

orously with 0.6 ml of a  $^3\text{H}$ -inulin solution and allowed to sit at room temperature for full hydration (about 4 h), then treated with 2 cycles of freeze-thawing followed by extrusion through a 0.2- $\mu\text{m}$ -pore membrane. Liposomes were collected by metrizamide gradient floatation.<sup>14</sup> This process also separated AF-liposomes from free AF and un-entrapped  $^3\text{H}$ -inulin solution. Plain liposomes, (-)LP, and (o)LP, were prepared by the same process, but palmitoyl-AF was omitted.

### Characterization of AF-liposomes

Vesicle size was analyzed by a particle analyzer (Photal LPA-3000 Photon Correlator, Otsuka, Japan). The zeta potential ( $\zeta$ ) of the liposomal vesicles was determined (Zetamaster version PCS v1.25, Malven, England). The phospholipid concentration of liposomes was determined by the method of Stewart.<sup>15</sup> AF-liposomes were identified by hemagglutination inhibition assay. Briefly, desialized red blood cells were incubated with N-acetyl-peanut agglutinin (PNA) in the absence and presence of serial concentrations (in double dilution) of AF-liposomes at room temperature for 1 h, and then hemagglutinations were compared.

### Distribution of Liposomes in vivo

Wistar rats (Experimental Animal Center, College of Medicine, National Taiwan Univ.) weighing 180 to 230 g were anesthetized with an i.p. injection of pentobarbital sodium (3.5% solution, 40 mg/kg) and given 1 ml liposomes (total lipid concentration 7.2  $\mu\text{mol/ml}$ ) by injection into the femoral vein. At time points of 1, 5, 15, 30 and 60 min after injection, blood (0.4 ml) was collected via a carotid artery cannula. After the final blood sampling, the animal was sacrificed, and its tissues were excised, cleaned, weighed, and in-

dividually homogenized with equal volumes of distilled water. For each 0.5 ml of plasma or tissue homogenate, 10 ml of aquasol-2 was added, and the radioactivities were determined in a liquid scintillation counter (Beckman LS-5801, Beckman Instruments, Fullerton, CA, USA). Counts were corrected for capillary blood contamination of the tissues under the assumption that the blood content by weight in the tissue fraction was the same as that in guinea pigs.<sup>16</sup>

### RESULTS

The vesicle size of liposomes was in the range of 161 to 185 nm and the content of AF in AF-liposomes for (-)AFLP and (o)AFLP was nearly equivalent ( $10.5 \pm 0.4$  vs.  $10.2 \pm 0.1$   $\mu\text{g}/\mu\text{mol}$  phospholipids). The values of mean diameters based on the number distribution (dn) and weight distribution (dw) were very close ( $\text{dn}/\text{dw} \approx 1$ , Table 1), which indicated that the size distribution of the liposomes was narrow; in other words, the size of liposomal vesicles was relatively uniform. The zeta potential for AF-labeled liposomes was slightly yet significantly lower than that of plain liposomes (Table 1).

The plasma elimination time profiles of liposomal radioactivity (percent of injected dose per milliliter of plasma) were shown in Fig. 1. For all tested liposomes, the radioactivity of either  $^{14}\text{C}$  or  $^3\text{H}$  remaining in plasma at 60 min was less than 1.5% of that at 1 min after injection. The AUCs of  $^{14}\text{C}$  and  $^3\text{H}$  from 1 to 60 min ( $\text{AUC}_{1-60}$ ) for AF-liposomes were smaller than those for plain liposomes (Table 2).

Fig. 2 shows the tissue distribution of  $^{14}\text{C}$  and  $^3\text{H}$ , in terms of the percentage of administered dose per gram of wet tissue, at the 60 min after i.v. injection of liposomes. Concentrations of both  $^{14}\text{C}$  and  $^3\text{H}$  in

**Table 1. Characteristics of Liposomes**

|         | Vesicle size (nm) | dn/dw | AF ( $\mu\text{g}/\mu\text{mol}$ lipid) <sup>a</sup> | $\zeta$ (mV) <sup>a</sup> |
|---------|-------------------|-------|------------------------------------------------------|---------------------------|
| (-)LP   | 161.1             | 1.12  |                                                      | $-44.0 \pm 2.6^*$         |
| (-)AFLP | 184.9             | 1.17  | $10.5 \pm 0.4$                                       | $-47.5 \pm 0.8$           |
| (o)LP   | 172.8             | 1.09  |                                                      | $-4.0 \pm 0.8^*$          |
| (o)AFLP | 161.4             | 1.14  | $10.2 \pm 0.1$                                       | $-6.0 \pm 0.6$            |

\* Significantly different from that of AF-labeled liposomes,  $p < 0.05$ .

<sup>a</sup> Data are mean  $\pm$  SD of 3 determinations.