Hepatic uptake and degradation of unilamellar sphingomyelin/cholesterol liposomes: A kinetic study

(lipid vesicles/kinetic modeling/lysosomal degradation/controlled release)

KARL J. HWANG, KUEN-FAI S. LUK, AND PAUL L. BEAUMIER

Department of Pharmaceutical Sciences, BG-20, University of Washington, Seattle, Washington 98195

Communicated by John D. Baldeschwieler, April 8, 1980

ABSTRACT The kinetics of hepatic uptake and degradation of sphingomyelin/cholesterol (2:1, M/M) small unilamellar liposomes were investigated in a BALB/c mouse. The tissue distribution of liposomes was determined by scintillation spectrometry. The percentage of intact liposomes in tissues was estimated by the technique of γ -ray perturbed angular correlation. A kinetic model was developed to analyze the above data. A remarkable agreement was noted between the experimental data and the corresponding theoretical values. Our results indicate that the sphingomyelin/cholesterol unilamellar liposomes had an unusually long half-life of 16.5 hr in the circulation after intravenous administration to mice. The hepatic degradation of the liposomes *in vitro* at 37°C followed first-order kinetics, with a half-life of 3.5 ± 0.2 (SEM) hr. Furthermore, the rate of the *in vivo* degradation of liposomes in the liver was found to be quite similar to that *in vitro*, with a half-life of 3.6 ± 0.4 hr. The rate of release of the liposome-encapsulated agent, indium-111, in the liver was not constant, and reached a maximum at about 8 hr after the administration of liposomes. The approach developed in the present study is general and can be applied to the investigation of factors that may control the release of pharmacologically active agents in any tissue.

The potential applications of liposomes as carriers for pharmacological agents has been increasingly recognized (1-6). However, before liposomes can be used therapeutically in humans, more must be known about the fate of the carrier liposomes *in vivo*. To achieve this goal, the kinetics of the uptake and degradation of liposomes in tissues are among the most important properties to be characterized. Recent studies (7, 8) suggest that it is possible to determine the percentage of intact liposomes remaining in circulation, in tissues, or even in a living organism by the technique of γ -ray perturbed angular correlation (PAC).

The PAC technique provides information on the tumbling rate of ^{111}In by measuring the angular correlation of the two successive γ rays emitted from ^{111}In . The PAC spectrometer provides a parameter, called the time-integrated perturbation factor or $\langle G_{22}(\infty) \rangle$, which has a value ranging from 0 to 1.0. A fast tumbling rate of ^{111}In is characterized by a high $\langle G_{22}(\infty) \rangle$ value close to 1, whereas a slow tumbling rate of ^{111}In is characterized by a low $\langle G_{22}(\infty) \rangle$ value close to zero. By use of PAC to study the breakdown of liposomes, a small rapidly tumbling complex, $^{111}\text{In}^{3+}$ -nitrilotriacetic acid, is encapsulated in liposomes. Upon the breakdown of the liposomes, the $^{111}\text{In}^{3+}$ is released. It rapidly binds to a macromolecule in the surrounding solution and consequently shows a decreased tumbling rate (7). Thus, from the measurement of the change in the tumbling rate or the $\langle G_{22}(\infty) \rangle$ of the encapsulated $^{111}\text{In}^{3+}$ it is possible to determine whether the liposomes taken up by tissues are still intact.

It appears that by combining the data of the structural integrity of liposomes in tissues with the distribution in various

organs, the rate constants involved in hepatic uptake and degradation of liposomes may be determined by the approach of least-squares analysis, with an appropriate model. The present study describes the application of a three-compartment model to determine the kinetics of the uptake and degradation of unilamellar liposomes in the liver, assuming first-order kinetics for all rate processes.

MATERIALS AND METHODS

Bovine brain sphingomyelin (SM), type I, L- α -dipalmitoyl phosphatidylcholine (DPPC), L- α -distearoyl phosphatidylcholine (DSPC), and cholesterol (CH) were purchased from Sigma. The trisodium salt of nitrilotriacetic acid was obtained from Aldrich. All buffers and solutions were prepared with sterile, deionized water purified with the Milli-Q Reagent Grade Water System (Millipore). Carrier-free indium-111 chloride, obtained from Medi + Physics (Emeryville, CA), was purified on an anion-exchange column by a slight modification of a described method (7). Briefly, the indium-111 chloride was acidified to 2 M HCl and applied to an AG1-X8 (Bio-Rad) column that had been equilibrated with 2 M HCl. The $^{111}\text{In}^{3+}$ was first eluted with two bed volumes of 2 M HCl and then with two bed volumes of 0.1 M HCl. Fractions were collected in Nalgene beakers and evaporated to dryness by a heat lamp. Rabbit serum was prepared as reported (7) and stored frozen until use.

Liposome Preparation. SM or DPPC or DSPC and CH at a molar ratio of 2:1 were dissolved in 2:1 (vol/vol) chloroform/methanol. The solvent was removed under a stream of nitrogen at 40°C. The dried film of lipid was further kept at reduced pressure overnight. A suspension of unilamellar liposomes in 1 mM nitrilotriacetic acid/106 mM isotonic phosphate solution, pH 7.4, was prepared by sonicating the lipid mixture with a Branson sonifier (model 350) at an output of 50 W for 15 min at 45°C (7). A preparation of unilamellar liposomes of homogeneous size was isolated by centrifugation at $180,000 \times g$ for 1 hr (9). Nonencapsulated nitrilotriacetic acid chelate was separated by passage over a Sephadex G-50 column equilibrated in 0.9% NaCl/5 mM acetate, pH 5.5. To encapsulate indium-111 ions, $^{111}\text{In}^{3+}$ was delivered to the encapsulated nitrilotriacetic acid chelate by means of a mobile ionophore, 8-hydroxyquinoline (oxine). The lipid-soluble $^{111}\text{In}^{3+}$ -oxine was prepared as reported (10). The details of this procedure will be described elsewhere. Briefly, 25 μl of $^{111}\text{In}^{3+}$ -oxine in ethanol was incubated with 1 ml of liposomes for 1 hr at room temperature. The loaded liposomes were passed over a small column of AG1-X8 (in phosphate form) to remove any remaining indium-111 that did not enter the interior of liposomes. From the analysis of the size of the loaded liposomes in a Separose 4B column, the size distribution of liposomes is not affected by the loading process (data not shown).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: PAC, γ -ray perturbed angular correlation; SM, sphingomyelin; DPPC, L- α -dipalmitoyl phosphatidylcholine; DSPC, L- α -distearoyl phosphatidylcholine; CH, cholesterol.

Blood Background Correction. About 1 ml of fresh mouse blood was collected in 0.1 ml of 114 mM citrate (pH 4.8). The erythrocytes were washed with 0.9% NaCl/5 mM sodium phosphate, pH 7.4 (P_i /NaCl) by centrifugation at $1000 \times g$ for 10 min at 4°C three times to remove the citrated plasma and buffy coat. The washed cells were incubated with $25 \mu\text{l}$ of $^{111}\text{In}^{3+}$ -oxine in ethanol for 1 hr at room temperature (11). The labeled erythrocytes were washed four times with P_i /NaCl and resuspended in the original citrated mouse plasma. The labeled erythrocytes were administered to mice via the tail vein. After 10 min, the mice were killed by cervical dislocation and immediate decapitation. The various organs and tissues of each mouse were isolated, rinsed with saline, blotted, and weighed; radioactivity was measured in a γ counter (Packard). The blood volume expressed as a percentage of the body weight was determined from the total administered activity and the specific activity of the sampled blood. A blood background correction factor was calculated for each organ or tissue and expressed as a ratio of radioactivity of the indium-111-labeled erythrocytes in the organ or tissue to the total radioactivity in the blood. In all tissue distribution studies, the radioactivity due to the blood background in each sample was subtracted.

In Vivo Studies. BALB/c mice (20–25 g) were weighed and given approximately 30–100 μCi of $^{111}\text{In}^{3+}$ -nitrilotriacetic acid encapsulated in 0.8–2.4 mg of SM/CH liposomes in a volume of 100–300 μl via the tail vein. The mice were killed at various times. The excised liver was placed in modified Ringer's solution (12) supplemented with 20% (vol/vol) rabbit serum, 100 units of penicillin per ml, and 100 μg of streptomycin per ml, and coincidence counts were determined immediately in the PAC spectrometer at room temperature (7). For investigation of the hepatic degradation of liposomes *in vitro*, the coincidence counts of ^{111}In in the liver removed from an animal killed 15 min after injection were measured in a sample holder at 37°C over a period of 20 hr. The remaining body parts were isolated and the radioactivity was measured.

KINETIC ANALYSIS

The uptake and degradation of the SM/CH unilamellar liposomes in tissues were analyzed by means of a kinetic model. For simplicity, the body is subdivided into three major compartments: blood, liver, and other organs.

The basic assumption of the analysis is that all rate processes involved are first order. Excretion is not considered in the present model because the renal and fecal excretion of radioactivity is negligible (about 0.5% of the injected dose in a 23-hr period). The amount of intact liposomes, expressed as the percentage of administered dose in the blood and in the liver, is denoted by $A(B)$ and $A(L)$, respectively. The amount of ra-

dioactivity released from the degraded liposomes to the circulating blood and the liver is represented by $D(B)$ and $D(L)$, respectively. The hepatic uptake of intact liposomes from the blood is described by a rate constant k_1 , whereas intact liposomes in the liver may return to the blood stream with a rate constant of k_{-1} . Radioactivity released from the degraded liposomes in the liver may return to the circulating blood with a rate constant of k_3 . The uptake of intact liposomes and the uptake of the radioactivity released from degraded liposomes by other tissues and organs are denoted by rate constants of k_4 and k_5 , respectively. The degradation of liposomes in the liver and in the blood is designated by rate constants of k_2 and k_6 , respectively.

Based upon the model, the rate equations for the four observed parameters of the radioactivity of intact and degraded liposomes in the circulating blood and liver are shown below.

$$\begin{aligned} dA(B)/dt = & -k_1(A(B)) + k_{-1}(A(L)) \\ & - k_4(A(B)) - k_6(A(B)) \end{aligned}$$

$$dA(L)/dt = k_1(A(B)) - k_{-1}(A(L)) - k_2(A(L))$$

$$dD(L)/dt = k_2(A(L)) - k_3(D(L))$$

$$dD(B)/dt = k_3(D(L)) - k_5(D(B)) + k_6(A(B))$$

The solutions at any time t were obtained by means of the IMSL (International Mathematical and Statistical Library) subroutine, DVERK, which uses a Runge-Kutta method based on Vernier fifth- and sixth-order pairs of formulas (13). From these solutions, the total amounts of radioactivity in the blood and in the liver and the time-integrated perturbation factor of the excised liver samples could be calculated. In calculating the $\langle G_{22}(\infty) \rangle$ values, the following equation (7) was used:

$$\langle G_{22}(\infty) \rangle_t = X_t \langle G_{22}(\infty) \rangle_{\text{intact}} + (1 - X_t) \langle G_{22}(\infty) \rangle_{\text{degraded}}, \quad [1]$$

in which X_t represents the percentage of intact vesicles in the liver at the time t after injection. The $\langle G_{22}(\infty) \rangle$ values, 0.59 and 0.12, corresponding to intact and completely disrupted vesicles, respectively, were used. These theoretical results were compared with the corresponding experimentally measured data at various times after injection. Because vesicles remained intact in the circulating blood for at least 24 hr, the rate constant for liposomal degradation in the blood, k_6 , was set at zero and was not varied. The other rate constants were then varied systematically until the sum of the squares of the deviation between the corresponding experimental and theoretical values was minimized. The instantaneous hepatic degradation rate of liposomes was evaluated by means of the final set of rate constants determined by the above least-squares fitting program with no weighting function.

To determine the *in vitro* hepatic degradation rate of liposomes in the excised liver sample at 37°C , we assumed the same kinetic model, whereas only the experimental PAC data were used in the fitting analysis. All computations were performed by means of the CDC 6400 computer system, maintained by the Academic Computer Center of the University of Washington.

RESULTS

Blood Background Correction Factors. Blood background correction factors for various organs and tissues, expressed as percentages $\pm$ SD of total radioactivity in the blood, are presented in Table 1. The blood volume was $8.0 \pm 0.1\%$ of the total body weight. This value agrees closely with previously reported values (14, 15). As illustrated in Table 1, a significant amount

Table 1. Blood background correction factors for organs and tissues in a BALB/c mouse

Tissue	Blood correction factors* (n = 6)
Liver	2.6 ± 0.6
Kidney	0.7 ± 0.1
Spleen	0.6 ± 0.1
Heart	0.7 ± 0.2
Lung	0.8 ± 0.1
Stomach	0.1 ± 0.1
Intestine	0.6 ± 0.2
Fat	0.3 ± 0.1
Tail	0.8 ± 0.2
Skin	1.9 ± 0.3
Brain	0.1 ± 0.04
Legs	1.6 ± 1.0
Carcass	10.1 ± 0.5

* Expressed as a percentage ± SD of radioactivity in the blood.

of radioactivity from the blood remained in certain tissues. To assess the actual amount of liposomes taken up by tissues, the blood background correction is necessary.

Stability and Biodistribution of SM/CH Unilamellar Liposomes. The stability of SM/CH unilamellar liposomes was determined by monitoring the amount of the encapsulated $^{111}\text{In}^{3+}$ -nitrilotriacetic acid complex released. By the technique of PAC, the $\langle G_{22}(\infty) \rangle$ value of freshly prepared SM/CH unilamellar liposomes was 0.59 ± 0.02 . No change in this value was noted upon incubation of the liposomes with an equal volume of rabbit serum at 37°C for up to 5 days. After addition of Triton X-100, $\langle G_{22}(\infty) \rangle$ dropped to 0.12, indicating the release of the encapsulated indium-111 and subsequent binding to serum proteins. These findings suggest that the release of encapsulated materials in SM/CH unilamellar liposomes is negligible in the presence of serum.

After intravenous injection of SM/CH unilamellar liposomes into mice, the distribution of the liposome-encapsulated radioactivity in 14 tissues at four representative time points was shown in Table 2. The $\langle G_{22}(\infty) \rangle$ value of the blood samples was 0.59 ± 0.02 regardless of when the mice were killed. This indicated that all the radioactivity observed in the blood samples was from intact liposomes. About 30% of the administered liposomes were cleared from the blood within 15 min after administration of liposomes. However, more than 50% of the administered liposomes still remained in the circulation 12 hr after injection. As calculated from the data in Fig. 2 by least-

Table 2. Time course of biodistribution of injected liposomal radioactivity in mice

Tissue	Percentage ± SD of administered dose at various times after injection			
	0.25 hr (n = 4)	3.0 hr (n = 4)	12.0 hr (n = 2)	23.0 hr (n = 3)
Blood	71.5 ± 7.0	65.6 ± 1.1	50.9 ± 7.0	28.4 ± 7.5
Liver	5.3 ± 2.2	6.6 ± 0.2	14.7 ± 1.1	27.5 ± 6.8
Kidney	1.1 ± 0.1	1.6 ± 0.4	1.5 ± 0.3	2.2 ± 0.3
Spleen	0.2 ± 0.1	0.3 ± 0.1	1.1 ± 0.4	1.9 ± 0.2
Heart	0.9 ± 0.3	0.9 ± 0.2	0.7 ± 0.2	0.7 ± 0.2
Lung	2.9 ± 0.9	2.1 ± 0.5	1.1 ± 0.3	1.2 ± 0.4
Stomach	0.4 ± 0.1	0.3 ± 0.1	0.6 ± 0.2	0.5 ± 0.1
Intestine	3.0 ± 0.7	3.9 ± 0.4	4.2 ± 0.2	6.8 ± 1.1
Fat	0.2 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	1.0 ± 0.1
Tail	2.5 ± 0.5	1.8 ± 0.6	1.6 ± 0.5	2.6 ± 0.6
Skin	2.6 ± 0.7	4.3 ± 0.3	6.2 ± 2.0	8.3 ± 3.1
Brain	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
Legs	2.3 ± 0.5	2.8 ± 0.3	3.3 ± 0.3	4.7 ± 1.5
Carcass	11.8 ± 2.1	10.0 ± 1.0	10.4 ± 0.4	12.1 ± 2.0

FIG. 1. *In vitro* degradation of SM/CH liposomes in an excised liver at two different temperatures. The liver was removed from a mouse killed 15 min after administration of liposomes. The $\langle G_{22}(\infty) \rangle$ values of the liver sample were measured at 37°C (X) and 24°C (Δ). Predicted $\langle G_{22}(\infty) \rangle$ values (O—O) were calculated from Eq. 1 and the first-order *in vitro* rate constant, k_2 , listed in Table 3. The percentage of intact liposomes corresponding to the $\langle G_{22}(\infty) \rangle$ values is shown on the right axis.

squares fitting, the elimination half-life of SM/CH liposomes was 16.5 hr.

***In Vitro* Hepatic Degradation of Liposomes.** The extent of degradation of SM/CH unilamellar liposomes taken up by liver was determined by PAC. The high $\langle G_{22}(\infty) \rangle$ values of the livers excised from mice killed 15 min after injection indicated that little degradation had occurred at that early time point. Hence, the continuing degradation of the vesicles in the excised liver could be followed by monitoring the change of the $\langle G_{22}(\infty) \rangle$ values. The percentage of intact liposomes that remained in the liver can then be determined by Eq. 1. The *in vitro* degradation of the intact vesicles in a single liver sample with time at two different temperatures is depicted in Fig. 1. The rate of degradation of liposomes in liver decreases as the temperature decreases. This may imply that the degradation is an enzymatic process. The kinetic analysis of the rate of the release of $^{111}\text{In}^{3+}$ from liposomes in the liver at 37°C (Fig. 1) suggests that hepatic degradation of liposomes follows first-order kinetics with a half-life of 3.5 ± 0.2 hr (Table 3).

***In Vivo* Hepatic Degradation.** Based upon the model described above and the least-squares kinetic analysis, the rate constants of the various processes of uptake and degradation of liposomes are shown in Table 3. To ensure that the global minimum was found, more than one set of initial guesses of the rate constants were used to check that a better fit had not been overlooked. It was found that the rate constants could vary as much as $\pm 10\%$, depending on the initial conditions used to solve the system of differential equations. The best fit was obtained

Table 3. Calculated rate constants*

Rate constant	<i>In vitro</i> , [†] 1/hr	<i>In vivo</i> , [‡] 1/hr
k_1	—	0.024 ± 0.005
k_{-1}	—	0.016 ± 0.016
k_2	0.200 ± 0.010	0.195 ± 0.015
k_3	—	0.000 ± 0.001
k_4	—	0.023 ± 0.003
k_5	—	0.000 ± 0.001

* Average of two independent determinations.

[†] The *in vitro* hepatic degradation rate constant, k_2 , was obtained by fitting the $\langle G_{22}(\infty) \rangle$ values of the excised liver at 37°C for the model while the rest of the rate constants were held equal to zero.

[‡] The rate constants were obtained by fitting all of the data obtained *in vivo* simultaneously to the model.

FIG. 2. Clearance of total radioactivity from intact and degraded liposomes from the blood. The experimental data (X) are compared with the predicted values (O—O) calculated from the rate constants listed in Table 3.

by using the values of the experimental data extrapolated to time zero [i.e., $A(B) = 74.1$, $A(L) = 2.57$, $D(B) = 0.0$, and $D(L) = 0.0$]. These values were similar to the blood background in the corresponding organ.

By using the rate constants in Table 3 it was possible to predict the total amount of radioactivity from intact and degraded liposomes present in the blood (Fig. 2) and in the liver (Fig. 3) and the $\langle G_{22}(\infty) \rangle$ values of the liver (Fig. 4). Excellent agreement between the predicted values and the experimental observations is apparent. It appears that the *in vivo* degradation of liposomes in the liver obeys the first-order kinetics as well. The half-life of the *in vivo* hepatic degradation of liposomes was 3.6 ± 0.4 hr. This is almost identical to the results obtained from *in vitro* measurements of an isolated liver. The instantaneous hepatic degradation rate of liposomes at various times after administration (Fig. 5) was calculated from the rate constants in Table 3. This is an extremely important parameter because it is related to the amount of the encapsulated agent available in the liver for pharmacological action at any time after injection. Our results indicated that the degradation rate of SM/CH small unilamellar liposomes is maximal 8 hr after injection.

DISCUSSION

We have demonstrated that important kinetic parameters of liposome degradation can be determined from a combination of PAC measurements and time-dependent tissue distribution data. The mathematical model used to fit the experimental data is very general and can be applied to the study of liposome degradation in any organs or tissues. The apparent good fit between the experimental data and the theoretically calculated values suggests that the present model is sufficient to describe the dynamics of the *in vivo* degradation of liposomes in mouse liver. Conceivably, the approach described above will be useful for investigating factors that control the release of pharmacologically active agents in tissues.

The recent exciting results on the dramatically improved efficacy of liposome-encapsulated antimonials in the chemotherapy of leishmaniasis (4–6) strongly suggest that liposome-entrapped drugs will be useful in the therapy of other intracellular parasitic diseases, such as Chagas disease (16), in which the causative agent is *Trypanosoma cruzi*. In using drug carriers as a means of chemotherapy of intracellular parasitic diseases, one of the most important factors is the bioavailability of the encapsulated drug. Presumably, only the released drugs will be effective. Thus, the present investigation of the kinetics of the cellular degradation of liposomes could provide a rational

FIG. 3. Accumulation of total radioactivity from intact and degraded liposomes of the liver. The experimental data (X) are compared with the predicted values (O—O) calculated from the rate constants listed in Table 3.

means of refining the conjectural approach to therapy, in which the response is contingent upon the dose and toxicity of the drugs and liposomes (17).

Zierenberg and Betzing (18) studied the hepatic uptake of labeled phospholipids in liposomes and found that the hepatic uptake rate constant for polyenyl phosphatidylcholine liposomes after intramuscular administration into rats was $0.1 \pm 0.02 \text{ hr}^{-1}$, which is about 5 times faster than the hepatic uptake rate of SM/CH liposomes (Table 3). It was also noted that both k_3 and k_5 are zero. The implication is that the released radioactivity remained within the liver and did not reenter the bloodstream presumably due to the rapid binding of released indium ion to macromolecules in the liver.

The apparent first-order rate constant, k_2 , for hepatic degradation of liposomes is, in fact, the sum of several rate constants. The breakdown of liposomes by cells involves a variety of mechanisms, such as phagocytosis, fusion, lipid exchange, and absorption (19). Isolation of individual processes *in vivo* is probably difficult. Further experiments will be needed to determine whether these rate constants can be determined under *in vitro* conditions, permitting a quantitative estimation of the relative contributions of these mechanisms to *in vivo* hepatic degradation of liposomes.

The remarkable similarity between the half-lives of the hepatic degradation of liposomes *in vitro* and *in vivo* was unex-

FIG. 4. Observed and predicted values of $\langle G_{22}(\infty) \rangle$ of liposomes in the livers of mice. The observed $\langle G_{22}(\infty) \rangle$ values (X) were determined as described in *Materials and Methods*. The predicted $\langle G_{22}(\infty) \rangle$ values (O—O) were calculated from Eq. 1 and the solutions of the rate equations using the rate constants listed in Table 3. The percentage of intact liposomes corresponding to the $\langle G_{22}(\infty) \rangle$ values is shown on the right axis.

FIG. 5. Predicted instantaneous degradation rate of SM/CH (2:1) liposomes in the liver of a BALB/c mouse at various times after administration of liposomes. The calculation was based on the rate constants listed in Table 3.

pected, given the very different circumstance of the livers in the two cases. DeDuve and Beaufray (20) found that the progressive release of lysosomal enzymes preceded general necrosis when liver lobes in rats were rendered ischemic by ligation. It is likely that under the conditions of ischemia in the excised liver, early acute intracellular release of lysosomal enzymes may occur. The similar rate constants of the *in vitro* and *in vivo* hepatic degradation of liposomes suggest that the processes of phagosome-lysosome fusion and subsequent enzymatic degradation of liposomes are not greatly affected by the present *in vitro* condition of incubation, whereas temperature seems to have a profound effect on these processes.

One of the most interesting findings of the present investigation is that SM/CH small unilamellar liposomes remained intact throughout the time of our study (at least 23 hr). This means that SM/CH unilamellar liposomes in the circulating blood are not affected by phagocytic leukocytes (21), by interaction with serum proteins (22), or by complement-mediated lysis (23, 24). The blood clearance half-life of about 16.5 hr observed in the present study is by far the longest clearance time ever reported for liposomes of natural sources.

In similar experiments, the blood clearance half-life of $^{111}\text{In}^{3+}$ -loaded DPPC:CH (2:1, M/M) unilamellar liposomes was 9.5 ± 1 hr. Mauk and Gamble found that DSPC/CH (2:1, M/M) unilamellar liposomes remained intact in serum at 37°C for at least 48 hr (8). To compare the difference in the blood clearance half-life, we encapsulated $^{67}\text{Ga}^{3+}$ -nitrotriacetic acid in DSPC/CH (2:1, M/M) unilamellar liposomes and injected them simultaneously with the $^{111}\text{In}^{3+}$ -loaded SM/CH (2:1, M/M) unilamellar liposomes into the same group of mice. The blood clearance half-life of the DSPC/CH liposomes was about twice as fast as that of the SM/CH liposomes. Furthermore, the control experiments showed that neither the loading procedure nor the difference in isotopes affected the tissue distribution of liposomes. Thus, in every case we examined, the half-life of SM/CH liposomes in circulation was longer than those of DPPC/CH or DSPC/CH liposomes.

Juliano and Stamp (25) reported that the plasma level of unilamellar bovine phosphatidylcholine/CH vesicles followed a simple exponential decay with a half-life of about 80 min. By using the relatively impermeant methotrexate, Kimelberg (26) showed that about 50% of the injected dose of methotrexate encapsulated in egg phosphatidylcholine/CH (59:41, M/M) unilamellar liposomes remained in the circulation of monkeys

4 hr after injection. Deshmukh *et al.* (27) found that liposomes composed of the dialkyl analogue of phosphatidylcholine and cholesterol, which are presumably semiresistant to catabolic enzymes, can prolong the clearance rate in mice of encapsulated maltose. However, about 40% of the initial level of liposomes in blood remained in 60 min. In our study, more than 90% of the initial amount of intact SM/CH liposomes remained in circulation 1 hr after injection. The long life time of SM/CH liposomes in circulation makes this system an ideal candidate for further investigation, especially in the area of *in vivo* targeting.

We thank Ms. Janet Merriam for valuable technical assistance. This work was supported by U.S. Army Medical Research and Development Command Contract DAMD 17-78-C-8049, American Heart Association of Washington Grant-in-Aid 78-W-517, and a new initiative fund from the University of Washington Diabetes Research Center Grant AM 17047 from the National Institutes of Health.

- Gregoriadis, G. (1976) *N. Engl. J. Med.* **295**, 704-710.
- Gregoriadis, G. (1976) *N. Engl. J. Med.* **295**, 765-770.
- Tyrrrell, D. A., Heath, T. D., Cooley, C. M. & Ryman, B. E. (1976) *Biochim. Biophys. Acta* **457**, 259-302.
- Alving, C. R., Steck, E. A., Chapman, W. L., Jr., Waits, V. B., Henricks, L. D., Swartz, G. M., Jr. & Hanson, W. L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2959-2963.
- Black, C. D. V., Watson, G. J. & Ward, R. J. (1977) *Trans. R. Soc. Trop. Med. Hyg.* **71**, 550-552.
- New, R. R. C., Chance, M. L., Thomas, S. C. & Peters, W. (1978) *Nature (London)* **272**, 55-56.
- Hwang, K. J. & Mauk, M. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4911-4955.
- Mauk, M. R. & Gamble, R. C. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 765-769.
- Barenholz, Y., Gibbes, D., Litman, B. J., Goll, J., Thompson, T. C. & Carlson, F. D. (1977) *Biochemistry* **16**, 2806-2810.
- Hwang, K. J. (1978) *J. Nuclear Med.* **19**, 1162-1170.
- Welch, M. J., Thakur, M. L., Coleman, R. E., Patel, M., Siegel, B. A. & Ter-Pogossian, M. M. (1977) *J. Nuclear Med.* **18**, 558-562.
- Krebs, H. A. (1950) *Biochim. Biophys. Acta* **4**, 249-269.
- Hull, T. E., Enright, W. H. & Jackson, K. R. (1976) *User's Guide for DVERK—A Subroutine for Solving Non-Stiff ODEs*, Technical Report No. 100 (Department of Computer Science, Univ. of Toronto).
- Mitruka, B. M., Rawusley, H. M. & Vodehra, B. V. (1977) in *Clinical, Biochemical, and Hematological Reference Values in Normal Experimental Animals* (Masson, New York), p. 6.
- McDougal, I. R., Dunick, J. K., McNamee, M. G. & Kriss, J. P. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3487-3491.
- Newton, B. A. (1974) *Ciba Found. Symp.* **20**, 285-307.
- Alving, C. R. & Steck, E. A. (1979) *Trend Biochem. Sci.* **4**, N175-N177.
- Zierenberg, O. & Betzing, H. (1979) *Drug Res.* **29**, 494-498.
- Pagano, R. E. & Weinstein, J. N. (1978) *Annu. Rev. Biophys. Bioeng.* **7**, 435-468.
- DeDuve, C. & Beaufray, H. (1959) *Biochem. J.* **73**, 610-616.
- Gregoriadis, G. & Bucklad, R. A. (1973) *Nature (London)* **244**, 170-172.
- Dawson, R. M. C. (1968) in *Biological Membranes*, ed. Chapman, D. (Academic, London), Vol. 1, pp. 203-232.
- Kinsky, S. C. (1972) *Biochim. Biophys. Acta* **265**, 1-23.
- Humphries, G. K. & McConnell, H. M. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1691-1694.
- Juliano, R. L. & Stamp, D. (1975) *Biochem. Biophys. Res. Commun.* **63**, 651-658.
- Kimelberg, H. K. (1976) *Biochim. Biophys. Acta* **448**, 531-550.
- Deshmukh, D. S., Bear, W. D., Wisniewski, H. W. & Brocheroff, H. (1978) *Biochem. Biophys. Res. Commun.* **82**, 328-334.