

## Distribution and Fate of Synthetic Lipid Vesicles in the Mouse: A Combined Radionuclide and Spin Label Study

(liposomes/tempocholine/pertechnetate)

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**ABSTRACT** Single compartmental spherules of various lipid constituents (vesicles), enclosing  $^{99m}\text{TcO}_4^-$  as a radioactive marker, were injected intravenously into  $\text{C}_3\text{H}$  mice, and the distribution of radioactivity was studied. About 25% of the administered radioactivity was present in the liver 5 min and 30 min after the injection of vesicles composed of phosphatidylcholine and gangliosides, which were sonicated for 5 min (standard preparation). About 10–20% of the radioactivity remained in the circulation. By use of a nonradioactive spin label (tempocholine) enclosed within vesicles, intact vesicles were demonstrated in the circulation for 46 min after intravenous injection. The distribution of radioactivity from  $^{99m}\text{TcO}_4^-$  inside vesicles is very different from that of free  $^{99m}\text{TcO}_4^-$  or of  $^{99m}\text{Tc}$  sulfur colloid.

Increase in the length of sonication or incorporation of cholesterol into the wall of the vesicles enhanced hepatic levels and reduced blood levels of radioactivity. These same manipulations also slowed the rate of transfer of  $^{99m}\text{TcO}_4^-$  out of vesicles in dialysis experiments *in vitro*. Addition of phosphatidic acid, phosphatidylethanolamine, or phosphatidylserine to the standard constituents did not greatly alter the distribution of radioactivity *in vivo* but did increase the number and type of active coupling sites on the outside of the vesicle. The results indicate that vesicles might be valuable as carriers of diagnostic or therapeutic agents.

Vesicles, single-compartment spheres bounded by a continuous lipid bilayer membrane, have provided an experimental model for biomembranes (1, 2). Despite the considerable knowledge of the properties of vesicles *in vitro* (3–5), there is no information of their behavior *in vivo*. This communication describes the distribution and fate of single-compartment vesicles injected intravenously in the mouse. A mixture of phosphatidylcholine and gangliosides has been used to make a standard preparation, and the distribution of standard vesicles has been compared with those made of phosphatidylcholine alone, or those whose composition was altered by various added ingredients. The vesicles were prepared so that spin label, tempocholine chloride, and/or a radioactive ion,  $^{99m}\text{TcO}_4^-$  (pertechnetate), were contained within their interior space. In some instances the studies *in vivo* have been paralleled by dialysis experiments to demonstrate *in vitro* the rate of transfer of  $^{99m}\text{TcO}_4^-$  across bilayers of different lipid compositions.

Abbreviation: EPR, electron paramagnetic resonance.

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## MATERIALS AND METHODS

Phosphatidylcholine (lecithin) was prepared by the method of Singleton *et al.* (6) and further purified by chromatography on silicic acid. Gangliosides, phosphatidic acid, and phosphatidylserine were purchased from Supelco, Bellefonte, Pa. (catalogue numbers 04-0632, 04-6012, and 04-6004, respectively). Phosphatidylethanolamine was bought from Calbiochem, La Jolla, Ca. and cholesterol from the Sigma Chemical Co., St. Louis, Mo. The intravesicular radionuclide marker was  $^{99m}\text{Tc}$  as pertechnetate ( $^{99m}\text{Tc}$ , half-life 6 hr) eluted from a  $^{99}\text{Mo}$ -Molybdenum generator (New England Nuclear Corp., Boston, Mass.). Spin label, tempocholine chloride [*N,N*-dimethyl-*N*-(2',2',6',6')-tetramethyl-4'-piperidyl]-2-acetoxyethyl ammonium bromide], was kindly supplied by Prof. H. M. McConnell. Free  $^{99m}\text{TcO}_4^-$  or  $^{99m}\text{Tc}$  sulfur colloid, prepared by the method of Patton *et al.* (7), were used as controls.

**Standard Vesicle Preparation.** Lecithin (44  $\mu\text{moles}$ ) in ethanol and 4  $\mu\text{moles}$  of gangliosides in chloroform-methanol (1:1) were mixed in a 50-ml round-bottomed flask and evaporated to dryness with a Buchler flash evaporator over a water bath at 38°–40°. The dried lipids were redissolved in 1 ml of toluene, and the mixture was evaporated to dryness again. Remaining traces of solvent were evaporated under a continuous stream of nitrogen. The dried lipids were suspended in 1.3 ml of salt buffer consisting of 3.5 mM  $\text{Na}_2\text{HPO}_4$ ; 1.5 mM  $\text{NaH}_2\text{PO}_4$ ; 145 mM  $\text{NaCl}$ ; 15 mM  $\text{KCl}$ ; 1 mM  $\text{MgSO}_4$ ; 1 mM  $\text{CaCl}_2$ ; and 10 mM dextrose. The pH of the buffer was adjusted to 7.1. The lipids were completely dispersed in the buffer solution with a Vortex mixer, producing a milky solution consisting of multilamellar liposomes. When  $^{99m}\text{TcO}_4^-$  was required as an intravesicular marker, it was added to the liposome mixture in a 0.1-ml volume (containing from 1 to 3 mCi) to give a total volume of 1.4 ml. The liposome solution was transferred to a 10-ml round-bottomed glass test tube, held in ice to maintain a constant cold temperature, for sonication, which was undertaken with a 4-mm diameter probe sonicator (Biosonik, Bronwill Scientific, Rochester, N.Y.). The change from multilamellar liposomes to bilayered vesicles was characterized by a clearing of the turbidity.

Vesicles were separated by passage through a Sephadex G25 (fine) column (11  $\times$  0.7 cm). When  $^{99m}\text{TcO}_4^-$  was present, the opalescent vesicles containing a proportion of the entrapped radiopharmaceutical were eluted from the column ahead of, and separate from, the free radiopharmaceutical. The collected fractions were placed on ice, and the radio-

activity in each fraction was determined in a well-type scintillation counter (Packard autogamma Spectrometer Series 410A or a Nuclear Chicago Radiation Analyzer model 1810). The vesicle fractions were pooled and kept on ice until use.

**Variations from Standard Preparation.** The chemical composition of the standard vesicle preparation was modified in five ways. First, phosphatidylcholine (88  $\mu$ moles) was used alone to make vesicles. The other four variations were the addition of one of the following: 32.8  $\mu$ moles of cholesterol, 2.8  $\mu$ moles of phosphatidic acid, 6.7  $\mu$ moles of phosphatidylethanolamine, or 6.3  $\mu$ moles of phosphatidylserine, to the standard preparation of 44  $\mu$ moles of phosphatidylcholine and 4  $\mu$ moles of gangliosides.

**Vesicles Containing Nonradioactive Spin Label.** Vesicles for spin label studies were prepared as described for the standard vesicle preparation, with the exception that 1 ml of 0.08 M tempocholine chloride in buffer solution was added to the lipids before sonication. This relatively high concentration of spin label was used to insure that a signal could be detected from vesicles recovered from the blood in experiments *in vivo*. Vesicles containing tempocholine were separated from the free spin label by gel filtration on a Sephadex G-25 (fine) column.

Electron paramagnetic resonance (EPR) spectra of the spin-labeled samples were obtained with a Varian model E-4 Spectrometer. Samples of vesicles, or blood containing vesicles, in glass capillary tubes were mounted in the variable temperature accessory of the spectrometer, and most spectra were taken at 0°. The relative intensity of the tempocholine signal was measured as the peak-to-peak height of the derivative line due to the tempocholine (8). Sodium ascorbate in a final concentration of 33 mM was added to selectively destroy, by reduction, any signal due to tempocholine outside the vesicles. Triton X-100, in a final concentration of 0.01% (w/v), was used to rupture vesicles, both in buffer solution and in mouse blood. Triton X-100, by rupturing vesicles, releases any enclosed tempocholine and changes the EPR signal from a protected to an unprotected one (8).

**Studies in Mice with Vesicles Containing  $^{99m}\text{TcO}_4^-$ .** Before each study, C<sub>3</sub>H mice (average weight, 25 g) bred at Stanford Medical Center were anesthetized with an intraperitoneal injection of 1.2 mg of pentobarbitone. Three mice of the same sex, age, and weight were used for each time point. An accurately measured volume of the vesicle preparation (prepared freshly immediately before each experiment) was injected into the tail vein of the mouse (volume range of injected material, from 0.05 to 0.4 ml). A measured sample of the vesicles was retained as a standard for radionuclide counting. Five minutes and 30 min after the vesicle injection, three mice were killed with an intravenous injection of pentobarbitone, which caused almost instantaneous death. Samples of blood, muscle, fat, liver, spleen, left and right kidney, heart, lung, and stomach were obtained from each mouse and weighed in preweighed plastic counting tubes. Urine was obtained whenever possible. After the radioactivity in the tubes was determined, results were expressed as percentage of the injected dose per organ and per g of tissue.

Control studies were undertaken with intravenous injections of  $^{99m}\text{TcO}_4^-$  in saline and  $^{99m}\text{Tc}$  sulfur colloid, to illustrate similarities to or differences from, the fate of intravesicular  $^{99m}\text{TcO}_4^-$ . Free  $^{99m}\text{TcO}_4^-$  is distributed in the extracellular fluid and is selectively concentrated in the thyroid, gastric

mucosa, salivary glands, and choroid plexus, and partially excreted into the urine and feces (9). The amount of radioactivity in the stomach was used as an index of the permeability of intravenously injected vesicles to the radiopharmaceutical contained therein. Intravenously administered radiocolloid is rapidly removed from the blood by reticuloendothelial phagocytic cells, predominantly in liver, spleen, and bone marrow.

The effect of varying the sonication time on the distribution of injected standard vesicles was also determined. A preparation of liposomes was divided into four equal volumes, which were sonicated for 1, 3, 7, and 12 min, respectively. After sonication, gel filtration separation was undertaken as described for each sample. Vesicle samples were injected intravenously into three mice, all of which were killed 5 min after injection. Tissue sampling, determination of radioactivity, and calculations of results were as described above.

**In Vitro Studies with Vesicles Containing  $^{99m}\text{TcO}_4^-$ .** As an independent test of vesicle permeability *in vitro*, diffusion dialysis was used to measure the rate of release of  $^{99m}\text{TcO}_4^-$  into the dialysate bath.  $^{99m}\text{TcO}_4^-$ -labeled vesicles (0.3–0.5 ml) were pipetted into dialysis bags fashioned from 0.5-inch (1.27-cm) Union Carbide cellophane tubing that had been boiled before use to prevent nonspecific adhesion of vesicles to the tubing. Each bag was placed in 50 ml of continuously stirred buffer. At intervals of 1, 2, 5, 10, 15, 20, 25, 30, 45, and 60 min, 1-ml samples of buffer were removed and placed in individual counting tubes. At 60 min, the dialysis bag was removed, dried, and placed in a counting tube. The radioactivity in each of the samples was then determined. For each withdrawal time, the percentage of radioactivity remaining in the dialysis bag (within the vesicles) was calculated.

**In Vivo Studies with Vesicles Containing Spin Label.** Vesicles containing tempocholine chloride and free tempocholine chloride were injected intravenously into two groups of mice, respectively. Serial blood samples of 100  $\mu$ l each were withdrawn from the cavernous sinus of each mouse with 100- $\mu$ l capillary tubes coated with heparin as an anticoagulant. EPR spectra were made on the blood samples before and after the addition of Triton X-100.

## RESULTS

The mice tolerated well the intravenous injection of vesicles containing either  $^{99m}\text{TcO}_4^-$  or tempocholine, and no experimental deaths could be attributed to the injections.

EPR spectra were used to characterize the integrity of the vesicles before injection and after their recovery in mouse blood. Tempocholine trapped within intact vesicles at a concentration of 0.08 M gives a broadened EPR signal of low amplitude, due to spin exchange (Fig. 1A). When the vesicles are ruptured by Triton X-100, the spin label is released into the external medium. The sharp three-line spectrum shown in Fig. 1B is characteristic of dilute tempocholine. The increase in signal amplitude after Triton X-100 treatment thus provides a sensitive measure of vesicle integrity. The relative signal height of the vesicle preparation was increased seven times by the addition of Triton X-100, proving that the vesicles were intact when they were administered. Spectra obtained from the blood of a mouse 5 min after the intravenous injection of vesicles containing spin label are shown in Fig.

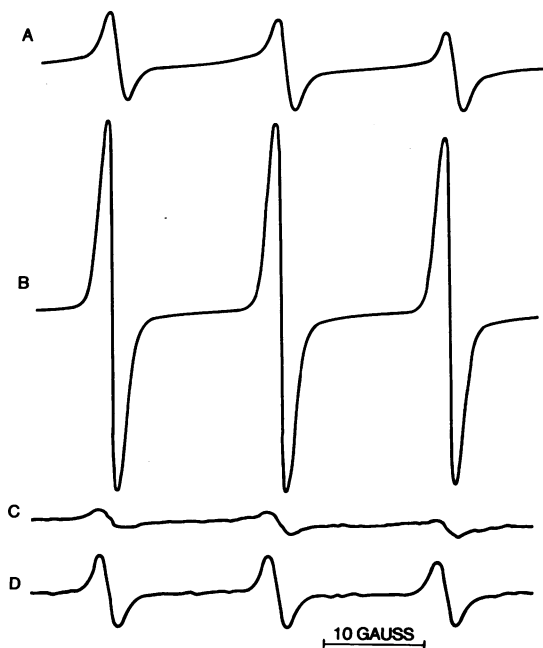


FIG. 1. EPR signals from: (A) standard vesicle preparation containing 0.08 M tempocholine before injection into mouse; (B) standard vesicles after treatment with Triton X-100 to release enclosed tempocholine (signal amplitude increased seven times); (C) blood from mouse 5 min after injection of vesicles containing tempocholine; (D) blood from mouse 5 min after injection of vesicles treated with Triton X-100 to release tempocholine (signal amplitude increased four times).

1C and D; the former is the signal from untreated blood and the latter the signal from blood to which Triton X-100 was added. The increase in signal amplitude after the addition of Triton X-100 verifies that a large proportion of the vesicles remained intact *in vivo* at the 5-min interval. By this technique, intact vesicles have been demonstrated in the circulation as long as 46 min after they were injected. Free tempocholine chloride could be detected in blood for as long as 2 hr after its administration to mice. The addition of Triton X-100 to such blood samples had no effect on the amplitude of the signal.

Table 1 shows the tissue distribution of intravenously injected vesicles containing  $^{99m}\text{TcO}_4^-$  measured 5 min and 30 min after the administration of the vesicles. Five minutes after injection about 25% of the injected dose was in the liver, 2% was in the stomach, and 7% per g of blood remained circulating. Measurements to ensure that all the injected radionuclide could be accounted for were not undertaken in every experiment, but whenever they were, more than 90% of the dose could be recovered. The tissue distribution of vesicles containing  $^{99m}\text{TcO}_4^-$  is quite different from that of free  $^{99m}\text{TcO}_4^-$ , the latter exhibiting a higher gastric accumulation and a lower hepatic uptake (Fig. 2). The figure also illustrates the difference in tissue distribution between vesicles containing  $^{99m}\text{TcO}_4^-$  and  $^{99m}\text{Tc}$  sulfur colloid. Radiocolloid is found predominantly in the liver, and blood activity is virtually zero within 5 min of the intravenous injection. The distribution of vesicles in animals anesthetized with barbiturate was not significantly different from that of unanesthetized controls.

In our earlier experiments the preparation of the vesicles was stopped as soon as the milky appearance of the liposome suspension cleared. However, using this method we noted

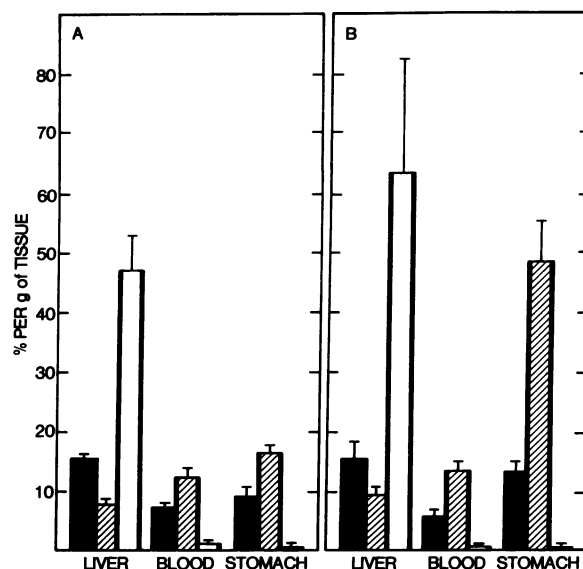


FIG. 2. Distribution of standard vesicles containing  $^{99m}\text{TcO}_4^-$  compared with free  $^{99m}\text{TcO}_4^-$  and  $^{99m}\text{Tc}$  sulfur colloid. Percentage of injected dose per g of liver, blood, and stomach, (A) 5 min, (B) 30 min after intravenous administration (mean value and SD shown).

small variations in the distribution pattern (Table 2). Experiments were done to determine whether the variations could be due to differences in sonication time. With progressive lengthening of the sonication time from 1 to 12 min, the hepatic uptake increased and the percentage of radionuclide recovered in the stomach decreased (Fig. 3). The results strongly suggest that the vesicles appeared to be made less permeable to  $^{99m}\text{TcO}_4^-$  by increasing the sonication time, an impression that was confirmed by dialysis studies *in vitro* (see below).

TABLE 1. Distribution of  $^{99m}\text{Tc}$  after injection of standard vesicles containing  $^{99m}\text{TcO}_4^-$

|            | % Injected dose*       |                    |
|------------|------------------------|--------------------|
|            | 5 min                  | 30 min             |
|            | <i>per g of tissue</i> |                    |
| Liver      | 15.5 ( $\pm 0.6$ )     | 15.6 ( $\pm 2.9$ ) |
| Blood      | 7.5 ( $\pm 0.8$ )      | 5.8 ( $\pm 1.2$ )  |
| Spleen     | 15.0 ( $\pm 2.3$ )     | 26.9 ( $\pm 7.2$ ) |
| Kidney (L) | 5.2 ( $\pm 0.5$ )      | 4.9 ( $\pm 0.6$ )  |
| Kidney (R) | 5.2 ( $\pm 0.7$ )      | 4.7 ( $\pm 0.6$ )  |
| Heart      | 3.1 ( $\pm 0.7$ )      | 2.0 ( $\pm 0.5$ )  |
| Lung       | 5.8 ( $\pm 1.4$ )      | 4.4 ( $\pm 0.6$ )  |
| Stomach    | 9.1 ( $\pm 1.5$ )      | 13.1 ( $\pm 1.9$ ) |
| Muscle     | 1.3 ( $\pm 0.6$ )      | 0.8 ( $\pm 0.3$ )  |
| Fat        | 0.5 ( $\pm 0.3$ )      | 1.1 ( $\pm 0.7$ )  |
|            | <i>per organ</i>       |                    |
| Liver      | 25.5 ( $\pm 0.5$ )     | 23.8 ( $\pm 1.0$ ) |
| Spleen     | 2.3 ( $\pm 0.3$ )      | 3.0 ( $\pm 0.5$ )  |
| Kidney (L) | 1.0 ( $\pm 0.1$ )      | 0.8 ( $\pm 0.1$ )  |
| Kidney (R) | 1.1 ( $\pm 0.2$ )      | 0.9 ( $\pm 0.1$ )  |
| Stomach    | 2.3 ( $\pm 0.5$ )      | 2.9 ( $\pm 0.1$ )  |
| Lung       | 1.8 ( $\pm 1.2$ )      | 1.8 ( $\pm 0.3$ )  |

\* Each point is the mean of three animals  $\pm$  standard deviation (SD).



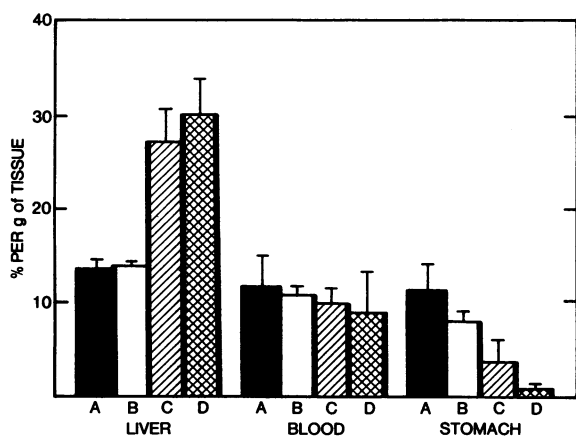


FIG. 3. Effect of sonicating liposomes (A) for 1 min, (B) for 3 min, (C) for 7 min, and (D) for 12 min, on the distribution in  $C_3H$  mice of vesicles containing  $^{99m}TcO_4^-$ . Percentage of injected dose per g of liver, blood, and stomach (mean and SD of three mice injected with each preparation).

The tissue distribution in liver, blood, and stomach for the standard preparation, phosphatidylcholine vesicles, and the standard preparation with the addition of cholesterol are shown in Fig. 4. Within 5 min of the injection of pure lecithin vesicles containing  $^{99m}TcO_4^-$ , the radionuclide was distributed in a pattern resembling free  $^{99m}TcO_4^-$ , with a lower hepatic and higher gastric distribution than the standard preparation. This pattern strongly suggests that there occurred rapid release of  $^{99m}TcO_4^-$  from the vesicle interior. When vesicles were made with cholesterol incorporated into the membrane and injected, the subsequent hepatic uptake of  $^{99m}TcO_4^-$  was augmented and the gastric distribution was reduced.

Addition of phosphatidic acid, phosphatidylethanolamine, or phosphatidylserine to the standard liposome constituents resulted in distribution of radioactivity that did not differ greatly from the standard preparation (Table 3).

Studies of the relative permeability of the vesicle membranes to  $^{99m}TcO_4^-$  *in vitro* are shown in Fig. 5. Vesicles composed of only phosphatidylcholine showed the greatest permeability to  $^{99m}TcO_4^-$ , while those composed of phosphatidylcholine, gangliosides, and cholesterol were least permeable. The rate of loss of  $^{99m}TcO_4^-$  from the standard preparation of vesicles was between these two extremes. Increasing the sonication

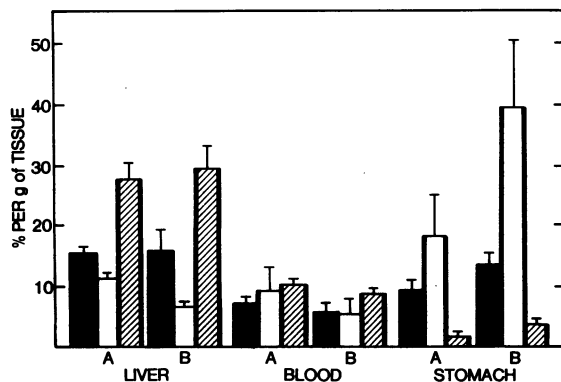


FIG. 4. Comparison of distribution of  $^{99m}Tc$  after injection of standard vesicles ■, lecithin vesicles ▨, and standard vesicles containing cholesterol □. Each preparation contained intravesicular  $^{99m}TcO_4^-$ . (A) 5 min and (B) 30 min after administration.

TABLE 2. Variation in distribution of  $^{99m}Tc$  after injection of standard vesicles containing  $^{99m}TcO_4^-$

|         | % Injected dose* per g of tissue |                    |                    |
|---------|----------------------------------|--------------------|--------------------|
|         | Exp. 1                           | Exp. 2             | Exp. 3             |
| Liver   | 15.5 ( $\pm 0.6$ )               | 12.4 ( $\pm 1.4$ ) | 10.8 ( $\pm 1.7$ ) |
| Blood   | 7.1 ( $\pm 0.8$ )                | 10.1 ( $\pm 1.6$ ) | 9.0 ( $\pm 0.4$ )  |
| Stomach | 9.1 ( $\pm 1.5$ )                | 10.6 ( $\pm 3.3$ ) | 9.0 ( $\pm 2.1$ )  |

Three separate experiments with same preparation; for each point, three mice were killed 5 min after administration.

\* Mean ( $\pm$  SD).

time of the standard preparation appeared to produce a "firmer" vesicle, which released intravesicular radionuclide less rapidly during dialysis.

## DISCUSSION

These investigations were undertaken to determine if single-compartment vesicles made of lecithin and gangliosides might serve as carriers for diagnostic agents (such as radionuclides) or therapeutic substances (drugs, radiopharmaceuticals, or enzymes), which might be introduced in the circulation and delivered selectively to target organs or tissues.

The distribution pattern of vesicles *in vivo* differs from that of colloidal particles. Within 5 min of injection of the latter, the blood was virtually completely cleared of radioactivity, and more than 80% of the activity was recovered in liver and spleen. Thirty minutes after intravenous administration of vesicles containing  $^{99m}TcO_4^-$ , 10–20% of the injected radioactivity still remained in the circulation, and only about 25% of the radioactivity was in the liver.

The amplitude of the EPR signal of vesicles containing tempocholine was increased by Triton X-100, and this response was found in blood from mice for as long as 46 min after the injection of vesicles. Such a response indicated that some of the vesicles were intact at that time.

Lengthening the sonication time appears to reduce vesicle size, an effect verified in preliminary experiments by laser

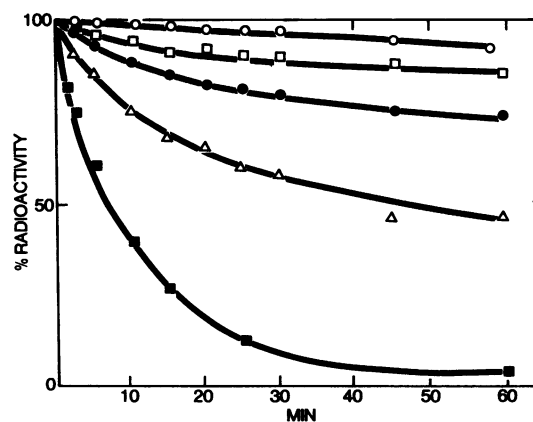


FIG. 5. Percentage of radioactivity in dialysis bag at time intervals up to 60 min. ○, Vesicles containing phosphatidylcholine, gangliosides, and cholesterol, sonicated for 5 min. □, Standard vesicle preparation sonicated for 12 min. ●, Standard vesicle preparation sonicated for 5 min. △, Vesicles consisting of only phosphatidylcholine, sonicated for 5 min. ■, Free  $^{99m}TcO_4^-$  (no vesicles).

TABLE 3. Effect of modification of vesicle composition on distribution of  $^{99m}\text{Tc}$  in vivo after injection of vesicles containing intravesicular  $^{99m}\text{TcO}_4^-$

|                                     | % Injected dose per g of tissue* |                   |                    |
|-------------------------------------|----------------------------------|-------------------|--------------------|
|                                     | Liver                            | Blood             | Stomach            |
| <i>At 5 min</i>                     |                                  |                   |                    |
| Standard preparation                | 15.5 ( $\pm 0.6$ )               | 7.1 ( $\pm 0.8$ ) | 9.1 ( $\pm 1.5$ )  |
| With added phosphatidic acid        | 14.2 ( $\pm 1.4$ )               | 9.1 ( $\pm 1.8$ ) | 9.1 ( $\pm 1.2$ )  |
| With added phosphatidylethanolamine | 15.6 ( $\pm 0.4$ )               | 9.4 ( $\pm 1.1$ ) | 13.5 ( $\pm 4.6$ ) |
| With added phosphatidylserine       | 19.7 ( $\pm 1.9$ )               | 7.4 ( $\pm 0.3$ ) | 7.3 ( $\pm 1.4$ )  |
| <i>At 30 min</i>                    |                                  |                   |                    |
| Standard preparation                | 15.6 ( $\pm 2.9$ )               | 5.8 ( $\pm 1.2$ ) | 13.1 ( $\pm 1.9$ ) |
| With added phosphatidic acid        | 14.0 ( $\pm 1.7$ )               | 6.7 ( $\pm 0.6$ ) | 18.9 ( $\pm 4.3$ ) |
| With added phosphatidylethanolamine | 14.0 ( $\pm 2.8$ )               | 6.7 ( $\pm 2.2$ ) | 21.8 ( $\pm 3.4$ ) |
| With added phosphatidylserine       | 16.8 ( $\pm 1.8$ )               | 5.0 ( $\pm 0.4$ ) | 19.0 ( $\pm 2.6$ ) |

\* Mean ( $\pm$  SD).

beam light scattering spectroscopy<sup>†</sup>. Since the sonication of liposomes was carried out in air, the alteration in distribution of radioactivity produced by differences in length of sonication might also be related to a progressive increase in oxidative saturation of the fatty acids (10, 11). Biomembranes containing saturated fatty acids are more ordered and less permeable than those containing kinked unsaturated fatty acids (12, 13). The longer the sonication, the higher the hepatic uptake and the lower the gastric uptake of radioactivity, findings strongly suggesting that the vesicles were indeed being made more rigid and less leaky. Similar, and even more marked, effects were noted when cholesterol was incorporated into the vesicle wall. The role of cholesterol in stabilizing lipid biomembranes has been the subject of considerable study (14–17). Cholesterol appears to decrease the movement of lipids in membrane, reducing membrane permeability by altering fluidity and thickness. When nonpermeable vesicles are desired for studies *in vivo*, either lengthening the sonication time or adding cholesterol is a simple and effective manipulation.

The addition of phosphatidic acid, phosphatidylethanolamine, and phosphatidylserine to the basic constituents, phosphatidylcholine and gangliosides, did not alter the pattern of distribution of the vesicles *in vivo*. However, the reason

that these additional constituents were studied was not simply to investigate possible differences in distribution, but to provide polar groups that might interact in coupling reactions. Through such groups, proteins, hormones, or antibodies may be attached to vesicles. Such a system might provide useful diagnostic *in vivo* tests or radioimmunoassay procedures *in vitro*.

Multilamellar liposomes have been studied as carriers for enzymes, with the eventual aim of supplying missing enzymes to patients with inborn errors of metabolism (18–21). Liposomes are larger than vesicles, are nonuniform in size, and are very permeable to anions like  $^{99m}\text{TcO}_4^-$ . For these reasons we believe that vesicles, which are simple to make and amenable to compositional alterations, are more attractive as carriers.

Although the constituent lipids of vesicles are natural components of the body and are by themselves not antigenic (22), intact vesicles, especially with proteins attached to their margins, may be. Long-term trials in experimental animals will have to be evaluated before clinical studies are undertaken.

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<sup>†</sup> I. R. McDougall, J. K. Dunnick, S. Aragonis, and J. P. Kriss, unpublished observations.