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# DISPOSITION OF INTACT LIPOSOMES OF DIFFERENT COMPOSITIONS AND OF LIPOSOMAL DEGRADATION PRODUCTS

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**Abstract**—Small unilamellar liposomes containing bovine serum albumin were prepared by a new double-emulsion technique and administered to mice and rats in intravenous injections. The elimination of intact liposomes, the association of phospholipid marker with lipoproteins, and the appearance of released internal marker and its degradation products were followed by column chromatography of plasma samples. In vitro labelled lipoproteins were administered to the animals in intravenous injections together with free bovine serum albumin and the elimination of the two substances was studied by closely related techniques. The clearance of intact PC : PS (4 : 1) liposomes from plasma was biphasic and much faster than that of labelled lipoproteins and bovine serum albumin either originating from liposomes or injected as such. The second elimination phase for these liposomes was barely detectable by our analytical methods. In contrast, DSPC : CHOL (2 : 1) liposomes showed a very significant second-phase elimination, with a half-life of 12 hr for the intact liposomes. In tissue distribution studies in mice, the major accumulation of liposomal markers was found in the liver and spleen, and less in the kidneys and intestinal wall. Uptake into liver and spleen appeared to be due to the uptake of intact liposomes, whereas the uptake into kidneys and gut wall was caused by the uptake of liposomal degradation products. The uptake of PC : PS (4 : 1) liposomes into the liver was higher than that of DSPC : CHOL (2 : 1) liposomes; the opposite was the case with their uptake into the spleen. In rats, too, liposomes of different compositions showed significant variations in stability and in plasma half-lives of intact liposomes. Generally, there was a considerable increase in the liposomal stability in the presence of cholesterol and when use was made of a phospholipid with a high transition temperature.

Much time has been devoted to the study of the elimination and tissue distribution of liposomes after i.v. injection in experiment a 1 animals. However, liposomes are generally unstable in the presence of plasma [1–10], and blood contains at any given time a mixture of intact liposomes, released internal marker, and liposomal degradation products. The composition of this mixture depends on the animal species, the time post injection and the liposomal properties, such as size, charge and lipid composition (cf. the recent review by Ryman and Tyrrell [11])

Liposomal integrity has been evaluated by several procedures [1–18], but on the whole no single method is superior to the other techniques. For entrapped substances with molecular weights below a few hundred thousand Dalton, column chromatography on Sepharose beads allows a simple study of liposomal intactness, phospholipid transfer, release of internal marker, and the metabolism of markers.

In tissue distribution studies, it is very important to use double-labelled liposomes and to subtract the radioactivity due to the blood background in each sample [18]. Furthermore, the tissue distribution pattern of liposomal transformation products should be known in order to distinguish between the uptake of liposome-associated markers and the uptake of transformation products [5, 6, 8–10, 13–15, 19–22].

In this paper we describe (1) the elimination of intact liposomes and metabolic products thereof from the plasma, and (2) the net tissue distribution of liposome markers following intravenous administration of liposomes prepared by a newly developed technique [18, 23, 24]. These new liposomes have a very high trapping capacity and a homogenous size distribution. BSA‡ was chosen as the entrapped marker to compare its uptake *in vivo* with our results on uptake mechanisms *in vitro* [18].

‡ *Abbreviations used:* BSA = bovine serum albumin; DSPC = distearoyl-L-α-phosphatidylcholine; HDL = high-density lipoprotein; PC = phosphatidylcholine; PS = phosphatidylserine; LMV = large multilamellar vesicles; SUV = small unilamellar vesicles; LUV = large unilamellar vesicles; CHOL = cholesterol.

## MATERIALS AND METHODS

*Materials.* Egg phosphatidyl choline (PC) and ox brain phosphatidyl serine (PS) were purchased from Lipid Products, Surrey, England; cholesterol

(CHOL) and distearoyl phosphatidyl choline (DSPC) were from Sigma, di[$^{14}C_1$]stearoyl-L-α-phosphatidyl choline ([$^{14}$C]DSPC) (spec. act. 60 mCi/mmol) was from Applied Science Laboratories, Pennsylvania, U.S.A., and [$^3$H]$NaBH_4$ (spec. act. 10–15 Ci/mmol) was from The Radiochemical Centre, Amersham, England. The phospholipids were > 99% pure (TLC). Bovine serum albumin (BSA), crystalline A grade, was from Calbiochem-Behring Corporation. Other chemicals and solvents were of analytical, or superior, grade.

$^3$H-labelled BSA was prepared essentially as described by Rice and Means [25], using [$^3$H]$NaBH_4$ as the radioactive agent instead of ($^3$H)HCHO. The specific activity obtained was 1–6 mCi/mg BSA, i.e. approx. 10% methylation of lysine groups [18].

*Preparation of liposomes.* Liposomes were prepared as described recently [23, 24]. Reversed micelles were formed in 5 : 1 dibutylether/cyclohexane for PC liposomes, and in 1 : 1 dibutyl ether/cyclohexane for DSPC liposomes [18, 24]. $^3$H-labelled BSA was diluted with unlabelled BSA and the buffers mentioned below to make 10 mg BSA/ml and 0.5–5 mCi/mg BSA, and used as the internal aqueous phase (30–40 μl/ml organic phase). [$^{14}$C]DSPC was added to phospholipid mixtures to obtain about 0.1 mCi/mmol phospholipid. The reversed micelles were dispersed in aqueous buffer in a volume ratio of 1 : 10: 140 mM NaCl 10 mM tris (pH 7.40) for PC containing liposomes and 10 mM tris (pH 7.40) for DSPC containing liposomes. After removal of organic solvents by evaporation, the phospholipid suspensions were centrifuged for 30 min at 37,000 *g* to remove any lipid aggregates, then passed through a DEAE-Sephadex or a Sepharose CL-6B column to remove non-entrapped BSA [18].

The entrapment yield was 60–100%, depending on the lipid composition, as determined by rocket immunoelectrophoresis after addition of 1% Triton X-100 to the liposomal suspension. Following centrifugation and chromatography the liposome preparations contained, typically, 10 μg BSA/ml and < 2 mol% lysolecithin (TLC). DPC liposomes were stored in the hypotonic buffer for stability reasons [24] and adjusted to isotonicity with 3 M NaCl prior to administration.

The size distribution of liposomes was calculated from electron micrographs of freeze-fracture replicas of spray-frozen liposome suspensions [18, 25]. Various batches of PC : PS (4 : 1), BSA liposomes showed size distributions with mean diameters of 36–44 nm, and typically with 70–80% of the liposomes having diameters of < 50 nm and 98–99% with diameters < 100 nm. DSPC : CHOL (2 : 1), BSA liposome preparations were somewhat larger, with mean diameters of 44–70 nm and with 10–60% below 50 nm, yet with 93–97% < 100 nm. These liposomes are stable with respect to size [24] and have now been followed for up to 2 years.

*Preparation of liposomal transformation products.* Liposomal transformation products, i.e. $^{14}$C-labelled high-density lipoprotein (HDL)-like structures and released $^3$H-labelled BSA, were prepared *in vitro* by incubating fresh mouse plasma with PC : PS (4 : 1), BSA liposomes at a final concentration of 2.5 μmol phospholipid/ml and 70% plasma for 16 hr at 37°. Transformation products were separated from intact liposomes by chromatography on a 1.5 × 32 cm Sepharose CL-6B column eluted with phosphate buffered saline [18]. Fractions containing $^{14}$C-labelled HDL-like lipoproteins and most of the released $^3$H-labelled BSA were pooled before administration to mice [18].

*Studies in vivo.* Female mice weighing 23–29 g were given i.v. injections of liposomes in a dose corresponding to 50 μmol phospholipid/kg body weight in a volume of 10 ml/kg body weight. The liposomal transformation products, $^{14}$C-labelled lipoproteins and free $^3$H-labelled BSA, corresponding to 8.2 μmol liposomal phospholipid and 56 μg/kg body weight, respectively, were injected as controls in a volume of 10 ml/kg body weight.

Three mice were anaesthetized with ether at various intervals for up to 24 hr, and as much blood as possible was drawn via a heart puncture into heparinized syringes. Blood samples were cooled on ice immediately, centrifuged within 5 min and then stored on ice until chromatography. The following organs/tissues were excised and sampled; cerebrum, cerebellum, thymus, lungs, heart, diaphragm, liver, kidneys, adrenals, stomach, and small intestines and colon freed of their contents.

Tissue samples were homogenized in a motor-driven Potter homogenizer, then sonicated 1 min in a Branson B-12 sonifier. The total tissue radioactivity was determined after incineration of homogenates in a Packard model 306 Oxidizer. Mouse serum albumin was determined in plasma and in the supernatants from tissue homogenates by immunodiffusion [26]. True tissue uptake was calculated after correction for radioactivity due to blood background in the tissue, as calculated from the serum albumin content [18]. An insignificant binding to blood cells (< 1%) was found at all sampling times.

In male rats weighing 145–155 g, elimination of liposomes of different compositions from the plasma was followed after the intravenous administration of 1 ml of liposomal suspension, corresponding to 50 μmol phospholipid/kg body weight.

Blood samples were drawn from the retro-orbital plexus into heparinized tubes prior to administration and at various intervals over 60 min after administration. Plasma radioactivity was counted directly and after chromatography at 4° on a 0.7 × 18 cm Sepharose CL-6B column with 140 mM NaCl, 10 mM tris (pH 7.4).

Double labelled samples of liposomes, plasma or chromatography fractions were counted in Aqualume (Lumac) in a Packard 3375 liquid scintillation counter in preset $^3$H/$^{14}$C channels. Correction for the quenching of samples and the contribution of $^{14}$C in the $^3$H channel was carried out by the external standard ratio method using 3 third-degree polynomial regression curves based on counting of radioactive standards containing increasing amounts of chloroform.

Samples from the Oxidizer recovered in Monophase-40 (Packard) and Carbo-Sorb + Permaflour (Packard) were counted in preset $^3$H/$^3$HQ and $^{14}$C/$^{14}$CQ channels. Quench correction was carried out by the external standard ratio method

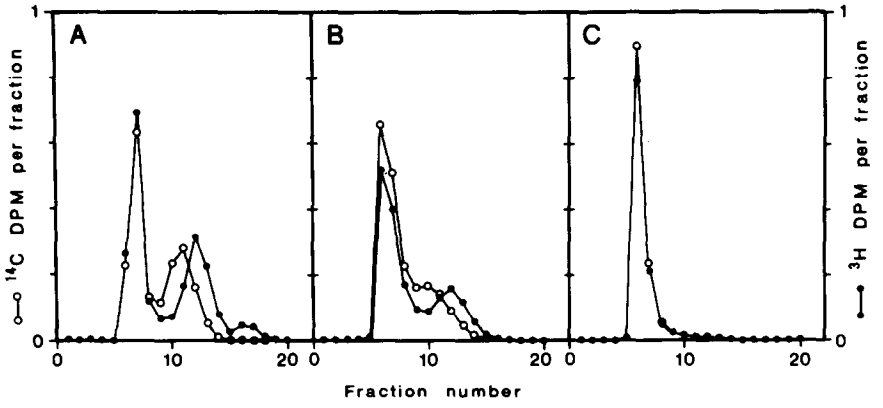


Fig. 1. Chromatography of plasma on Sepharose CL-6B. The Sepharose CL-6B (0.7 × 18 cm) columns were eluted with 140 mM NaCl, 10 mM Tris (pH 7.4). The columns were loaded with: (a) 100 μl of plasma obtained 15 min after i.v. administration of PC : PS (4 : 1) liposomes to a NMRI mouse. Blood was cooled in ice immediately, and all subsequent procedures were carried out at 0–4°. (b) 100 μl of plasma incubated *in vitro* with 1 μmol PC : PS (4 : 1), BSA liposomes per ml for 15 min at 37°, then cooled to 0–4°. (c) 100 μl of plasma incubated *in vitro* as in (b) but at 4°. 0.5 ml fractions were recovered and radioactivity was determined after addition of the scintillation cocktail. ○——○, $^{14}$C from DSPC; ●——●, $^{3}$H from BSA. Maximum ordinates for $^{14}$C/$^{3}$H were: 2000/30,000, 4000/60,000 and 5000/75,000 dpm fraction for (a), (b) and (c) respectively.

as above. The recovery of labelled standards incinerated with tissue homogenates was 98 ± 2% for both isotopes.

## RESULTS

After intravenous administration to mice of small unilamellar PC : PS (4 : 1) liposomes (mean diameter: 36 nm) containing $^{3}$H-labelled BSA and $^{14}$C-labelled phospholipids, chromatography of plasma samples showed that the liposomes rapidly lost their integrity. A typical elution profile is shown in Fig. 1(A). Both types of radioactivity associated with intact liposomes eluted in the void volume and the transformation products—HDL-like lipoproteins ($^{14}$C), free BSA and low-molecular-weight metabolites of BSA ($^{3}$H)—eluted in subsequent fractions. The liposome preparation contained neither free BSA nor low-molecular-weight BSA metabolites before injection. After 15 min of *in vitro* incubation at 37° at 1 μmol phospholipid/ml and at 85% serum concentration, the phospholipid transfer amounted to 20–23% and the BSA release was 20–30% (Fig. 1(B)) whereas at 4° no transfer or release was observed (Fig. 1C). During *in-vitro* incubations no low-molecular-weight metabolites of BSA were formed.

A similar loss of integrity has been shown repeatedly for corresponding markers in both multilamellar and unilamellar vesicles following intravenous injection or *in-vitro* incubation with serum proteins or whole blood [1, 11, 17, 18, 24, 30].

Intact PC : PS (4 : 1), BSA liposomes were rapidly eliminated from the circulation and were barely detectable 2 hr after the intravenous injection, as revealed by chromatography of plasma samples. The half-life of both liposome markers was 7 min, as determined from their concentrations in plasma 2–30 min after injection (Fig. 2). This value is considerably lower than the half-lives based on total radioactivity measured for negative SUV [5, 14, 15], except for the 1.2 min half-life reported for pure PS vesicles [20], and it approaches the half-lives of LMV and LUV [12–15, 17].

Free BSA and $^{14}$C associated with HDL-like lipoprotein could be seen already 2 min after injection.

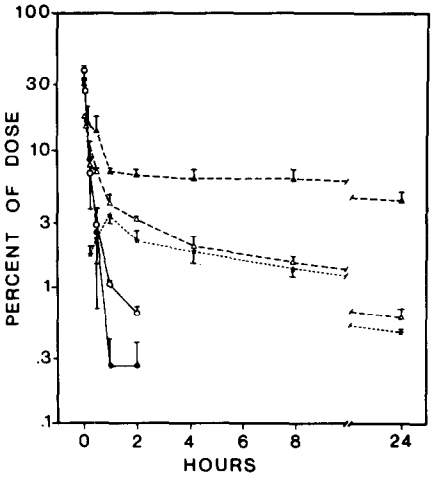


Fig. 2. Elimination of liposomes and their transformation products from mouse plasma after i.v. administration of 50 mol phospholipid/kg body weight of PC : PS (4 : 1), BSA liposomes containing [$^{14}$C]DSPC and [$^{3}$H]BSA. Intact liposomes were separated from phospholipid labelled HDL, released BSA and low molecular weight (LMW) metabolites of BSA by column chromatography as shown in Fig. 1. ○——○, $^{14}$C in intact liposomes; ●——●, $^{3}$H in intact liposomes; △——△, $^{14}$C in HDL-like particles; ▲——▲, $^{3}$H as intact BSA; X——X, $^{3}$H LMW metabolites of BSA. Mean values for 3 animals, with bars exceeding the symbols indicating S.D. Total plasma volume estimated at 42 l per g body weight.

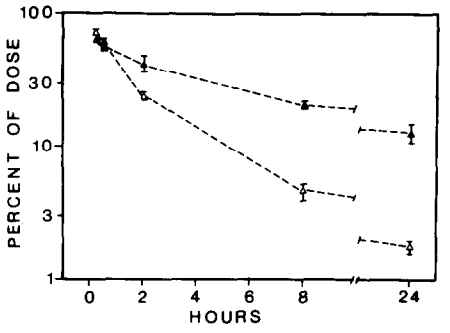


Fig. 3. Elimination of injected $^{14}C$ phospholipid-labelled HDL and $^{3}H$-labelled BSA from plasma. The dose administered corresponded to 8.2 μmol $^{14}C$ phospolipid and 56 μg $^{3}H$-labelled BSA per kg body weight. Chromatography and symbols as in Fig. 2.

BSA metabolites appeared after 15 min, attained their maximum proportion at 60 min, and were subsequently eliminated slightly faster than the released intact BSA (Fig. 2).

When liposomal transformation products, prepared *in vitro* from PC : PS (4 : 1), BSA liposomes, were injected in mice, column chromatography of plasma showed only one peak for $^{14}C$ eluting as HDL-like lipoproteins. The major part of tritium eluted throughout as free BSA, but a small second peak of low-molecular-weight metabolites was found 8 hr after injection. The disappearance of injected transformation products is shown in Fig. 3. The initial half-lives of labelled HDL and BSA were approx. 30 min, increasing to 12 and 24 hr, respectively, in agreement with our results with PC : PS (4 : 1), BSA liposomes (Fig. 2) and the results reported by others [28, 29].

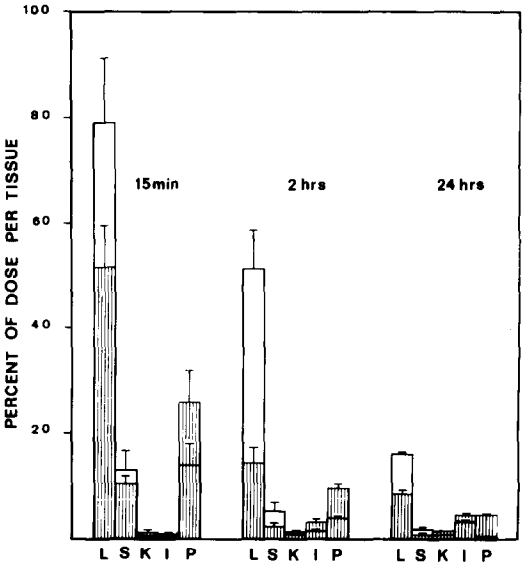


Fig. 4. Net distribution of radioactivity in tissue after i.v. administration to mice of PC : PS (4 : 1), BSA liposomes labelled with $^{14}C$-phospholipid in the membrane and containing entrapped $^{3}H$-labelled BSA. The per cent dose found per tissue as mean value for 3 animals. Total plasma content calculated from a plasma volume of 42 μl per g body weight. Open columns: $^{14}C$, hatched columns: $^{3}H$, L: liver, S: spleen, K: kidneys, I: intestines, P: plasma. Bars indicate the S.D. exceeding 0.2% of total.

Analysis of tissue distribution after administration of liposomes showed low tissue affinities in organs other than liver and spleen (Table 1). Because of their significant contribution to total body weight, the uptake by kidneys and intestines was also significant in terms of total uptake. Table 1 also shows

Table 1. Plasma retention by tissue and relative liposome affinities

| | μl plasma / g tissue | Relative affinity dpm recovered/g tissue / dpm injected/g body weight | |
|---|---|---|---|
| | $\bar{X} \pm$ S.D. ($n = 8$) | $^{14}C$ $\bar{X} \pm$ S.D. ($n = 3$) | $^{3}H$ $\bar{X} \pm$ S.D. ($n = 3$) |
| Cerebellum | 16 ± 10 | 0.06 ± 0.01 | 0.34 ± 0.02 |
| Cerebrum | 9 ± 4 | 0.06 ± 0.01 | 0.35 ± 0.06 |
| Thymus | 66 ± 20 | 0.18 ± 0.06 | 0.36 ± 0.09 |
| Heart | 125 ± 50 | 0.37 ± 0.29 | 0.86 ± 0.18 |
| Lungs | 149 ± 54 | 0.67 ± 0.07 | 0.42 ± 0.06 |
| Diaphragm | 73 ± 25 | 0.32 ± 0.16 | 0.43 ± 0.15 |
| Liver | 67 ±38 | 10.17 ± 1.27 | 2.82 ± 0.52 |
| Spleen | 76 ± 34 | 12.35 ± 4.85 | 4.65 ± 1.54 |
| Adrenals | 99 ± 49 | 0.80 ± 0.22 | 0.60 ± 0.14 |
| Kidneys | 107 ± 40 | 0.58 ± 0.09 | 0.96 ± 0.08 |
| Stomach | 51 ± 21 | 0.22 ± 0.16 | 0.47 ± 0.09 |
| Small intestines | 45 ± 22 | 0.22 ± 0.08 | 0.46 ± 0.08 |
| Colon | 58 ± 27 | 0.26 ± 0.12 | 0.59 ± 0.31 |

Blood background correction factors for organs and tissues calculated from the amount of mouse serum albumin found in tissue homogenates by immunodiffusion. Relative affinities of liposome labels calculated as the ratio of net dpm recovered per g tissue to net dpm injected per g body weight in animals examined 2 hr after i.v. injection of PC : PS (4 : 1) liposomes in doses corresponding to 50 μmol phospholipid per kg body weight.

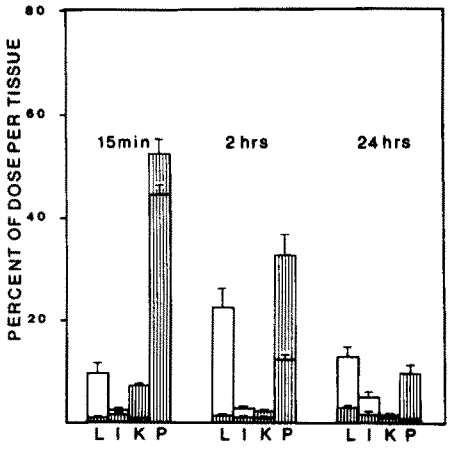


Fig. 5. Net tissue distribution of radioactivity after i.v. administration to mice of $^{14}$C-phospholipid labelled HDL and $^{3}$H-labelled BSA. Other details as in Fig. 4. The spleen contained less than 0.5% of the injected dose of both labels in all the animals examined.

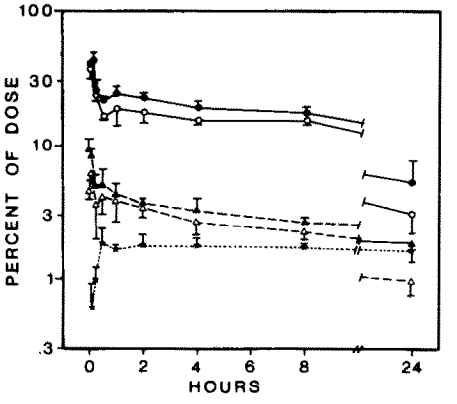


Fig. 6. Elimination of liposomes and transformation products from mouse plasma after i.v. administration of 50 μmol phospholipid per kg body weight of DSPC : CHOL (2 : 1), BSA liposomes containing [$^{14}$C]DSPC and [$^{3}$H]BSA. Chromatography carried out as in Fig. 1. Symbols as in Fig. 2.

the contents of plasma in tissues which were used for the individual calculations of the net uptake of liposomal markers by tissues. The variability of plasma content in tissues was high (30–50%).

Time-dependent tissue distribution of liposome markers calculated as the total amount of radioactivity due to intact liposomes and degradation products corrected for the radioactivity present in the plasma content of the tissue is shown in Fig. 4. The maximum uptake in liver and spleen was found 15 min after administration, whereas the uptake in kidneys and in intestines kept increasing for up to 24 hr.

Following administration of HDL and free BSA, the tissue distribution of markers showed a completely different pattern (Fig. 5). $^{14}$C-radioactivity from HDL showed a maximal uptake in liver of 22% after 2 hr. Tritium from BSA did not accumulate to any large extent, only kidneys showed an initial uptake > 5%. In spleen, the net uptake of both markers was < 1% at any time.

In an attempt to obtain more stable liposomes, we prepared small unilamellar liposomes of different lipid composition and studied them after intravenous administration to rats. The variation in their initial stability and their elimination from plasma are summarized in Table 2. Neutral fluid and solid liposomes containing cholesterol showed longer initial half-lives and an improved stability with respect to both phospholipid transfer and the release of internal marker.

The DSPC : CHOL (2 : 1), BSA liposomes (mean diameter 44 nm) were injected intravenously in mice and their elimination from plasma and tissue distribution examined for periods of from 2 min to 24 hr after injection.

Table 2. *In vivo* stability and plasma clearance of intact liposomes of various compositions

| Liposome composition* (molar ratio) | | PC : PS (4 : 1) | DSPC : PS (4 : 1) | PC : CH (2 : 1) | DSPC : CH (2 : 1) |
|---|---|---|---|---|---|
| Number of batches (number of rats) | | 2 (7) | 1 (3) | 1 (3) | 3 (10) |
| % initial transfer/release at 2 min (mean ± S.D.) | $^{14}$C-DSPC | 36 ± 7 | 32 ± 7 | 11 ± 3 | 1 ± 2 |
| | $^{3}$H-BSA | 40 ± 8 | 79 ± 4 | 27 ± 5 | 11 ± 3 |
| Initial half-life of intact liposomes (2–15 min after inj.) | | 6 min | 9 min | 45 min | 30 min |

* All liposomes contained entrapped BSA.

Liposomes injected in rats in doses corresponding to 50 μmol phospholipid per kg body weight as described in Materials and Methods. Sampling times: – 5, 2, 5, 15, 30 and 60 min after injection. Intact liposomes, transfer of phospholipid marker to HDL, and release of BSA determined by chromatography on Sepharose CL-6B columns, as illustrated in Fig. 1.

Figure 6 shows that, just as in rats (Table 2), the plasma stability of DSPC : CHOL (2 : 1), BSA liposomes was superior to the stability of PC : PS (4 : 1), BSA liposomes following intravenous administration to mice. Most of the radioactivity found in plasma was accounted for by the intact liposomes. The initial distribution/tissue uptake phase over the first 30 min was followed by an elimination phase, with a half-life of 12 hr estimated from concentrations at 1–24 hr after administration. Released BSA and phospholipid-labelled HDL accounted for 12–18% of the total plasma radioactivity and were eliminated in parallel to the intact liposomes. DSPC : CHOL (2 : 1) liposomes prepared by ultrasonication have previously been shown to be rather stable in plasma after intravenous injection [5], and so were sphingomyelin/cholesterol liposomes [16].

The net uptake of liposomal markets in whole organs/tissues was negligible in organs other than liver, spleen, kidneys and intestines [18] when appropriate corrections were made for the radioactivity due to blood background in each sample. The plasma contents of tissues were similar to the values given in Table 1 (data not shown). Fig. 7 shows the uptake of liposomal markers in liver, spleen, kidneys and intestines 15 min, 2 hr and 24 hr after injection. The spleen showed maximal uptake at 15 min, the liver at 2 hr [18], whereas the uptake in kidneys and intestines kept increasing over 24 hr. In the liver and spleen, the uptake of the $^{14}C$ lipid label generally equalled, or exceeded, that of tritium from BSA, which was also observed after intravenous injection of PC : PS (4 : 1), BSA liposomes.

## DISCUSSION

For an appropriate comparison of the elimination of different types of liposomes from plasma it is necessary to separate intact liposomes from their transformation products. Throughout the present studies we have chromatographed plasma samples on Sepharose CL-6B columns. This permitted the separation of plasma $^{14}C$-radioactivity into two fractions: intact liposomes and HDL-like lipoproteins, and the separation of plasma $^{3}H$-radioactivity into three fractions: intact liposomes, free BSA and low-molecular-weight BSA degradation products. We have thus reduced the uncertainty about the nature of the plasma radioactivity prevalent in earlier studies [5–7, 9, 12–16, 19, 20].

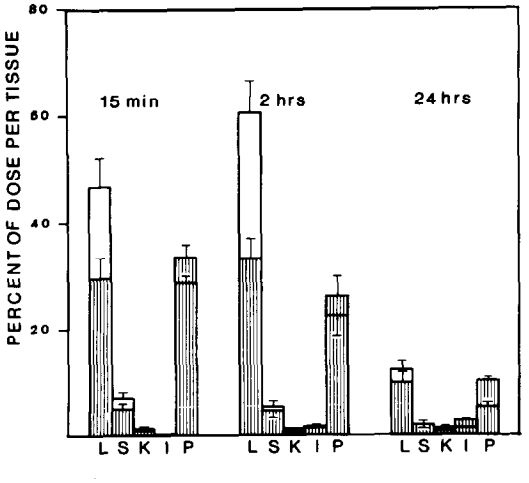


Fig. 7. Net tissue distribution of radioactivity after i.v. administration to mice of DSPC : CHOL (2 : 1), BSA liposomes containing [$^{14}C$]DSPC and [$^{3}H$]BSA. Ordinates and abbreviations as in Fig. 4.

To study the tissue specific uptake of liposomes after intravenous injection in mice, the distribution patterns of liposomal radioactive markers after administration of liposomes were compared to the distribution patterns obtained after injection of the transformation products, i.e. free BSA and lipoprotein-bound phospholipid. Since the uptake of tritium from free BSA in liver and spleen remained very low for up to 24 hr post injection, it was concluded that the significant $^{3}H$ accumulation in liver and spleen was due to the uptake of intact liposomes. After i.v. injection, there was a considerable uptake of both liposomes in the parenchymal cells of rat liver [18], as judged by the uptake of entrapped BSA. The accompanying paper [31] shows in detail the uptake of DSPC : CHOL (2 : 1), BSA liposomes at different intervals post i.v. injection.

Likewise, the uptake by liver of the liposome membrane marker was much more rapid from liposomes than from *in vitro* labelled lipoproteins. This shows that, contrary to what has been suggested earlier [14], the phospholipid marker does not need to be transferred to lipoprotein-like structures to be taken up in liver. In spleen, the phospholipid label found after HDL administration was in fact negligible.

With increasing time intervals after injection some of the excess uptake of phospholipid label, as compared to the uptake of label from the entrapped BSA, might have been due to uptake by labelled lipoproteins. However, most of the difference might have been caused by (1) direct exchange of phospholipids between liposomes and cells and (2) loss of entrapped BSA during cell-liposome intraction. These two mechanisms have been shown to take place *in vitro* during incubation of cell cultures (hepatocytes, macrophages, fibroblasts or endothelial cells) with these liposomes [18].

Low-molecular-weight degradation products from the entrapped $^{3}H$-labelled BSA were found in plasma already 15 min post injection, indicating a rapid extravascular/intracellular degradation of liposomes even after administration of the more stable DSPC : CHOL (2 : 1), BSA liposomes. Any similar metabolites were virtually absent from plasma for up to 8 hr post injection of free BSA.

In organs other than liver, spleen, kidneys and intestines, the net uptake of liposome markers following injection either of liposomes or of their transformation products was very insignificant, though they did contain appreciable amounts of radioactivity before correction, which advocates the necessity of subtracting the radioactivity due to blood background.

The net uptake found in kidneys and intestines continued to rise after the complete disappearance of intact PC : PS (4 : 1), BSA liposomes from plasma

(at around 1–2 hr), and it is therefore concluded that the major portion of radioactivity taken up by these organs stems from the labelled transformation products and only a minor portion (if any) from the intact liposomes.

Following intravenous injection of both PC : PS (4 : 1), BSA and DSPC : CHOL (2 : 1), BSA liposomes to mice, the uptake of the BSA label exceeded that of the lipid label in the kidneys and in the intestines, whereas the ratio of uptake of the two labels was the reverse in the liver and spleen, indicating a similar mechanism of tissue uptake of labels for the two types of liposomes.

We have studied the dose dependency of plasma and tissue contents of labels from DSPC : CHOL (2 : 1), BSA liposomes in mice at 7 dose levels, from 1 to 100 μmol phospholipid per kg [18]. With increasing dose levels, the relative amount of intact liposomes in plasma increased 3-fold whereas there was a 60–40 percent decrease in liver uptake when the dose was increased from 10 to 100 μmol phospholipid per kg. The uptake in spleen showed a broad maximum around 10 μmol phospholipid per kg while the uptake in kidneys and intestines showed no consistent dose dependency.

The nature of the liposome labels in tissues has not been examined. The tritium from BSA was slowly eliminated via the kidneys after injection of both types of liposomes, whereas more than one-half of the lipid label was recovered within the first day in the breath as $^{14}CO_2$ [18].

Our *in vitro* studies included transfer of phospholipids to lipoproteins and release of sucrose, inulin, insulin or BSA from liposomes of 8 different lipid compositions (DSPC or PC × with/without CHOL × with/without PS). We found no correlation between the extent of transfer and the release [24]. Four of the more stable types of these liposomes were injected intravenously in rats. The results led to the conclusion that neutral cholesterol-containing liposomes are relatively stable and their elimination is slow [5] whereas negative cholesterol-free liposomes are relatively unstable and their clearance rapid [15]. Like the results *in vitro* [24], there was no correlation between transfer and release, or between these and the disappearance rate, although the more stable neutral cholesterol-containing liposomes disappeared at a slower rate.

The transfer of labelled DSPC from intravenously administered DSPC : CHOL (2 : 1), BSA liposomes was less pronounced in rats than in mice both shortly after injection (Fig. 6 vs Table 2) and later on [31]. Preliminary data on PC : PS (4 : 1), BSA liposomes have not shown the same trend. In mice, the phospholipid transfer from DSPC : CHOL (2 : 1), BSA liposomes seemed to take place throughout the time they were in circulation, since the disappearance of labelled lipoproteins was slower after administration of liposomes than after injection of prelabelled lipoproteins (cf. Figs. 3 and 6). This might also explain why more BSA seemed to be recovered with the fraction containing intact liposomes than with the fraction containing labelled phospholipids.

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