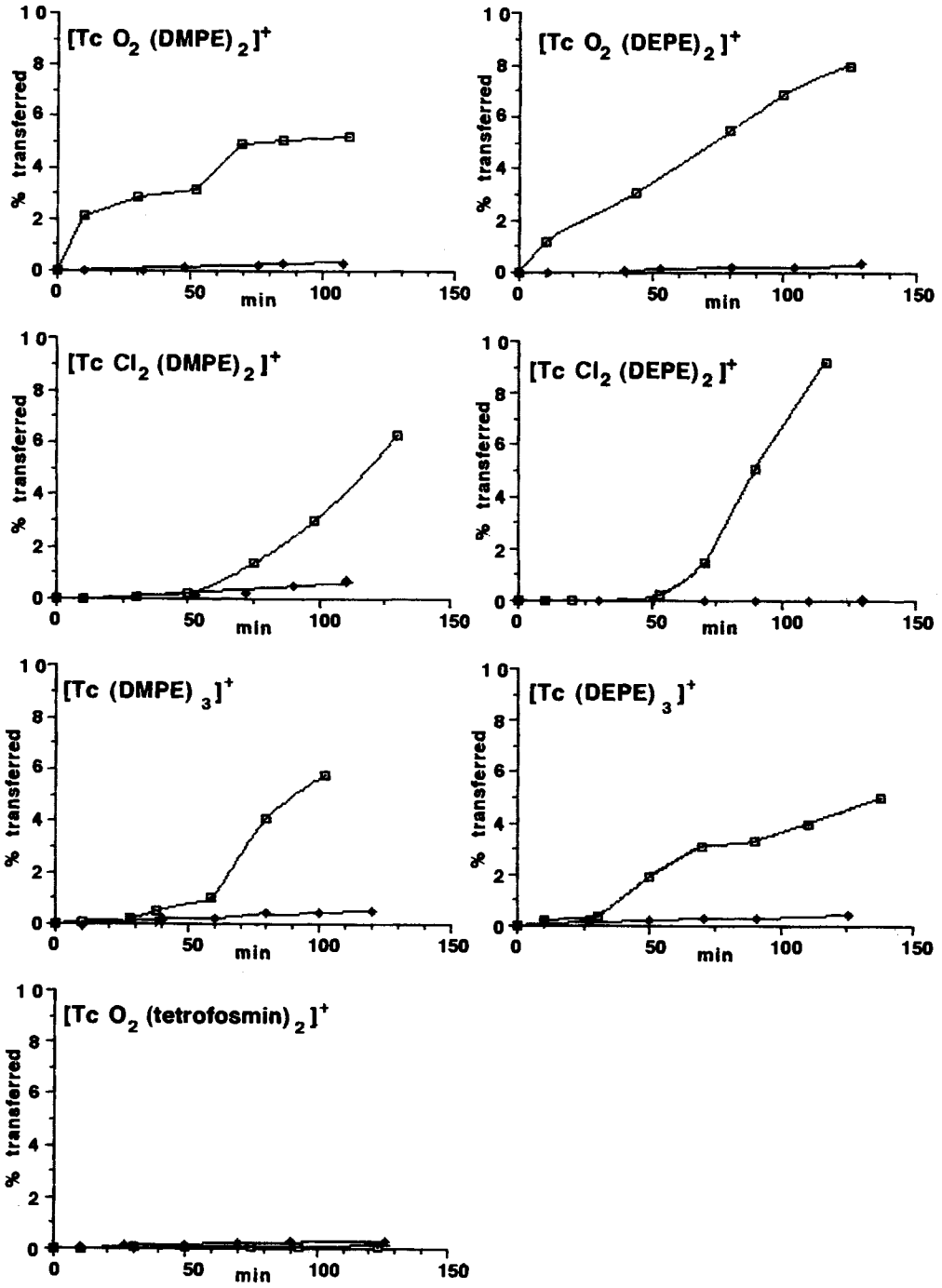

Fig. 3. Percent complex transferred to receptor phase: diamond, IPM; square, 5% oleic acid in IPM. Average of 2 determinations.

presence of oleic acid. The complex [^{99m}Tc O_2 (tetrofosmin)$_2$]$^+$ exhibited no partitioning into the IPM membrane, but in the presence of oleic acid, demonstrated the greatest degree of partitioning. Table I shows distribution coefficient at room temperature and 37°C. Also shown are first-order rate constants are for donor phase depletion, where diffusion into the IPM membrane results can be seen to generally reflect the diverse lipophilicities determined for these complexes. No significant temperature effects were found.

Figure 3 shows the appearance of each complex in the receptor compartment over a period of 150 min as a percentage of the initial radioactivity added to the system. With the exception of complex [^{99m}Tc O_2 (tetrofosmin)$_2$]$^+$ all other complexes demonstrated greater transfer into the receptor compartment in the presence of an IPM/oleic acid membrane than an IPM membrane. Overall, Figure 3 shows that the amount transferred to the receptor compartment from a membrane containing IPM only was not more than 1.5% over the run time. Indeed, for most complexes the amount transferred was a lot less than this value. Using an IPM/oleic acid membrane, transfer into the receptor compartment was generally of significant magnitude, with steady state flux being attained. The complexes [^{99m}Tc

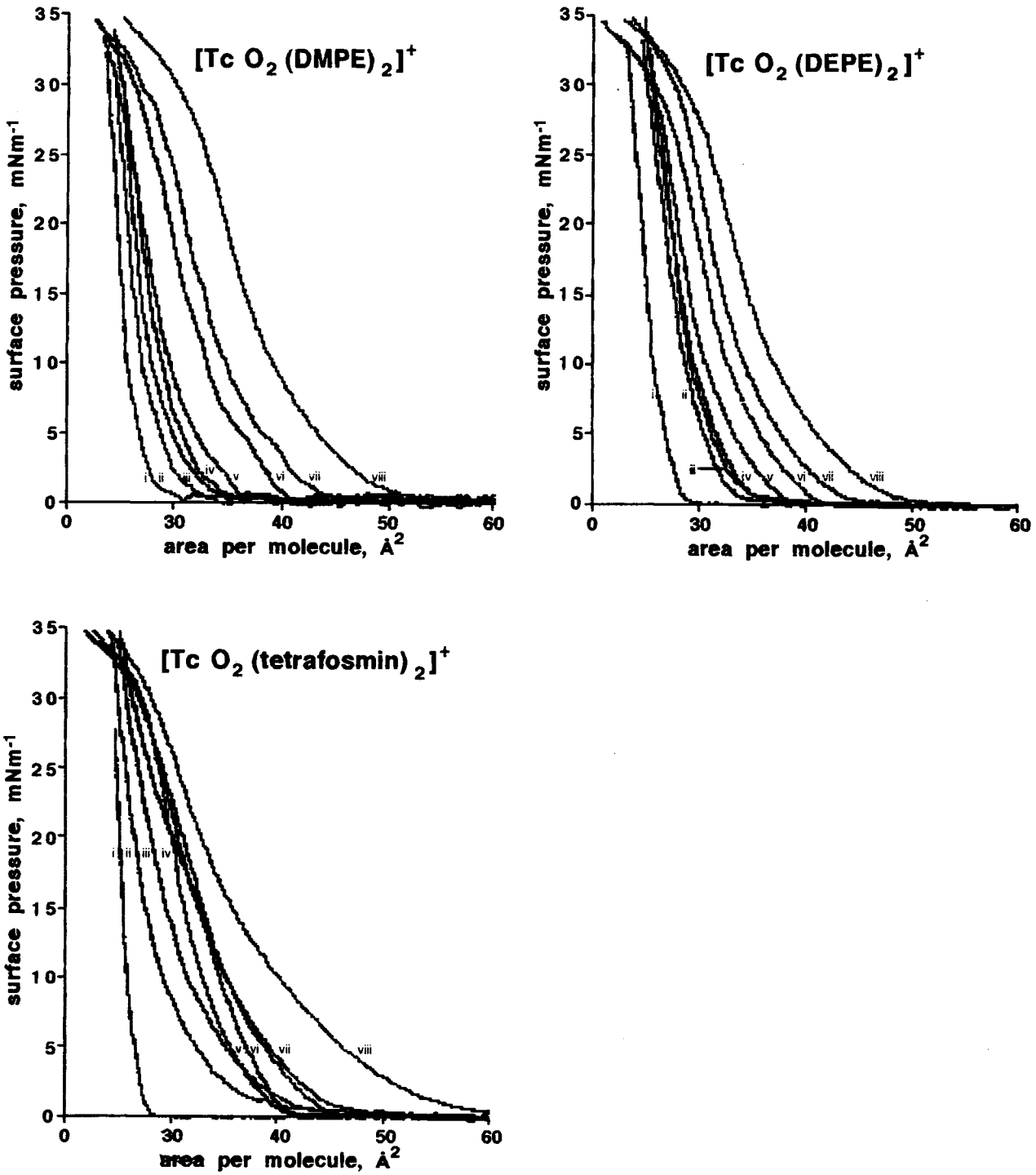

Fig. 4. Compression/area isotherms for monolayers of hexadecanol (i-iv) and 10% oleic acid in hexadecanol (v-viii) in the presence of different concentrations of [Tc O_2 $(DMPE)_2]^+$, [Tc O_2 $(DEPE)_2]^+$, and [Tc O_2 $(tetrofosmin)_2]^+$. (i & v: 0, ii & vi: 15.4, iii & vii: 46.3, iv & viii: 77.1 mmol/700ml). Average of 2 determinations.

O_2 $(DMPE)_2]^+$ and [^{99m} Tc O_2 $(DEPE)_2]^+$ demonstrated very short lag times, whereas the other complexes had lag-times of up to 50 minutes. The complex [^{99m} Tc O_2 $(tetrofosmin)_2]^+$ differed from the other complexes in that the transfer into the receptor was equally low using either membrane. The effect of the excess of ligand remains unclear, although at 37°C it may have evaporated.

Monolayer Studies

Figure 4 shows isotherms constructed in the presence of varying concentrations of subphasic [Tc O_2 $(DMPE)_2]^+$, [Tc O_2 $(DEPE)_2]^+$ and [Tc O_2 $(tetrofosmin)_2]^+$ respectively. Isotherms i–iv were formed from hexadecanol only; isotherms v–viii were formed from 10% oleic acid in hexadecanol. In each case there

Table II. Complex Distribution after 100 min and Lag Times using an IPM/5% Oleic Acid Membrane

Complex	% donor depletion	sd	% in receptor	sd	% in membrane	sd	lag time (min)
$[Tc\ O_2\ (DMPE)_2]^+$	13.0	2.44	5.2	0.88	7.8	2.59	< 1
$[Tc\ O_2\ (DEPE)_2]^+$	6.0	0.96	5.5	1.41	0.50	1.71	< 1
$[Tc\ Cl_2\ (DMPE)_2]^+$	40.0	3.33	3.2	0.56	36.8	3.38	50
$[Tc\ Cl_2\ (DEPE)_2]^+$	49.0	3.46	8.6	4.3	40.4	5.52	50
$[Tc\ (DMPE)_3]^+$	43.5	2.83	5.7	3.1	37.8	4.20	45
$[Tc\ (DEPE)_3]^+$	36.0	2.80	3.7	0.40	32.3	2.83	25
$[TcO_2$ (tetrofosmin)$_2]^+$	54.0	4.40	0.2	1.3	53.8	4.59	–

was a concentration—dependent relationship between complex concentration which was indicative of interactions between hexadecanol headgroups and technetium complex both in the presence and absence of oleic acid. The DMPE and DEPE complexes behaved similarly in showing small but consistent expansion of hexadecanol isotherms (i–iv) with increasing complex concentration, which is indicative of uptake into the bulk monolayer[15]. The same effect, although much more marked was seen in the presence of oleic acid. The tetrofosmin complex concentration-dependent expansion of both monolayer types, but in the presence of oleic acid the effect was somewhat reduced, indicating less uptake. The excess ligand present had minimal influence on isotherm formation[11], possibly as a result of evaporation.

DISCUSSION

The charge associated with the complexes investigated is carried by the central technetium atom and overall complex lipophilicity may be affected by the lipophilicity of the ligands. This might explain the large distribution coefficient of the complex $[^{99m}$ Tc (DEPE)3$]^+$, where the charge is effectively shielded from the external environment. In general, the considerable energy barrier for a charged species to partition into a non-polar environment may be reduced if a suitable counterion is present and ion-pairs are formed. The presence of oleic acid in the membrane enhanced the partitioning of each complex into the membrane to varying degrees. The exception again was $[^{99m}$ Tc $(DEPE)_3]^+$ which was not enhanced, again suggesting shielding of the central charge. The transfer of $[^{99m}$ Tc O_2 $(DMPE)_2]^+$ was enhanced the least and the tetrofosmin complex was enhanced the most. Such differences in tendency for ion-pair formation may explain why $[^{99m}$ Tc O_2 $(DMPE)_2]^+$ fails to target the myocardium, whereas $[^{99m}$ Tc O_2 (tetrofosmin)$_2]^+$ does, despite the two complexes being comparably hydrophilic (Table I).

The mechanism which exists within the myocyte cell to transfer fatty acids from the inner membrane interface, via the aqueous intra-cellular medium, to the site of metabolism was not present in the simple model system used. In the absence of such sink conditions, dissociation at the receptor interface would be unfavourable due to the overall lipophilicity of the ion-pair, explaining the considerable lag-times observed for most complexes and particularly the very slow release of tetrofosmin.

Table II shows the percentage of the initial activity present in the donor compartment and in the receptor compartment at a timepoint of 100 minutes using the IPM/oleic acid membrane. The difference between these two values is the percentage of complex resident in the membrane at that particular timepoint. From this in addition to lag time data, inferences can be made about the stability of the ion-pair and the kinetics of ion-pair diffusion through the membrane. Firstly, there was very little difference for $[^{99m}Tc\ O_2\ (DMPE)_2]^+$ and $[^{99m}Tc\ O_2\ (DEPE)_2]^+$, indicating that their passage through the membrane and ion-pair dissociation is rapid: the rate-limiting step being the ion-pairing interaction at the donor compartment/membrane interface. This was further supported by the small lag-times of these complexes. The high percentage of $[^{99m}Tc\ Cl_2\ (DMPE)_2]^+$, $[^{99m}Tc\ Cl_2\ (DEPE)_2]^+$ and $[^{99m}Tc\ (DMPE)_3]^+$ resident in the membrane and their long lag-times demonstrate stable ion-pairs. The complex $[^{99m}Tc\ (DEPE)_3]^+$ is anomalous in that there was no apparent ion-pairing with oleic acid and thus, the amount in the membrane is the same as for an IPM membrane; although the presence of the oleic acid did enhance transfer into the receptor compartment. The $[^{99m}Tc\ O_2$ (tetrofosmin)$_2]^+$ complex demonstrated the greatest ion-pair stability, with only 0.2% of the complex detectable in the receptor compartment after 100 minutes.

The monolayer work provided further evidence of ion-pair interaction between the three complexes studied and oleic acid. Each was found to expand a monolayer of hexadecanol and 10% oleic acid in hexadecanol in the solid phase indicating a level of uptake into the monolayer. The complex $[^{99m}Tc\ O_2$ (tetrofosmin)$_2]^+$ demonstrated the *least* solid phase expansion in the presence of oleic acid, despite liquid phase expansions comparable with the other two test complexes, a difference which is even more apparent in view of the much larger size of this complex. This is indicative of greater retention at the lipid/aqueous interface, probably due to stronger interaction between this complex and oleic acid.

Although the problems associated with investigating very small concentrations of ^{99m}Tc are manifest, our results demonstrated a capability for ion-pair formation with oleic acid, which varied depending upon the nature of the complex. The clinically successful complex $[^{99m}Tc\ O_2$ (tetrofosmin)$_2]^+$ demonstrated particularly strong interaction, suggesting that whether or not a complex is taken up by myocardial tissue may depend on its ability to 'hitch a ride' by ion-pairing with the energy source of the myocyte—a molecule of long chain fatty acid. Why $[^{99m}Tc\ O_2$ (tetrofosmin)$_2]^+$ interacts uniquely with oleic acid remains to be elucidated and the potential importance of ion-pair formation with fatty acid in the myocardium warrants further investigation, as indeed do ion-paired transport processes in other tissues.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the Science and Engineering Research Council and to Amersham International for supporting this research. We are also grateful to the Medical Physics Department of the University Hospital of Wales for the donation of pertechnetate eluate.

REFERENCES

1. E. A. Deutsch, K. A. Glavan, V. J. Sodd, H. Nishiyama, D. L. Ferguson and S. J. Lukes. Cationic Tc-99m complexes as potential myocardial imaging agents. *J. Nucl. Med.* **22**(10):897-907 (1981).
2. D. M. Wieland and E. A. Deutsch. Cationic radiotracers as myocardial radiopharmaceuticals. In Colombetti (Ed.) *Radiopharmaceuticals: Progress and Clinical Perspectives* Chapter 1 CRC Press, Boca Raton, USA (1984) p.p. 1–20.
3. M. L. Thakur, C. H. Park, F. Faxio, P. Gerundini, A. Margonato, G. Vicedomini, R.Colombo, F. Colombo, M. Gilardi, F. Fregoso, R. Bencivelli and Taddei G. Preparation and evaluation of [^{99m}Tc]DEPE as a cardiac perfusion agent. *Int. J. Appl. Radiat. Isot.* **23**:1102-1110 (1984).
4. J. D. Kelly, B. Higley, C. M. Archer, L. R. Canning, K. W. Chiu, B. Edwards, A. M. Forster, H. K. Gill, I. A. Latham, R. D. Pickett, P. Webbon, P. G. Edwards, A. Imran, D. V. Griffiths, D. C. York, P. M. Mahoney, D. J. Tonkinson, J. R. Dilworth and A. Lahiri. Technetium-99m complexes of functionalised diphosphines for myocardial imaging. In M. Nicolini, G. Bandoli and U. Mazzi (Eds.) *Technetium & Rhenium in Chemistry, Nuclear Medicine Vol. 3* (1990) p.p. 405-412.
5. R. C. Hendel, M. A. Parker, F. J. Wackers, P. Rigo, A. Lahiri and B. L. Zaret. Reduced variability of interpretation and improved image quality with a technetium-99m myocardial perfusion agent: Comparison of thallium-201 and technetium99m-labelled tetrofosmin. *J. Nucl. Cardiol.* **1(6)**:509-514 (1994).
6. H. Sands, M. L. Delano, L. L. Camin and B. M. Gallagher. Comparison of the transport of $^{42}K^+$, $^{22}Na^+$, $^{201}Tl^+$ and [^{99m}Tc Cl_2 $(DMPE)_2$ $]^+$ using human erythrocytes. *Biochem. Biophys. Acta.* **812**:665-670 (1985).
7. S. T. Dahlberg and J. A. Leppo. Myocardial kinetics of radiolabelled perfusion agents: Basis for perfusion imaging. *J. Nucl. Cardiol.* **1**(21):189-197 (1994).
8. E. Bing. Cardiac metabolism. *Phys. Rev.* **45**:171-180 (1965).
9. P. G. Green and J. Hadgraft. Facilitated transport of cationic drugs across a lipoidal membrane by oleic acid and lauric acid. *Int. J. Pharm.* **37**:251–255 (1987).
10. B. J. Aungst , J. A. Blake and M. A. Hussain. Contributions of drug solubilisation, partitioning, barrier disruption and solvent permeation to the enhancement of skin permeation of various compounds with fatty acids and amines. *Pharm. Res.* **7**(7):712-718 (1990).
11. C. M. Heard. Site targeting of imaging agents. *PhD Thesis* Department of Pharmaceutical Chemistry, University of Wales, College of Cardiff (1991) p.p. 36-41. Contact Bute Library, UWC, Cardiff, U.K. CF1 3AP.
12. N. Barker, J. Hadgraft and P. K. Wotton. Facilitated transport across liquid/liquid interfaces and its relevance to drug diffusion across biological membranes. *Farad. Disc.* **77**:97-104 (1984).
13. D. Ellis and R. C. Thomas. Direct measurement of the intracellular pH of mammalian cardiac muscle. *J. Physiol.* 262:755-771 (1976).
14. B. Sims and S. L. Holder. Surface activity of prostaglandins E2, $F2_\alpha$, A1 and B1 in the presence of insoluble monomolecular films. *J. Pharm. Sci.* **63**:1540-1545 (1974).
15. C. M. Heard and K. R. Brain. Stereoselective interaction between (+)- and (−)-ephedrine with ceramides can be demonstrated, so why was this not manifested as a difference in percutaneous penetration rate? In K. R. Brain, V. James and K. A. Walters (Eds.) *Prediction of Percutaneous Penetration Vol. 4b* STS Publishing, Cardiff, U. K. (in press).