Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 9191–9195, October 1992
Cell Biology

Phagocytosis of liposomes by human platelets

ROXANNE MALE, WILTON E. VANNIER, AND JOHN D. BALDESCHWIELER

California Institute of Technology, Division of Chemistry and Chemical Engineering, Pasadena, CA 91125

Contributed by John D. Baldeschwieler, May 15, 1992

ABSTRACT **We have shown that platelets are capable of phagocytosing liposomes rather than simply sequestering particles as previously postulated. Incubation of human platelets with small neutral unilamellar liposomes (≈74 nm) resulted in uptake of the liposomes and retention of the lipid with rapid release of the aqueous-phase components. The lipid label [^{3}H]-cholesterylhexadecyl ether and water-soluble [^{3}H]inulin were used to study the fate of the liposome components. Uptake of liposomes was proportional to the number of liposomes added and to the incubation time. Approximately 250 liposomes per platelet were taken up within a 5-hr incubation period. Uptake of the liposomes occurred through the open-channel system, as evidenced by thin-section electron microscopy, and was followed by accumulation and degradation in acid- and esterase-containing vesicles, as determined by changes in fluorescence of the pH-sensitive probe, pyranine (1-hydroxypyrene-3,6,8-trisulfonic acid), and hydrolysis of the cholesteryl [^{14}C]oleate membrane marker. Uptake was inhibited by the addition of EDTA, cytochalasin B, or 2,4-dinitrophenol and iodoacetate to the medium. Results from the serotonin release assay, microaggregation assay, fluorescein diacetate membrane integrity assay, and electron microscopy indicate that neither the conditions for loading nor phagocytosis of liposomes significantly alter platelet function or morphology.**

Platelets have been shown to take up a variety of particles *in vitro* (1–6) and evidence is accumulating which suggests that removal of foreign materials from the blood may be an important physiologic role for these cells (7–10). However, it remains to be definitively demonstrated whether platelets are truly phagocytic or simply sequester the particles within the open-channel system (OCS).

In prior work, the platelet–latex sphere system has been used as a model for foreign particle ingestion by these cells (2, 3, 11–13). Various studies have shown progressive accumulation of latex spheres in the OCS, a system of channels believed to be formed by invagination of the plasma membrane. Lewis *et al.* (13) noted subsequent localization of the particles in acid phosphatase-positive electron-opaque vacuoles and concluded that platelets phagocytose latex particles. However, White (12) concluded that platelets sequester latex particles within membrane invaginations, which are in communication with the exterior of the cell. We report the use of small neutral unilamellar liposomes to study possible phagocytosis of particles by platelets. Liposomes have been used extensively to model microorganism interactions and, unlike latex beads, are more suitable for the study of uptake, metabolism, and exocytosis.

MATERIALS AND METHODS

Purification of Platelets. Platelets were prepared by two methods; experiments measuring uptake using either platelet preparation gave similar results. Platelet-rich plasma packs obtained from the Red Cross were diluted in a 1:1 ratio with 0.9% saline solution. A volume of 4.5% dextran (M_r, 250,000) in saline solution was added to an equal volume of the diluted packs and stirred; erythrocytes were allowed to sediment for 30 min at room temperature. Fifteen-milliliter aliquots of the supernatant were pipetted into centrifuge tubes. Equal volumes of a solution containing 6.35% Ficoll and 10% Hypaque (wt/wt) were gently layered on the bottom of the tubes. The tubes were spun at 400 × *g* for 15 min at room temperature. The cloudy band containing the platelets and lymphocytes located directly above the Ficoll/Hypaque gradient was gently removed, diluted in a 1:1 ratio with saline solution, and centrifuged at 300 × *g* for 15 min at room temperature to sediment the lymphocytes. The supernatant was spun at 800 × *g* for 20 min; the platelet pellet formed was washed twice in saline solution and finally resuspended in Hanks' balanced salt solution (HBSS).

Platelets purchased from the American Blood Institute (Los Angeles) were centrifuged at 1000 × *g* for 15 min in an IEC Centra-8 centrifuge. The pellet was resuspended in either 0.9% saline solution or Tyrode's buffer and was spun at 110 × *g* for 5 min to remove excess erythrocytes. The supernatant was spun at 450 × *g* for 10 min. The pellet was resuspended in HBSS or modified Tyrode's buffer (Tyrode's buffer plus 1.26 mM $CaCl_2$).

The final purities of both platelet preparations were >95% platelets as assessed by a Coulter S+4 Cell Counter; the main contaminants were erythrocytes. Platelets were used within 24 hr of blood collection.

Determination of the Number of Platelets per mg of Protein. Cell counts were performed with a Coulter S+4 Cell Counter to determine the number of platelets. The Peterson modification of the Lowry protein assay (14) with a bovine serum albumin standard and without trichloroacetic acid precipitation was used to determine the amounts of protein in the same samples. One milligram of protein was found to correspond to 3×10^8 platelets.

Preparation of Liposomes. *[^{3}H]Cholesterylhexadecyl ether-labeled liposomes.* A 2:1 (mol/mol) mixture of distearoylphosphatidylcholine (Avanti Polar Lipids) and cholesterol (cell culture tested; Sigma) in chloroform was made such that the total lipid content was ≈20 mg. Five microcuries (1 Ci = 37 GBq) of [^{3}H]cholesterylhexadecyl ether (New England Nuclear) in chloroform was also added. The solution was then taken to dryness in a 100-ml round bottomed flask with a Büchler "Rotovap" apparatus and was dried under vacuum overnight. The resulting phospholipid mixture was resuspended in 5 ml of phosphate-buffered saline (PBS; 0.90% NaCl/0.12% $Na_2HPO_4 \cdot 7H_2O$/0.013% $NaH_2PO_4 \cdot H_2O$ in water, pH 7.3) and Vortex mixed, and 2.5-ml portions were probe sonicated for 15 min with a Heat Systems-Ultrasonics sonicator with a microprobe.

[^{3}H]Inulin-labeled liposomes. Liposomes were prepared exactly as described except that no [^{3}H]cholesterylhexadecyl ether was added and the resuspension buffer contained 5 μCi of [^{3}H]inulin (Amersham). Excess [^{3}H]inulin (M_r 5200) was

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: OCS, open-channel system; HPTS, 1-hydroxypyrene-3,6,8-trisulfonic acid; FDA, fluorescein diacetate.

separated from the liposomes by gel filtration with a Sephadex G-50-100 column.

Nonradiolabeled liposomes. Liposomes were prepared as described but without the tritiated labels.

Pyranine (1-Hydroxypyrene-3,6,8-trisulfonic acid; HPTS)-labeled liposomes. Liposomes were prepared as described above except that the resuspension solution was 35 mM in HPTS (Molecular Probes) and 75 mM in NaCl. Excess HPTS was removed by gel filtration with a Sephadex G-50-100 column equilibrated with 150 mM NaCl.

Cholesteryl [^{14}C]oleate-labeled liposomes. Liposomes were prepared as described for the [^{3}H]cholesterylhexadecyl ether-labeled liposomes except 5 μCi of cholesteryl [^{14}C]-oleate (Amersham) in chloroform was added instead of [^{3}H]cholesterylhexadecyl ether.

Incubation Procedure. Platelets and liposomes were incubated at 37°C in 1.5-ml polypropylene centrifuge tubes for the desired incubation times, after which the mixtures were centrifuged at 5700 × *g* for 5 min in a Beckman Microfuge 11. The pellets were washed twice in buffer and finally resuspended in 0.5 M NaOH/0.5% SDS and incubated overnight at room temperature. The solution was transferred to liquid scintillation vials with 10 ml of Safety Solve (Research Products), mixed, and allowed to sit at least 2 hr before scintillation counting. For each experiment, all samples were done in duplicate; the number of independent experiments (*n*) is noted in the figure legends or in the text. Bars on graphs represent range of data.

Exocytosis Studies. Platelets and liposomes were incubated as described above for 12 hr. The mixtures were centrifuged and platelets were washed by centrifugation twice in buffer to remove excess liposomes and then further incubated at 37°C for various periods of time. Both the pellets and supernatants were collected by centrifugation, solubilized in the NaOH/SDS solution overnight, and assayed.

Determination of Liposome Diameters and Estimation of Liposome Numbers. Liposome diameters for different preparations were determined by photon correlation spectroscopy at 480 nm with a Malvern Instruments PCS 100 system. The numbers of [^{3}H]cholesterylhexadecyl ether-labeled liposomes were calculated by assuming that the counts per weight of lipid were constant for all preparations, the average surface area for a phospholipid molecule is 0.7 nm^{2} (15), and the average molecular weight for the lipid is 656 g/mol. The numbers of [^{3}H]inulin-labeled and nonradiolabeled liposomes were determined by performing Böttcher phosphate assays (16) on the samples and assuming a 0.7-nm^{2} phospholipid surface area. The light scattering data indicated that liposome diameters were 65–83 nm. The numbers of liposomes reported have all been normalized assuming that the diameters of the liposomes for all experiments were 74 nm.

Fluorimetry of HPTS. After incubation with HPTS-labeled liposomes for various times, the cells were washed twice with buffer. Fluorescence excitation spectra (λ_{ex}, 395–465 nm; bandwidth, 4 nm) were measured at 510-nm emission (bandwidth, 4 nm) using a SLM 4800 (SLM, Urbana, IL) outfitted with a stirred, temperature-controlled cuvette (20°C). HPTS is a pH-dependent dye that exhibits two major fluorescence maxima (403 and 460 nm), which have a complementary pH dependence in the range pH 5–9; the peak at 403 nm is maximal at low pH values, while the peak at 460 nm is maximal at high pH values. The fluorescence at 413 nm is relatively pH independent and is used to standardize the concentration of dye associated with the cells. The fraction of liposomes taken up by phagocytosis and delivered to an acidic (pH 6.0) environment was calculated by using the 460/413 nm ratio and the equation

$$\text{fraction phagocytosed} = (\text{ratio}_{\text{pH } 7.4} - \text{ratio}_{\text{measured}})/(\text{ratio}_{\text{pH } 7.4} - \text{ratio}_{\text{pH } 6.0}),$$

where $\text{ratio}_{\text{measured}}$ is the 460/413 ratio of liposome-treated cells and $\text{ratio}_{\text{pH } 7.4}$ and $\text{ratio}_{\text{pH } 6.0}$ are the 460/413 ratios of liposomes in buffer and acidified buffer, respectively (17). In the original paper by Daleke *et al.* (17), the second peak occurred at 450 nm; however, according to our data the peak maximum is at 460 nm.

Intralysosomal Degradation of the Cholesteryl [^{14}C]oleate-Labeled Liposomes. Platelets (3 × 10^{8}) were preincubated with [^{3}H]cholesterylhexadecyl ether or cholesteryl [^{14}C]-oleate-labeled liposomes (7 × 10^{11}) for 0.5 hr at 37°C. The mixtures were washed twice in buffer by centrifugation. The pellets were collected, resuspended in buffer, and incubated for 10–60 min at 37°C. After incubation, the mixtures were centrifuged to collect the pellets that were then solubilized in NaOH/SDS solution overnight and assayed.

Upon intralysosomal degradation, cholesteryl [^{14}C]oleate is hydrolyzed to form [^{14}C]oleate, which is released from cells. Conversely, [^{3}H]cholesterylhexadecyl ether is retained within the cells. Therefore, by monitoring the ^{3}H/^{14}C ratio of the pellets, the degree of degradation can be determined (18).

Thin-Section Electron Microscopy. Samples of 3 × 10^{8} platelets were incubated with 7 × 10^{11} nonradiolabeled liposomes for 6 hr at 37°C, fixed in a standard fashion, and embedded in LR white.

In addition, platelets suspended in HBSS or modified Tyrode's buffer incubated with or without liposomes for 1 hr at 37°C prepared as described above were studied to determine changes in platelet morphology under the conditions used for studying the liposome interaction.

***In Vitro* Studies to Assess Platelet Function.** *Serotonin release assay.* The serotonin assay was performed with 3 × 10^{8} platelets suspended in HBSS or in modified Tyrode's solution and incubated with or without 1.4 × 10^{12} liposomes for 1–9 hr as described (19).

Microaggregation assay. Platelets suspended in HBSS or modified Tyrode's solution were incubated at 37°C, with or without liposomes, for 1–9 hr and then fixed with 1% glutaraldehyde. The percentages of aggregated platelets were determined by examining the cells in a hemacytometer.

Fluorescein diacetate (FDA) assay. After incubation of platelets suspended in HBSS or in modified Tyrode's solution at 37°C, with or without liposomes, for 1–9 hr, FDA (Sigma) was added and incubated (final concentration, 4 μg/ml) with the cells for 10 min at room temperature. The platelets were then kept on ice for 1 min and pelleted at 4°C. The fluorescence was measured at 520-nm emission while exciting at 489 nm.

FDA is a nonfluorescent probe that readily crosses the cell membrane. Within the cell the probe is hydrolyzed by esterases to form fluorescent fluorescein, which does not readily traverse the cell membrane at 4°C but can leak out of the cells through membrane lesions. Therefore, the leakage of fluorescein is a measure of loss of membrane integrity (20).

RESULTS

[^{3}H]Cholesterylhexadecyl Ether-Labeled Liposome Uptake. To model the liposome uptake by platelets, [^{3}H]cholesterylhexadecyl ether-labeled liposome uptake was studied for various incubation times and incubation concentrations of both the liposomes and platelets. Platelet uptake of [^{3}H]cholesterylhexadecyl ether-labeled liposomes increased with increasing incubation times (Fig. 1). After incubation of 3 × 10^{8} platelets with 7 × 10^{11} liposomes for 5 hr, ≈250 liposomes were associated with each platelet. Liposome uptake by platelets also increased with increasing numbers of incubated liposomes; two incubation times are shown (Fig. 2). Incubation for 12.5 hr resulted in greater uptake values than the 2.0-hr incubation. As the number of platelets incubated with a constant amount of liposomes was increased, the overall

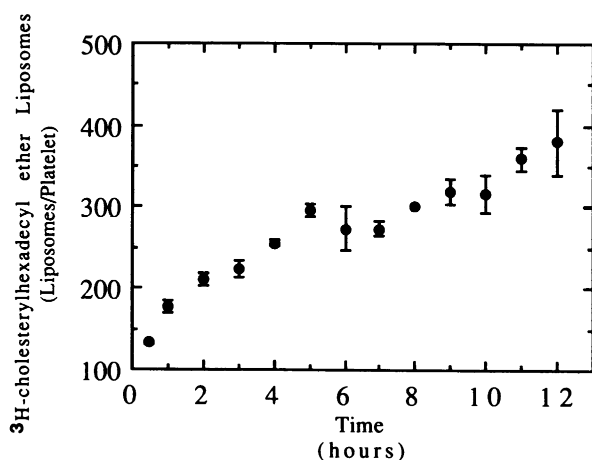

FIG. 1. Incubation time dependence of platelet uptake of [^{3}H]cholesterylhexadecyl ether-labeled liposomes. Platelets (3×10^8) were incubated with [^{3}H]cholesterylhexadecyl ether-labeled liposomes (7×10^{11}) at 37°C for various incubation times (n = 8).

number of liposomes incorporated increased (Fig. 3); however, the ratio of liposomes to platelets remained constant at ≈475 liposomes per platelet for a 12.0-hr incubation period.

[^{3}H]Inulin-Labeled Liposome Uptake. Uptake of the aqueous components of the liposomes was studied by following the uptake of [^{3}H]inulin-labeled liposomes versus various incubation times and liposome concentrations. Although the number of [^{3}H]inulin-labeled liposomes taken up increased with time (Fig. 4), the kinetics of uptake were different than that for the [^{3}H]cholesterylhexadecyl ether-labeled liposomes. Incubation of 2×10^{12} liposomes with 3×10^8 platelets resulted in uptake of ≈150 liposomes per platelet after 5 hr. The uptake of [^{3}H]inulin-labeled liposomes versus the concentration of liposomes incubated is shown for two different incubation times (Fig. 5). Uptake was proportional to both the number of liposomes incubated and the incubation time. The difference in kinetics of uptake as assessed by the lipid label and the aqueous label suggests that there is a difference in the fate of the lipid and aqueous components after platelet uptake. Therefore, the values reported for the uptake of the [^{3}H]inulin-labeled liposomes are given as "apparent liposomes per platelet."

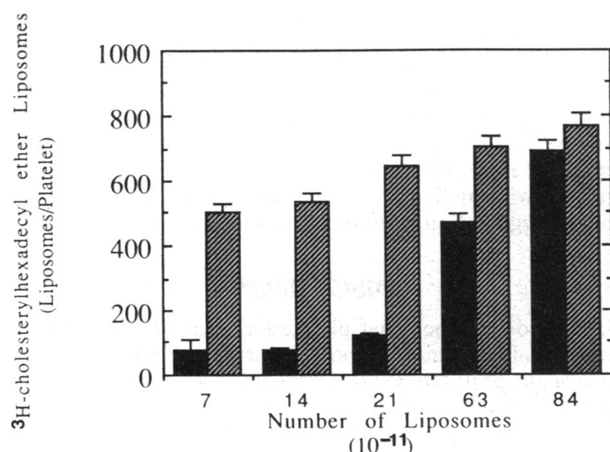

FIG. 2. Liposome concentration dependence of platelet uptake of [^{3}H]cholesterylhexadecyl ether-labeled liposomes. Platelets (3×10^8) were incubated with various amounts of [^{3}H]cholesterylhexadecyl ether-labeled liposomes at 37°C for 2 hr (■) or 12.5 hr (▨) (n = 10 for 2 hr; n = 6 for 12.5 hr).

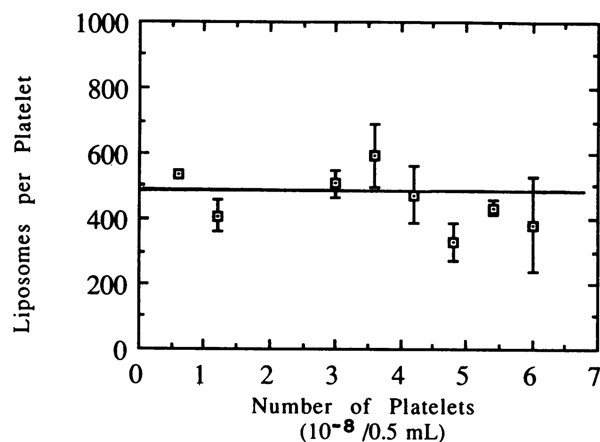

FIG. 3. Platelet concentration dependence of platelet uptake of [^{3}H]cholesterylhexadecyl ether-labeled liposomes. [^{3}H]Cholesterylhexadecyl ether-labeled liposomes (8.3×10^{11}) were incubated with various numbers of platelets (final volume, 0.5 ml) at 37°C for 2.0 hr. The line representing 475 liposomes per platelet is shown (n = 6).

Release of Tritiated Radiolabels. To study the possible exocytosis of liposomes, the amounts released of the tritiated radiolabels [^{3}H]cholesterylhexadecyl ether and [^{3}H]inulin probes were examined (Fig. 6). After 12 hr no membrane-associated probe was released. However, up to 60% of the aqueous-phase probe was released during this period.

HPTS-Labeled Liposome Uptake. To determine the percentage of liposomes phagocytosed and delivered to acidic compartments, the uptake of fluorescently labeled (HPTS) liposomes was measured by fluorimetry versus time (Fig. 7). Initially, the liposomes were associated with the platelets, either bound to the surface or contained in the OCS, but were not phagocytosed. Within minutes the liposomes began to accumulate in acid-containing vesicles. The percentage of platelet-associated liposomes phagocytosed increased to 80% within 1 hr.

Cholesteryl [^{14}C]oleate-Labeled Liposome Degradation. Liposome degradation was monitored by studying the time dependence of the relative abundances of ^{3}H and ^{14}C within the platelets after preincubating the cells with [^{3}H]cholesterylhexadecyl ether- or cholesteryl [^{14}C]oleate-labeled liposomes for 0.5 hr, washing the pellets twice, and resuspending in buffer (n = 10). During a 60-min period, the ^{3}H/^{14}C ratios within the cells increased linearly from 1.00 at 0 min, 1.028 at 10 min, 1.130 at 20 min, 1.212 at 30 min, and 1.246

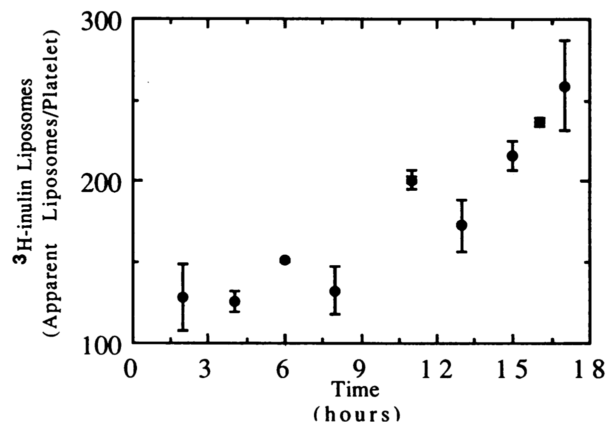

FIG. 4. Incubation time dependence of platelet uptake of [^{3}H]inulin-labeled liposomes. Platelets (3×10^8) were incubated with 2×10^{12} [^{3}H]inulin-labeled liposomes at 37°C for various incubation times (n = 6).

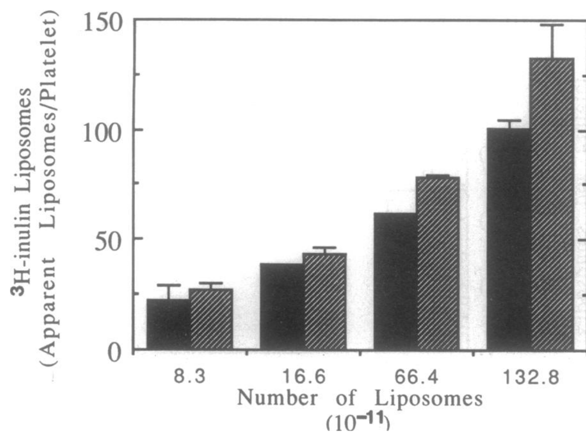

FIG. 5. Liposome concentration dependence of platelet uptake of [^{3}H]inulin-labeled liposomes. Platelets (3×10^8) were incubated with various amounts of [^{3}H]inulin-labeled liposomes at 37°C for 6.0 hr (■) and 12.5 hr (▨) (n = 6).

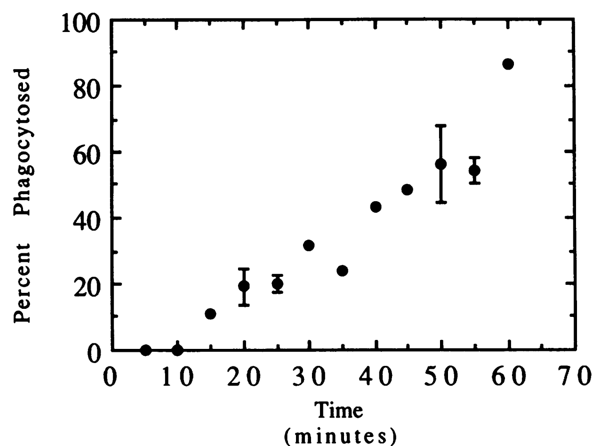

FIG. 7. Incubation time dependence of the percentage of platelet-associated HPTS-labeled liposomes phagocytosed. Platelets (3×10^8) were incubated with 7×10^{11} HPTS-labeled liposomes at 37°C for various incubation times. Cells were then pelleted, washed twice in buffer, and resuspended in buffer. Percentages of platelet-associated liposomes phagocytosed were calculated (n = 6).

at 50 min, to 1.294 at 60 min. [^{3}H]Cholesterylhexadecyl ether was retained within the platelets; however, the cholesteryl [^{14}C]oleate was hydrolyzed to form [^{14}C]oleate, which was released by the cells. Therefore, the increase in the ^{3}H/^{14}C ratio in the cells was indicative of degradation of the liposomes. The amount of liposome degradation is probably underestimated since incubations were done in the absence of serum, which is unfavorable for the release of oleate (21).

Electron Microscopy. Electron microscopy was performed to study the effects of HBSS and modified Tyrode's solution and the phagocytosis of liposomes on platelet morphology (n = 4). The effects of incubation of up to 2.1×10^{12} liposomes were studied.

Platelets maintained a discoid shape when suspended in modified Tyrode's buffer. However, platelets suspended in HBSS tended to have irregular shapes and approximately half had pseudopodia. Therefore, we believe that incubation in modified Tyrode's buffer is superior. Incubation with liposomes did not significantly alter platelet morphology.

Competitive Assay. A competitive assay was performed to establish whether liposome uptake could be saturated. Preincubation of platelets with 0–3.4×10^{12} nonradiolabeled liposomes prior to two centrifugation washes and incubation with 7.9×10^{11} tritiated liposomes for preincubation times of

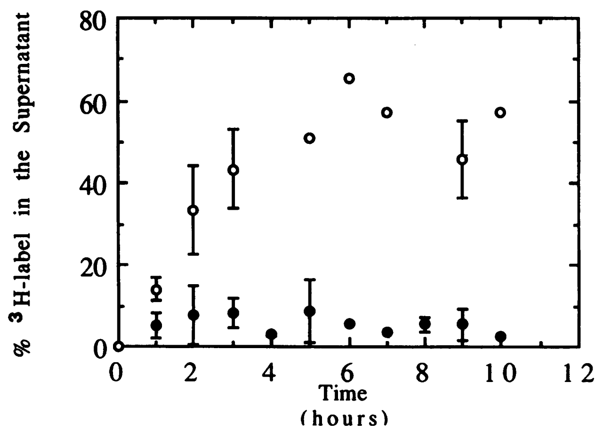

FIG. 6. Release studies of lipid and aqueous probes. Platelets (3×10^8) were preincubated with 8.3×10^{11} [^{3}H]cholesterylhexadecyl ether-labeled (●) or 2×10^{12} [^{3}H]inulin-labeled (○) liposomes at 37°C for 12.0 hr. Cells were then pelleted, washed twice in buffer, resuspended in buffer, and incubated for various incubation times at 37°C. Percentages of the [^{3}H]cholesterylhexadecyl ether and the [^{3}H]inulin labels in the supernatants were determined (n = 6).

0.5, 2, and 6 hr and incubation times of 0.17, 0.5, 2, and 6 hr gave similar results (data not shown; n = 4). The amount of [^{3}H]cholesterylhexadecyl ether liposomes taken up was independent of the amount of nonradiolabeled liposomes previously added. In addition, uptake was relatively independent of the preincubation time, while uptake increased with increasing incubation times. Simultaneous incubation of nonradiolabeled and ^{3}H-labeled liposomes resulted in a linear decrease in uptake of the ^{3}H-labeled liposomes as expected.

Inhibition of Liposome Uptake. The inhibitory effects of a variety of reagents known to decrease platelet uptake of latex spheres were examined during platelet incubation with liposomes. Incubation for 12 hr in the presence of 5 mM EDTA inhibited the uptake of liposomes by 48% ± 9%; however, incubation for 1 hr showed no significant inhibition of uptake (n = 10). The addition of cytochalasin B (10 μg/ml) (n = 8) or 0.25 mM 2,4-dinitrophenol with 50 μM iodoacetate (n = 8) also inhibited uptake by 46% ± 16% and 66% ± 12%, respectively.

***In Vitro* Assays to Assess Platelet Function.** Although the numbers of liposomes phagocytosed by platelets suspended in HBSS or modified Tyrode's buffer are identical, the *in vitro* assays showed that platelets, incubated with or without liposomes, spontaneously secrete serotonin, aggregate, and lose membrane integrity more while suspended in HBSS. However, phagocytosis of liposomes did not significantly alter platelet secretion of serotonin or induce additional platelet aggregation or loss of membrane integrity. This was not surprising since it had been previously shown that the uptake of Intralipid, a lipid emulsion used in treatment of patients with deficient caloric intake, does not increase platelet aggregation or induce morphological changes (22).

DISCUSSION

We have determined that platelets are capable of phagocytosing small unilamellar liposomes rather than sequestering particles as postulated (12). Liposomes are taken up through the OCS, localize within acid- and esterase-containing vesicles, and are degraded. The lipid is retained; the aqueous components are exocytosed. Therefore, all uptake values reported using [^{3}H]inulin-labeled liposomes really reflect a consecutive first-order kinetic scheme of uptake and exocytosis.

The values reported in this paper for platelet uptake do not differentiate between incorporated and bound liposomes.

However, according to electron micrographs, the vast majority of the liposomes appear to be within the OCS. In addition, the use of a pH-dependent fluorescent liposome probe confirms that a substantial portion of the liposomes are phagocytosed and are in acid-containing compartments. These data suggest that the liposomes are incorporated in surface invaginations, which pinch off their connections to the cell membrane and migrate into the cytoplasm as sealed vesicles, rather than being sequestered in the OCS and in contact with the outside medium. Degradation of the liposomes, which probably occurs in the acid phosphatase- and esterase-containing vacuoles, has also been demonstrated by utilizing cholesteryl [^{14}C]oleate-labeled liposomes. The mechanism for release of the aqueous components to the outside of the cell probably involves migration of these vacuoles to the periphery of the cell and exocytosis of their contents; however, this has not been confirmed.

Although the space available for uptake within the cell must be limited, exocytosis of the aqueous components of the liposomes increases the amount of liposome lipid capable of being stored as detected by the [^{3}H]cholesterylhexadecyl ether label. With the amounts of liposomes and platelets used and on the time scale of the preincubation experiments, the space available for uptake is not saturable. The uptake is independent of the amount of nonradiolabeled liposomes added and of preincubation times. Simultaneous incubation of nonradiolabeled and radiolabeled liposomes demonstrates a dilution effect, which results in decreased uptake of the radiolabeled liposomes.

According to Mustard and Packham (3), the addition of EDTA inhibits phagocytosis of particles and platelet aggregation, but it does not interfere with particle adherence. Liposome uptake is inhibited by the presence of EDTA at long time intervals but not at short intervals when the predominant process of uptake in the absence of Ca^{2+} may be binding. This evidence suggests that divalent cations are involved in the phagocytosis process; however, the effects of different divalent cations have not been investigated. The inhibitory effect of cytochalasin B with respect to platelet uptake has been attributed to its effect on microfilaments and the plasticity of the platelet membrane (23). The addition of 2,4-dinitrophenol and iodoacetate before incubation blocks aggregation and phagocytosis by interfering with glycolysis and oxidative phosphorylation (2). Liposome uptake is Ca^{2+} dependent; however; platelets tend to aggregate and secrete more in the presence of Ca^{2+}. Therefore, we studied the effects of HBSS and modified Tyrode's buffer on the morphology and function of platelets, which both contained 1.26 mM $CaCl_2$. The serotonin release assay, microaggregation assay, and FDA membrane integrity assay all show that platelet function and morphology are better preserved in modified Tyrode's buffer than in HBSS. However, even in modified Tyrode's buffer, the loss of membrane integrity is significant. This loss may be due to incubation of the platelets in small polypropylene centrifuge tubes at 37°C. It is preferable to store platelets at room temperature, but phagocytosis is maximized at 37°C. The assays also indicated that phagocytosis of liposomes does not adversely affect the platelets.

Although we began this research to define the mechanisms involved in platelet–liposome interactions, we realize the ability to encapsulate a variety of drugs or radioactive labels *in vitro* in platelets and to target specific locations *in vivo* such as areas of inflammation or infection, neoplastic tumor cells, or thrombosis would be of great medical importance. The exocytosis process is comparatively rapid; the half-life of the aqueous compartment calculated assuming a first-order reaction is 3.9 hr. Therefore, it may be necessary to use inhibitors that would slow the digestion of the liposomes or the release of the liposomes' aqueous components. Alternatively, lipid analogs of the drugs could be used for longer platelet retention. In some cases, rapid release of drugs from the aqueous compartments may be desirable. An example of this might be the delivery of thrombolytic agents to a coronary thrombus in the initial treatment of myocardial infarction.

We are indebted to Dr. Dudley Moon (Albany College of Pharmacy) and Dr. Pramod Lad and his staff (Kaiser Regional Research Laboratory, Los Angeles) for their advice during the course of the work. We thank Dr. G. L. Scherphof for suggesting the use of the cholesteryl [^{14}C]oleate hydrolysis method. Funding for this work was provided by Army Research Office Grant DAAL-03-87-K-0044 and the Caltech Consortium in Chemistry and Chemical Engineering (founding members: E.I. du Pont de Nemours and Company, Inc., Eastman Kodak Company, Minnesota Mining and Manufacturing Company, Shell Oil Company Foundation).

1. David-Ferreira, J. F. (1961) *Z. Zellforsch. Mikrosk. Anat.* **55**, 89–103.
2. Movat, H. Z., Weiser, W. J., Glynn, M. F. & Mustard, J. F. (1965) *J. Cell Biol.* **27**, 531–543.
3. Mustard, J. F. & Packham, M. A. (1986) *Ser. Haematol.* **1**, 168–184.
4. Schulz, H. (1961) *Folia Haematol.* **5**, 195–205.
5. Clawson, C. C. (1973) *Am. J. Pathol.* **70**, 449–464.
6. Movat, H. Z., Mustard, J. F., Talchman, N. S. & Urihara, T. (1965) *Proc. Soc. Exp. Biol. Med.* **120**, 232–237.
7. Zawilska, K. & Izrael, V. (1973) *Pathol. Biol.* **21**, 771–780.
8. Fajardo, L. F. & Tallent, C. (1974) *J. Am. Med. Assoc.* **229**, 1205–1207.
9. Hovig, T. & Grøttum, K. A. (1973) *Thromb. Diath. Haemorrh.* **29**, 450–460.
10. Kaplan, J. E. & Moon, D. G. (1984) in *The Reticuloendothelial System*, eds. Filkins, I. P. & Reichard, S. M. (Plenum, New York), Vol. 7, pp. 237–266.
11. Glynn, M. F., Herren, R. & Mustard, J. F. (1966) *Nature (London)* **212**, 79–80.
12. White, J. G. (1972) *Am. J. Pathol.* **69**, 439–450.
13. Lewis, J. C., Maldonado, J. E. & Mann, K. G. (1976) *Blood* **47**, 833–840.
14. Peterson, G. L. (1977) *Anal. Biochem.* **83**, 346–356.
15. Rand, R. P. & Parsegian, V. A. (1989) *Biochim. Biophys. Acta* **988**, 351–376.
16. Böttcher, C. J. F., Van Gent, C. M. & Pries, C. (1961) *Anal. Chim. Acta* **24**, 203–204.
17. Daleke, D. L., Hong, K. & Papahodjopoulos, D. (1990) *Biochim. Biophys. Acta* **1024**, 352–366.
18. Derksen, J. T. P., Morselt, H. W. M. & Scherphof, G. L. (1988) *Biochim. Biophys. Acta* **971**, 127–136.
19. Holmsen, H. & Dangelmaier, C. A. (1989) *Methods Enzymol.* **169**, 205–210.
20. Pesidsky, M. D. & Baillie, G. S. (1977) *Cryobiology* **14**, 322–331.
21. Derksen, J. T. P., Morselt, H. W. M. & Scherphof, G. L. (1987) *Biochim. Biophys. Acta* **931**, 33–40.
22. Hovig, T. (1970) *Ser. Haematol.* **3** 4, 47–67.
23. White, J. G. & Krumweide, M. (1973) *Blood* **41**, 823–832.