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TISSUE DISTRIBUTION OF EDTA ENCAPSULATED WITHIN LIPOSOMES OF VARYING SURFACE PROPERTIES

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SUMMARY

Liposomes containing ethylenediaminetetraacetic acid (EDTA) were prepared with different surface properties by varying the liposomal lipid constituents. Positively charged liposomes were prepared with a mixture of phosphatidylcholine, cholesterol, and stearylamine. Negatively charged liposomes were prepared with a mixture of phosphatidylcholine, cholesterol, and phosphatidylserine. Neutral liposomes were prepared with phosphatidylcholine alone, dipalmitoyl phosphatidylcholine alone, or with a mixture of phosphatidylcholine and cholesterol. Distributions of ^{14}C-labeled EDTA were determined in mouse tissues from 5 min to 24 h after a single intravenous injection of liposome preparation. Differences in tissue distribution were produced by the different liposomal lipid compositions. Uptake of EDTA by spleen and marrow was highest from negatively charged liposomes. Uptake of EDTA by lungs was highest from positively charged liposomes; lungs and brain retained relatively high levels of EDTA from these liposomes between 1 and 6 h after injection. Liver uptake of EDTA from positively or negatively charged liposomes was similar; the highest EDTA uptake by liver was from the neutral liposomes composed of a mixture of phosphatidylcholine and cholesterol. Liposomes composed of dipalmitoyl phosphatidylcholine produced the lowest liposomal EDTA uptake observed in liver and marrow but moderate uptake by lungs. Tissue uptake and retention of EDTA from all of the liposome preparations were greater than those of non-encapsulated EDTA. The results presented demonstrate that the tissue distribution of a molecule can be modified by encapsulation of that substance into liposomes of different surface properties. Selective delivery of liposome-encapsulated drugs to specific tissues could be effectively used in chemotherapy and membrane biochemistry.

INTRODUCTION

Direction of biologically active molecules to specific target tissues is an impor-

Abbreviation: DTPA, diethylenetriaminepentaacetic acid.

tant problem in chemotherapy. Many drugs are metabolized and eliminated from the animal body, often before reaching the desired site of action. Some substances, particularly the antibiotic and anti-tumor agents, may also cause systemic toxic effects. Furthermore, not all drugs can penetrate cellular membranes. Encapsulating a particular drug within a specific carrier could reduce these difficulties by increasing cellular penetrability and greater selectivity of tissue interaction.

The polyaminopolycarboxylic acid chelating agents ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA), which are used in treatment of heavy metal poisoning, do not appreciably penetrate cell membranes [1, 2]. Attempts have been made to increase their cellular penetration, primarily by esterification. However, the esterified chelating agents were toxic and almost insoluble in aqueous media [3, 4]. Artificial lipid spherules, liposomes, have been recently used to encapsulate EDTA and DTPA. The liposome-encapsulated chelating agents were retained longer and in higher concentrations in mouse tissues than the corresponding non-encapsulated EDTA or DTPA; they were also more effective in removing toxic metals from mouse tissues [5–7]. Intracellular penetration of liposomes containing EDTA has been demonstrated in mouse liver, spleen and lung [8, 9]. Increased cellular penetration and, in some cases, increased biological activity have also been shown for liposome-encapsulated actinomycin D [10, 11], penicillin [11], and cyclic AMP [12].

A drug can be incorporated either into the lipid phase or into the aqueous phase of liposomes, depending on its solubility in organic or aqueous media. In the case of a water-soluble substance, such as EDTA, a phospholipid film is dispersed in an aqueous solution of the drug, and the dissolved drug molecules are trapped in the aqueous compartments enclosed by the lipid bilayers. The surface properties of liposomes, such as charge or bilayer fluidity, can be varied by addition of lipids bearing charged functional groups, by alteration of liposomal phospholipid fatty acid content, or by addition of cholesterol.

Effects of liposomal membrane charge and fluidity have been studied *in vitro* by a number of other workers. For example, aggregated ("membrane active") immunoglobulin G has been shown to interact preferentially with positively charged liposomes [13]. A calcium-dependent fusion of cells in culture has been induced by negatively charged liposomes that possess fluid bilayers [14]. However, no *in vivo* examination of interactions between specific, competing tissues and liposomes of various lipid compositions has been made. In this investigation, the specificities of *in vivo* uptake by various mouse tissues of EDTA encapsulated in the aqueous compartments of liposomes with varying lipid compositions and surface characeristics are presented.

MATERIALS AND METHODS

Phosphatidylcholine was obtained as Type VII-E egg lecithin from Sigma Chemical Co., St. Louis, Mo., or as the chromatographically pure preparation from Grand Island Biological Co., Grand Island, N. Y. Synthetic dipalmitoyl-L-α-phosphatidylcholine was purchased from Schwarz/Mann, Orangeburg, N. Y. Phosphatidylserine (bovine; chromatographically pure) was obtained from Applied Science Laboratories, Inc., State College, Pa. Stearylamine (95–99 % pure) was a product of K and K Laboratories, Inc., Plainview, N. Y. Cholesterol (chromatographically pure) was purchased from Sigma. Ethylenediaminetetra[2-^{14}C]acetic acid, with a specific radio-

activity of 19.8 Ci/mol, was purchased as the tetrasodium salt from Amersham/Searle Corp., Arlington Heights, Ill. Unlabeled EDTA (free acid)was obtained from Fisher Scientific Co., Fair Lawn, N. J. Reagent grade chemicals and "Spectranalyzed" (Fisher) organic solvents were used in all preparations.

Preparation of liposomes containing ^{14}C-labeled EDTA

Multilamellar liposomes were prepared as described previously [7] by mechanical stirring of dried thin films of phospholipids, to which an aqueous solution containing 80 μCi of 132 mM (5% wt) ^{14}C-labeled $CaNa_2EDTA$ (pH 7.4) had been added. Multilamellar liposomes were used because lower incorporation of encapsulated drugs has been consistently found with sonicated (unilamellar) liposome preparations (Rahman *et al.*, unpublished); this low incorporation would provide insufficient amounts of encapsulated drug for injection [5–7, 10]. All liposomes were prepared at 37°C, except for those made of dipalmitoyl phosphatidylcholine, which were prepared at 60°C (above the transition temperature for this phospholipid). Each preparation contained a total of approx. 30 mg of lipid and 250 mg EDTA. Lipids were used as obtained from the manufacturer; however, care was taken to use only fresh stock solutions and to protect lipids from oxygen and heat. Phospholipid stock solutions were monitored for evidence of breakdown by thin-layer chromatography.

Unincorporated ^{14}C-labeled EDTA was removed from the liposomes by two successive centrifugation and resuspension procedures. Centrifugation (800 × g, 5 min) was used because it does not dilute the liposome preparations (as does the gel filtration method), it is rapid, and it also conserves costly or scarce drugs, which can be recovered undiluted and re-used after appropriate extraction of residual lipid. Neutral liposomes were washed in unlabeled $CaNa_2EDTA$, pH 7.4, or in 154 mM NaCl (physiological saline). Charged liposomes were usually washed in unlabeled $CaNa_2EDTA$ because a large proportion of these liposomes did not sediment in saline. The washed liposomes were resuspended in physiological saline and used without prior filtration. Liposomes were used for injection between 1 and 6 h after preparation. No special precaution was taken to maintain aseptic conditions because bacterial growth was not observed in similar liposome preparations in this laboratory [7]. The morphology of liposomes in each preparation was examined using a light microscope (400 × magnification) with a polarized light source. There was relatively little influence of lipid composition upon the sizes of liposomes formed. Previous measurements have indicated that 85% of multilamellar liposomes containing EDTA are less than 2 μm in diameter [7]. Any preparation in which the liposomes had aggregated into small clumps was discarded. (See Rahman *et al.* [7] for discussion of phospholipid quality and formation of liposomes). Surface charges of liposomes were confirmed by cell electrophoresis, using the technique of Bangham *et al.* [15] with apparatus manufactured by Grant Instruments, Cambridge, U. K.

Stearylamine was used to produce a positive charge on liposomal surfaces; phosphatidylserine was used to produce a negative charge. Lipid compositions are given in Table I. Lipid weights were used in preparing liposomes because of the indeterminate nature of molecular weights of phospholipids from natural sources. Proportions of lipids in the charged liposomes were chosen empirically to give the greatest amount of charged species compatible with liposome stability, as measured by tests of EDTA incorporation into the washed liposome preparations.

Injection of liposomes

A total of 160 female CF 1 (Carworth Farms) mice were used; mice had an average age of 113 days and an average weight of 27.5 g. Each mouse was given a single injection, in a tail vein, of 0.40 ml of liposome suspension containing 31 000–113 000 dpm per mouse. Radioactivity injected was 120–550 nmol $CaNa_2EDTA$ per mouse and 26–68 mg EDTA per kg of body weight. Liposome preparations with lipid compositions that consistently produced low yields and low EDTA incorporation (such as phosphatidylcholine-cholesterol-phosphatidylserine liposomes) were always prepared in several batches, which were then pooled before injection. Groups of mice were killed, as previously described [7], at intervals of 5 min to 24 h after injection: samples of liver, spleen, bone marrow, lungs, brain, kidneys, and blood, tissues previously shown to accumulate measurable amounts of EDTA [7], were removed for determination of ^{14}C-labeled EDTA activity. Whole organs or standard samples [7, 16] of liver, bone marrow, and blood were taken.

The tissue samples were prepared for ^{14}C analysis by automatic combustion as described [7]. The amount of ^{14}C-labeled EDTA injected was calculated from triplicate samples of each injection solution taken with the same injection syringe; these samples were prepared for combustion by the same method as the blood and bone marrow samples. The ^{14}C radioactivity recovery of the Packard 305 sample oxidizer used was determined to be greater than 97 % for standard samples in the activity range analyzed. Scintillation fluid contained 1.5 % 2,5-diphenyloxazole and 0.1 % *p*-bis-*O*-methylstyrylbenzene in toluene. Samples were counted in a Beckman liquid scintillation counter, either Model LS-200 B or LS-333.

RESULTS

Incorporation of ^{14}C-labeled EDTA into liposome preparations

The average EDTA incorporation into neutral liposomes ranged from 1.2 to 1.8 mg per preparation (Table I). Positively and negatively charged liposomes incorporated 0.9 and 0.4 mg per preparation, respectively. Lower incorporation of EDTA into charged liposome preparations probably reflects loss of liposomes during washing.

TABLE I

LIPOSOMAL LIPID COMPOSITION AND INCORPORATION OF ^{14}C-LABELED EDTA

Liposomal lipid composition*	Weight ratio of lipids	Approximate molar ratio of lipids	Liposomal surface charge	Incorporation of ^{14}C-labeled EDTA (average mg/preparation)
PC (only)	(1)	(1)	0	1.5
DpPC (only)	(1)	(1)	0	1.2
PC-chol	1 : 1	1 : 2	0	1.8
PC-chol-StAm	103 : 52 : 10	35 : 17 : 10	+	0.9
PC-chol-PS	53 : 19 : 10	52 : 38 : 10	−	0.4

* Abbreviations: PC, phosphatidylcholine (egg); DpPC, dipalmitoylphosphatidylcholine (synthetic); chol, cholesterol; StAm, stearylamine; PS, phosphatidylserine (bovine).

TABLE II

PERCENT OF TOTAL INJECTED RADIOACTIVITY IN MOUSE TISSUES 5 MIN AFTER INTRAVENOUS INJECTION OF LIPOSOMES CONTAINING ^{14}C-LABELED EDTA

Values are mean percent of injected radioactivity ± the standard error of the mean. Each group contained four mice. The abbreviations used as in Table I.

Liposomal lipid composition	Liver	Spleen	Bone marrow*	Lungs	Brain	Kidneys	Blood**	Total in tissues shown
PC (only)	31.12	2.25	5.28	5.89	0.13	2.67	14.98	62.29
	± 2.83	0.20	± 0.83	± 0.28	± 0.01	0.32	± 2.96	± 1.31
DpPC (only)	12.15	1.99	1.28	13.31	0.17	5.08	24.56	58.10
	± 2.34	0.27	± 0.14	± 0.78	± 0.01	± 0.46	± 1.81	± 1.40
PC-chol	21.34	6.58	3.48	12.43	0.24	3.48	34.53	82.09
	± 4.18	± 2.02	± 0.88	0.52	± 0.02	0.36	5.22	± 1.85
PC-chol-StAm	17.88	2.22	4.38	19.89	0.29	3.69	27.85	76.13
	± 2.40	± 0.17	0.73	0.36	± 0.03	± 0.22	3.61	± 2.21
PC-chol-PS	23.08	4.82	6.23	4.47	0.41	8.14	35.71	82.85
	± 1.48	± 0.69	± 0.85	± 0.14	± 0.02	0.55	± 2.12	1.24

* Activity in the estimated total bone marrow per mouse, calculated by multiplying activity in a standard bone marrow sample taken from both tibia by a factor of 44 [16].

** Activity in the estimated total blood volume per mouse, 2 cm^3, calculated from activity in duplicate 100-μl blood samples.

TABLE III

PERCENT OF TOTAL INJECTED RADIOACTIVITY IN MOUSE TISSUES 1 H AFTER INTRAVENOUS INJECTION OF LIPOSOMES CONTAINING ^{14}C-LABELED EDTA

Values are mean percent of injected radioactivity ± the standard error of the mean. Each group contained four mice. The abbreviations used as in Table I.

Liposomal lipid composition	Liver	Spleen	Bone marrow*	Lungs	Brain	Kidneys	Blood**	Total in tissues shown
PC (only)	37.25	3.21	5.17	2.41	0.06	0.43	0.90	49.43
	± 1.91	0.29	± 0.36	± 0.18	± 0.01	0.03	± 0.07	1.66
DpPC (only)	26.63	4.20	1.58	5.60	0.05	0.91	2.56	41.53
	± 3.90	± 1.01	± 0.14	0.42	± 0.00	± 0.04	0.58	2.41
PC-chol	43.39	7.02	5.47	5.24	0.09	0.67	0.89	66.47
	± 1.33	1.13	± 0.33	± 0.38	± 0.01	± 0.11	0.18	4.10
PC-chol-StAm	36.15	4.89	6.61	10.49	0.21	1.02	1.96	61.32
	± 1.80	± 0.47	± 0.54	± 1.35	± 0.03	0.12	± 0.84	± 1.11
PC-chol-PS	39.62	8.24	7.61	3.10	0.10	1.22	0.36	60.17
	± 2.05	± 2.50	± 1.38	± 0.22	± 0.02	0.04	± 0.02	± 2.12

* Activity in the estimated total bone marrow per mouse, calculated by multiplying activity in a standard bone marrow sample taken from both tibia by a factor of 44 [16].

** Activity in the estimated total blood volume per mouse, 2 cm^3, calculated from activity in duplicate 100-μl blood samples.

Distribution of liposome-encapsulated ^{14}C-labeled EDTA and recovery of radioactivity in mouse tissues

The percentages of ^{14}C-labeled EDTA taken up by individual tissues and the total amount of EDTA recovered in the tissues analyzed 5 min and 1 h after injection are given in Tables II and III. These tables permit correlation of the percent uptake of injected liposomal EDTA by an entire tissue with the specific radioactivity (dpm/mg) found in that tissue (Figs. 1–7). The recovery of labeled EDTA in the tissues examined 5 min after injection of the liposome preparations ranged from 58 to 83 % (Table II), depending upon liposomal lipid composition.

The liver took up the highest percentages of injected liposomal EDTA, reaching peak concentrations 1 h after injection, although the total amount of EDTA recovered had decreased. Of the tissues analyzed at 1 h, the liver, spleen, marrow, and lungs retained the largest amounts of liposomal EDTA (Table III). Both the lowest recovery (42 %) and the greatest proportional decrease in recovery were observed with liposomes composed of dipalmitoylphosphatidylcholine alone (Tables II and III).

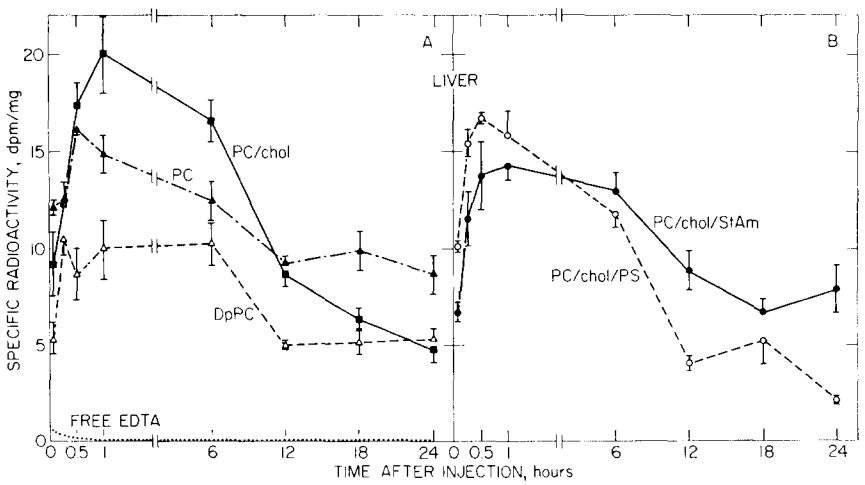

Fig. 1. Uptake and retention in mouse tissues of ^{14}C-labeled EDTA at intervals between 5 min and 24 h after intravenous injection of liposome-encapsulated EDTA. Figures designated A show distribution of neutral liposome preparations: phosphatidylcholine (PC) (▲– – –▲), dipalmitoylphosphatidylcholine (DpPC) (△– – –△), and phosphatidylcholine-cholesterol (■–■). Also included for comparison in the figures A are distribution curves of non-encapsulated EDTA (· · ·), which have been calculated from the experiments of Rahman et al. [7]. Figures designated B show distribution of charged liposome preparations: PC-chol-stearylamine (StAm) (●–●) and PC-chol-phosphatidylserine (PS) (○– – –○). Liposome preparations are described in Materials and Methods; abbreviations are as in Table I. Each point represents the mean value from four mice. Vertical bars represent two standard errors of the mean. In the case of bone marrow and blood (Figs 3 and 7), total radioactivity was calculated as described in Table II. For clarity of presentation, data points are not connected for phosphatidylcholine and dipalmitoylphosphatidylcholine liposome preparations in graphs of brain, kidney, and blood distributions (Figs 5–7). Correction was made for differences in amounts of ^{14}C-labeled EDTA administered in different experiments in the calculations of specific activity.

Specific radioactivity of ^{14}C-labeled EDTA in mouse tissues observed for 24 h

Uptake of ^{14}C-labeled EDTA from the different liposome preparations varied among the mouse tissues examined:

Liver. Liver uptake of EDTA from charged liposomes was relatively non-specific (Fig. 1). Uptake of EDTA from neutral liposomes was highest in liver after injection of phosphatidylcholine-cholesterol liposomes, and lowest after injection of dipalmitoylphosphatidylcholine liposomes. However, even the lowest level of liposomal EDTA uptake in liver, as well as in spleen, marrow and lungs, was significantly above the level of non-encapsulated EDTA uptake (Figs 1–4).

Spleen. The spleen accumulated the highest concentrations of EDTA from the negatively charged phosphatidylcholine-cholesterol-phosphatidylserine liposomes during the first 30 min after injection (Fig. 2). Intermediate EDTA concentrations, which changed little between the 6- and 24-h intervals, were observed after injection of phosphatidylcholine-cholesterol liposomes and positively charged phosphatidylcholine-cholesterol-stearylamine liposomes; the EDTA retained following injection of negatively charged liposomes decreased during the same period. Uptake of liposomal EDTA was lowest in spleen after injection of phosphatidylcholine liposomes.

Marrow. Bone marrow also showed the highest uptake of EDTA from negatively charged liposomes (Fig. 3). Similar changes of spleen and marrow EDTA levels were observed with these phosphatidylcholine-cholesterol-phosphatidylserine liposomes during the first 6 h after injection. Marrow uptake of EDTA from positively charged liposomes and from neutral liposomes composed of phosphatidylcholine alone or phosphatidylcholine-cholesterol was at an intermediate level. Unlike spleen, marrow accumulated the lowest amount of liposomal EDTA from liposomes composed of dipalmitoylphosphatidylcholine.

Lungs. In lungs, the uptake of EDTA from positively charged liposomes was significantly higher than that from all other liposome preparations (Fig. 4). Moderate elevation of lung EDTA concentrations was observed after injection of dipalmitoylphosphatidylcholine liposomes.

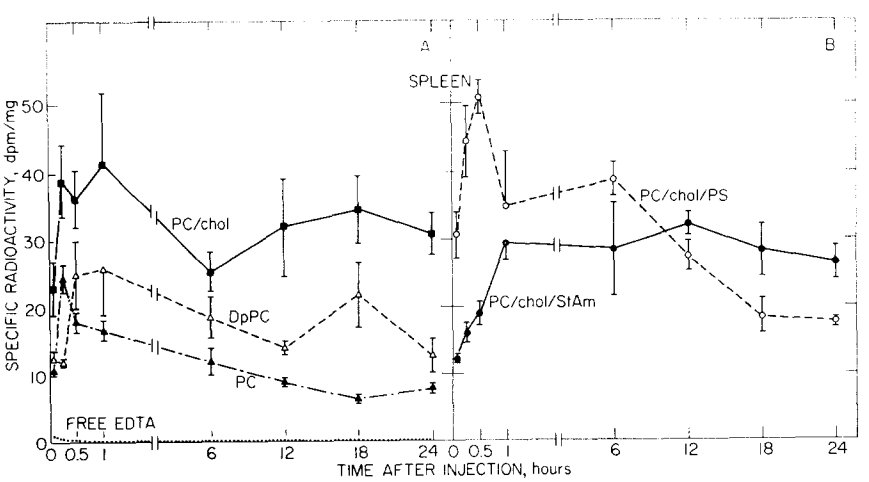

Fig. 2. See legend for Fig. 1 for details.

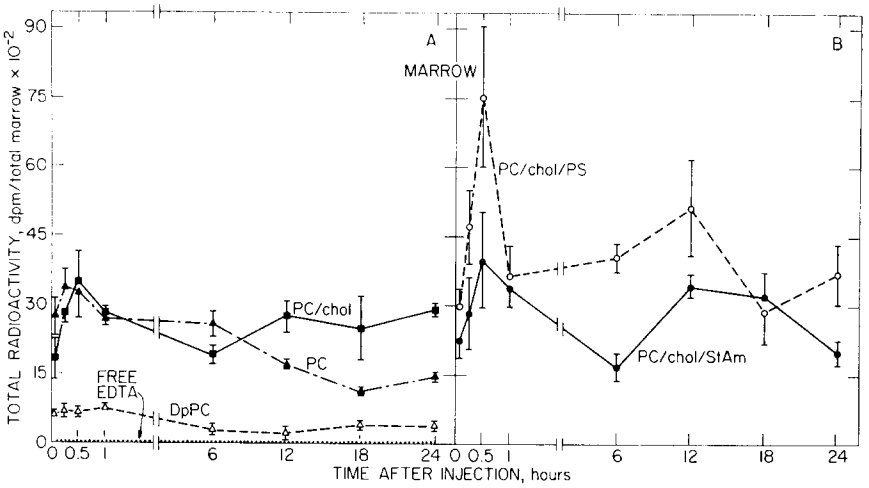

Fig. 3. See legend for Fig. 1 for details.

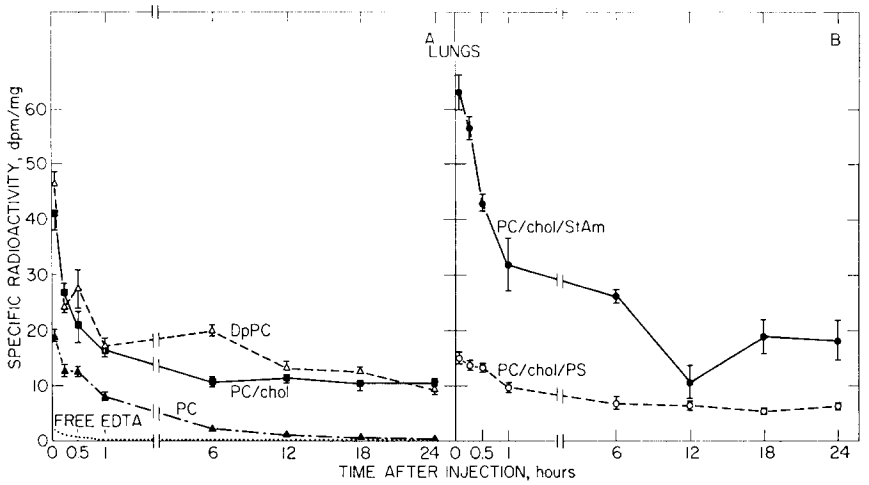

Fig. 4. See legend for Fig. 1 for details.

Brain. Brain uptake of EDTA from all liposome preparations was relatively low (Fig. 5). The highest brain EDTA levels from the phosphatidylcholine-cholesterol-stearylamine liposomes showed a similar retention pattern to that of the lungs in the same group of mice. An initial high uptake of EDTA from phosphatidylcholine-cholesterol-phosphatidylserine liposomes was observed in brain, but these levels decreased rapidly, whereas the uptake of encapsulated EDTA from liposomes composed either of dipalmitoylphosphatidylcholine or phosphatidylcholine alone was very low, similar to that of non-encapsulated EDTA.

Kidneys and blood. Kidney and blood levels of EDTA followed similar, rapid decay curves (Figs 6 and 7). High EDTA uptake by kidneys and blood from charged liposomes were found 5 min after injection. Liposomes composed of phosphatidylcholine-cholesterol also produced high initial EDTA uptakes in blood, while non-en-

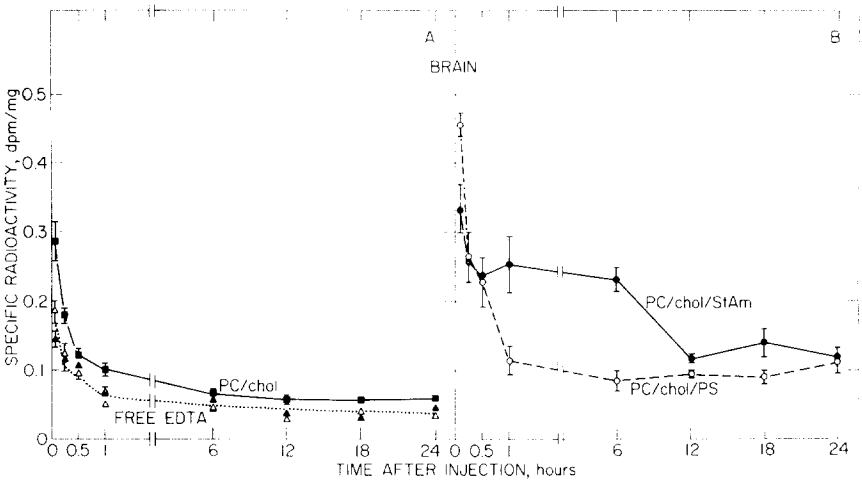

Fig. 5. See legend for Fig. 1 for details.

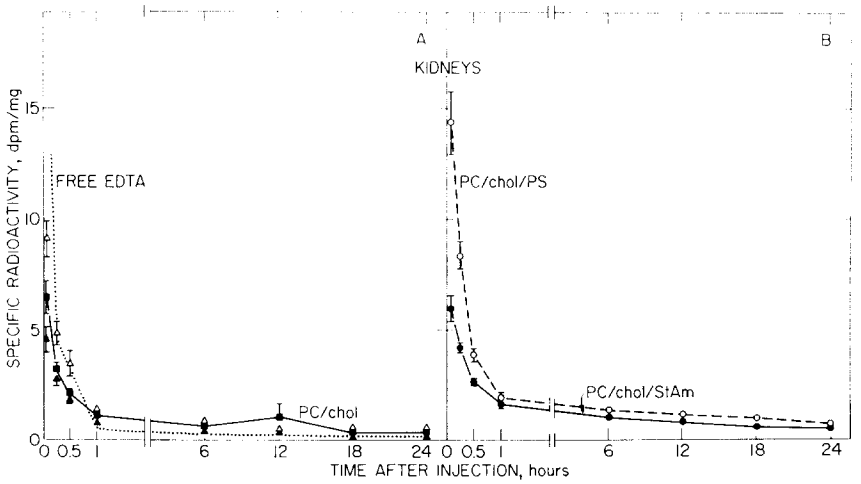

Fig. 6. See legend for Fig. 1 for details.

capsulated EDTA produced high initial EDTA uptakes in kidneys. The blood and kidney levels of EDTA produced by the different preparations reflected changes in circulating liposomal EDTA as it was either incorporated into other tissues or excreted in the urine.

DISCUSSION

Liposomes with different liposomal lipid compositions have produced different distribution patterns of EDTA in mouse tissues. These results demonstrate the influence of liposomal membrane surface properties upon interactions of liposomes with tissues.

The most striking findings of this investigation were the differences in tissue

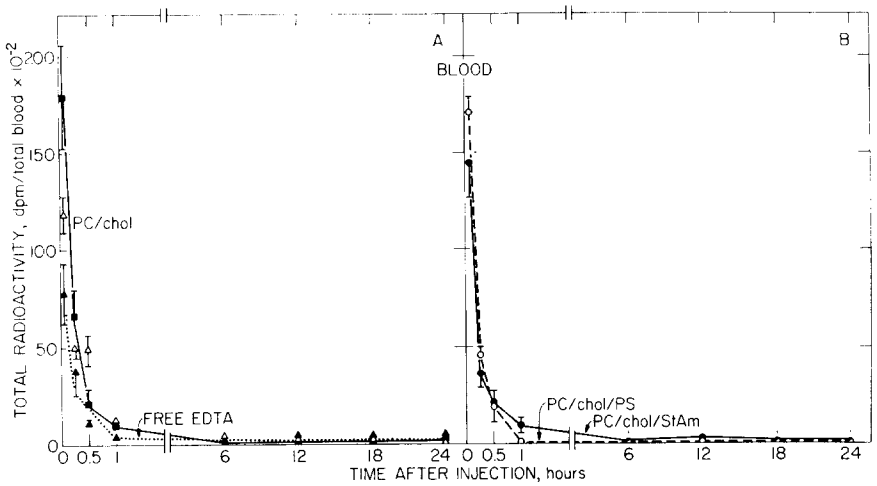

Fig. 7. See legend for Fig. 1 for details.

uptake of EDTA between the positively and negatively charged liposomes, especially in spleen, bone marrow, lungs, and brain. For example, the blood-forming tissues, spleen and marrow, rapidly accumulated high EDTA concentrations after injection of negatively charged liposomes, while lungs accumulated high concentrations from positively charged liposomes. The lungs incorporated very little EDTA from either negatively charged liposomes or neutral liposomes composed of phosphatidylcholine-cholesterol or phosphatidylcholine alone. This selectivity of EDTA uptake from charged liposomes observed in spleen, marrow, and lungs suggests different types of interactions between liposomes and tissues.

Other possible examples of tissue selectivity are found in these experiments. During the first hour after injection of positively charged liposomes, the slow increase in EDTA levels in spleen and marrow, and the concomitant decrease in EDTA levels in lungs, suggests some translocation of intact, positively charged liposomes. Because none of these liposomes were large enough to be entrapped [7], their temporary retention in the lungs may be caused by a lung-specific interaction with the positively charged liposomal membrane.

The higher brain uptake of EDTA from positively charged liposomes than that from the other preparations may represent increased crossing of the blood-brain barrier, due to a combination of the hydrophobicity and positive charge of these liposomes. Penetration of the blood-brain barrier by small molecules is generally considered to depend more upon lipid solubility than charge; however, positively charged dyes have been shown to have greater ability to cross the blood-brain barrier [17]. This apparent specific uptake of positively charged liposomes by brain requires further investigation.

The greater uptake by liver and spleen of EDTA from phosphatidylcholine-cholesterol liposomes than from the other neutral liposomes may indicate a preferential interaction of these tissues with liposomal membranes stabilized by cholesterol. The other tissues did not show a pronounced affinity for neutral liposomes containing cholesterol.

A specific interaction between the lung and dipalmitoylphosphatidylcholine in liposomal surfaces may exist. Analyses performed in Van Deenen's laboratory [18] showed that dipalmitoylphosphatidylcholine was the principal phosphatidylcholine in lungs of all animals examined. Lung uptake of EDTA from liposomes composed of dipalmitoylphosphatidylcholine alone was similar or slightly higher than that observed with phosphatidylcholine-cholesterol liposomes, although the dipalmitoylphosphatidylcholine liposomes produced the lowest liposomal EDTA uptake observed in liver and marrow. Liposomes prepared with all-saturated phospholipids may have decreased capacity to interact with some membrane systems, as has been observed *in vitro* [14, 19], but such liposomes, although of decreased fluidity, have been shown to be taken up by cells in culture [12]. In the present study, their degree of uptake appears to vary among the different tissues examined.

The rapid excretion and low recovery of non-encapsulated EDTA [7] indicates that if a small amount of free EDTA were associated with the liposome preparations, it did not significantly contribute to the labeled EDTA levels observed in tissues after injection of liposome-encapsulated EDTA. Since EDTA is not appreciably metabolized by animal tissues [2, 20], the radioactivity found in these studies should represent the true concentrations of EDTA in the tissues analyzed. Previous investigations of tissue distribution and retention of EDTA encapsulated in neutral liposomes composed of phosphatidylcholine-cholesterol (in a somewhat different molar ratio) showed 96 % of injected labeled EDTA could be recovered in mouse liver, spleen, marrow, lungs, brain, kidneys, and blood 5 min after injection [7]. The lower recoveries of injected liposomal EDTA in the present study may possibly be explained by an altered tissue distribution, resulting in incorporation of EDTA into tissues which were not examined. Because the two liposome preparations which showed the lowest recovery (dipalmitoylphosphatidylcholine and phosphatidylcholine) also lacked cholesterol, a component known to impart greater membrane stability, tests of liposomal leakage were performed. However, there was no correlation between *in vitro* release of liposomal EDTA and the observed *in vivo* uptake and recovery for the different liposome preparations. No measurable degradation in lipids of these liposomes was detected by thin-layer chromatography.

Bangham and coworkers [21] measured greater interlamellar aqueous volumes within negatively charged liposomes than within neutral, phosphatidylcholine liposomes. Greater interlamellar spaces should result in a greater incorporation of EDTA into charged liposomes; however, lower EDTA radioactivity was found in our preparations of charged liposomes (Table I). As mentioned in Results, large losses of charged liposomes occurred during our washing procedure by repeated centrifugations. Therefore, the EDTA incorporation reported in Table I is a practical indication of EDTA radioactivity yields per liposome preparation, and is not related to the innate volume differences between charged and neutral liposomes.

Because cell surfaces bear a net negative charge, greater uptake of a drug encapsulated in positively charged liposomes might be expected, but this has not been consistently observed in the present study, or in *in vitro* studies [13, 14, 19, 22, 23]. There is evidence, however, for non-homogeneous distribution of negatively charged groups of both normal and tumor cell surfaces [24, 25], as well as for inaccessibility to organic reagents of charged groups of liposomal surfaces [26]. A mediating influence of Ca^{2+} has been observed for fusion of negatively charged liposomes [19] and

for cell fusion induced by negatively charged liposomes [14]. Preferential interaction of immunoglobulins with positively charged liposomes requires a prior conformational change in the protein [13]. Specific interaction of a tissue with liposomes of a certain surface charge may therefore be a specific property of particular cell surfaces and cell environments. Thus, lungs and brain have an apparent affinity for positively charged liposomes, but spleen and marrow show affinity for negatively charged liposomes. The uptake patterns of the blood-forming tissues may reflect leucocyte surface interactions with liposomes; preliminary evidence has indicated that liposomal EDTA in spleen is entirely in the leucocyte fraction (Rahman and coworkers, unpublished).

Variations in kinetics of tissue uptake of EDTA from liposomes of different charges also suggest different interactions between liposomes and cell membranes. In all tissues examined but lungs, there is rapid and high EDTA accumulation from the negatively charged liposomes, but the highest EDTA levels decrease within 1 h after injection, while EDTA accumulated by liver, spleen, lungs, and brain from positively charged liposomes decreases at a relatively slow rate.

Liposome-encapsulated drugs show greater tissue uptake and prolonged retention than the non-encapsulated forms, allowing improved dosage regulation and increased therapeutic efficiency. Avoidance of undesirable side effects and drug inactivation by enzymatic degradation or other reactions are additional advantages of the use of liposome-encapsulated drugs. It is clear that the liposomal surface is an important determinant of liposome interactions with cellular membranes, and that modification of the liposomal surface is a potential method of directing liposome-encapsulated drugs to specific tissues. The variations in specificities of liposomal uptake observed in these investigations indicate a need for additional study of *in vivo* and *in vitro* interactions of liposomes with other membrane systems. The specificities of drugs encapsulated in liposomes of a particular bilayer composition could be used effectively both in therapeutic applications and in studies of membrane receptor sites. Knowledge of specific cellular-liposomal interactions and ability to prepare liposome-encapsulated molecules with desired surface properties would provide powerful tools for chemotherapy and basic biochemistry.

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