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BIOMEDICAL APPLICATIONS OF MAGNETIC FLUIDS. II.[1] PREPARATION AND MAGNETIC GUIDANCE OF MAGNETIC ALBUMIN MICROSPHERE FOR SITE SPECIFIC DRUG DELIVERY *IN VIVO**

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Magnetic guidance of magnetic albumin microsphere for site specific delivery was investigated in mice and rats. After intravenous injection in mice, magnetic microspheres with 1 and 3 μm diameter size were localized and retained in the target-site (lung) by application of two permanent magnets to the lungs. Injection into the renal artery in rats also indicated that the 1-μm microspheres were concentrated in the kidney by a magnetic field. When the magnets were not applied, however, the microspheres following intravascular injection were concentrated mainly in the liver, regardless of the route of administration. Such preferential localization by magnetic means suggested that magnetic albumin microspheres could become effective drug carriers with site specificity for the delivery of chemotherapeutic agents in cancer therapy.

Keywords—magnetic albumin microsphere; magnetic fluid; drug carrier; site specificity; drug delivery; magnetic guidance; tissue distribution

One of the goals in cancer chemotherapy has been to find means for directing drugs selectively to tumor sites. The most interesting approach to this problem today is to entrap potent drugs in some drug carriers which are non-toxic and biodegradable. Various investigators have attempted to develop useful drug carriers, based on evaluation *in vitro* and *in vivo* experiments.[2,3] Recent examples include antitumor agents into liposomes,[4] albumin microspheres,[5] and microspheres in oil emulsion.[6] We recently reported that 5-fluorouracil entrapped in albumin microspheres was present in high levels in the liver of mice after intravenous injection,[7,8] and suggested sustained release and prolonged action of entrapped drug occurred in Ehrlich ascites[9] and solid carcinoma.[10] More recently, we reported that adriamycin entrapped in albumin microspheres showed pronounced antitumor activity on AH 7974 liver metastasis in rats as a model with an experimental tumor.[11] However, intravenous injection of those drug carriers as mentioned above results in their uptake predominantly by the reticuloendothelial system,[7,8,12] especially the Kupffer cells in the liver.[13]

Recently, Widder *et al.*[14] suggested that since magnetic microspheres injected into the ventral caudal artery could be localized to some extent to a predetermined tail segment by an externally applied magnetic field, magnetically guided albumin microspheres entrapped in drugs would be useful as a drug delivery system with site specificity. Kato *et al.*[15] also reported the possibility of magnetic control of antitumor drug and preparation of the ferromagnetic mitomycin C microcapsules with sustained release property, responding to necessity of selective cancer chemotherapy. If site specific drug delivery of antitumor agents could be

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achieved with magnetic means, this delivery system would eliminate adverse side effects that are often the sequelae of generated systemic drug distribution. This paper describe the utility of the magnetic albumin microsphere (ferro-colloid-entrapped albumin microsphere) as a drug carrier with target specificity by measuring microsphere levels in the lung and kidney after intravenous and intra-renal-arterial administration, respectively. A short communication of this work has been published.[1)]

MATERIALS AND METHODS

Materials—Bovine serum albumin (BSA), fraction V powder (Seikagaku Kogyo Co. Ltd.) and ^{125}I-human serum albumin (^{125}I-HSA, Japan Radioisotope Association) were used. A part of water-based magnetic fluid which was a suspension containing Fe_3O_4 fine particles, that is magnetites, was obtained from Taiho Industries Co. Ltd. and colloidal magnetite was prepared by the method of Shimoiizaka *et al.*[16)] Permanent magnet (Super Disc Magnet, No. 30730, inner radius 5 mm, outer radius 9.5 mm, thickness 6 mm, Edmund Scientific) with a magnetic introduction of about 3000 Gauss was used.

Animals—Male mice of ICR strain weighing about 30 g and male rats of Wistar strain weighing 210—250 g were used. The animals were maintained in a environment of controlled temperature at 24 ± 1œC and provided with Oriental regular solid diet and tap water *ad libitum*.

Preparation of Magnetic Albumin Microsphere—Magnetic albumin microspheres were prepared with BSA, ^{125}I-HSA, and magnetic fluid by a modification of the method of Widder *et al.*[17)] Two hundred mg of BSA labeled with 2 mg ^{125}I-HSA and 0.5 ml magnetic fluid were added to 2 ml distilled water. This was added to 200 ml of 10 % (v/v) Span 85 in cottonseed oil and the mixture was emulsified with Ultra Turrax (Ika Werk) at 8000 or 20000 rpm for 30 min at 10œC. Heat hardening of microspheres was accomplished by exposure to temperature of 180œC for 10 min. Since the range of particle size of microspheres prepared at 8000 rpm was 1—7 μm, the 1—2 and 5—7 μm microspheres

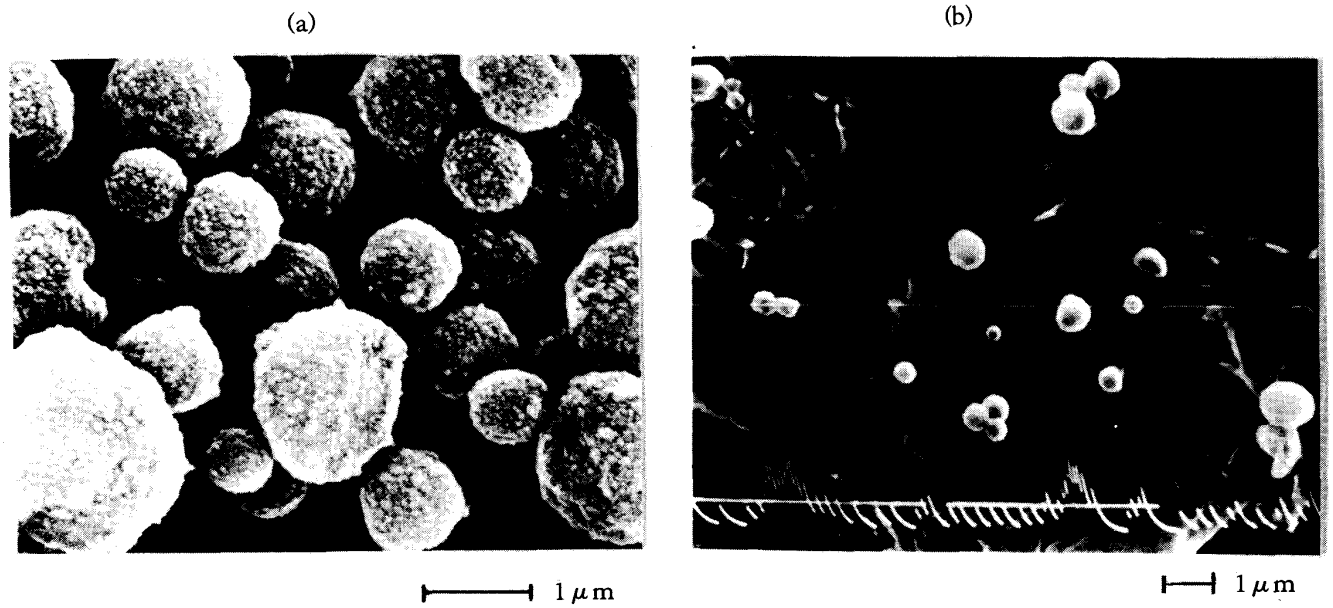

FIG. 1. *Scanning Electron Micrograph of Magnetic Microspheres and Spectrogram obtained with an Energy Dispersive-type X-Ray Microanalysis*

were separated from the 2—4 μm microspheres by centrifugation of the microsphere suspension. When the range of particle size of microsphere prepared at 20000 rpm was 0.4—1.5 μm, the microspheres were used without further separation. The mean particle size of microspheres was determined by a photomicrographic method. The magnetic microspheres are shown in the scanning electron microsphere photomicrograph in Fig. 1a and the presence of magnetites on the surface of microspheres was observed with the energy dispersive-type X-ray microanalysis (Fig. 1b). The final microsphere contain about 50 % magnetites by dry weight.

In Vivo Model for Carrier Targeting—In the first experiments, the mouse lung was selected as a model for *in vivo* testing of microspheres for two reasons: (i) The lung tumor occupies high ratios in many malignancies, and (ii) the microspheres after intravenous injection pass through the lung until sequestration in the liver. Each mouse was fixed on its back without anesthesia. One mg of the 1 or 3 μm microsphere was injected into the tail vein in mice in 0.2 ml of a suspension. Two magnets were directly applied to the side of each mouse, that is, the breast and back of mice, throughout the experiment so as to concentrate the microspheres into the lung. The mice were killed 10 or 60 min after administration, and ^{125}I-labeled microspheres in various tissues were determined by an auto-gamma scintillation spectrometer (Type 5110, Packard).

The kidney was selected as a model in the second experiments. It is difficult to collect the microspheres in other tissues except the lungs or heart after intravenous injection, so the route of

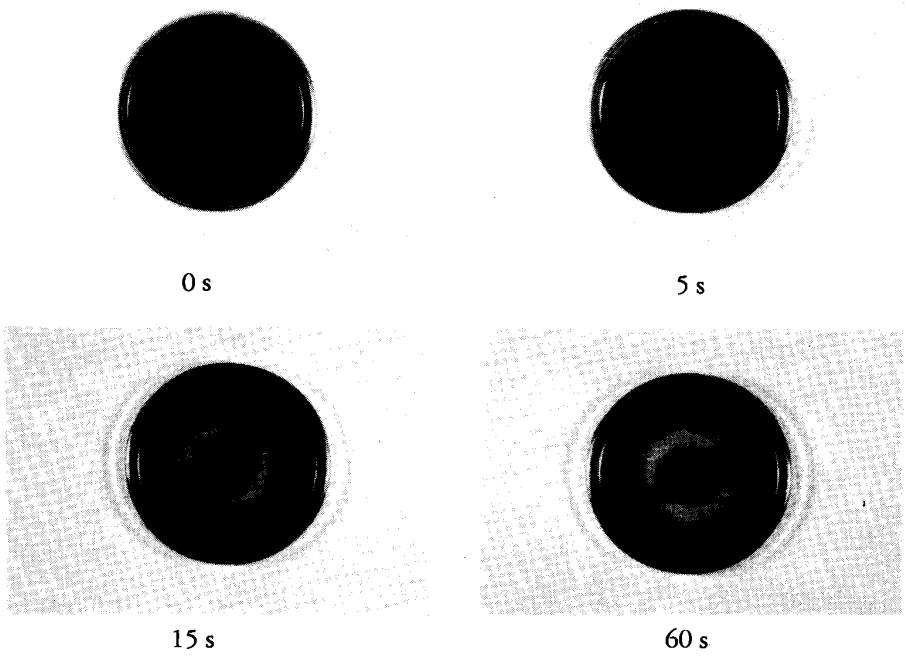

FIG. 2. *Sequential Time Sequence Photographs of Magnetic Microspheres in Aqueous Suspension after a Disc Magnet is applied*

administration was changed to intra-arterial injection. Each rat was anesthesized with pentobarbital sodium at a dose of 60 mg/kg *i.p.*, and was fixed on its back. Administration of the microspheres through the renal artery was carried out by cannulating a polyethylene tubing and forming a new vessel bypassing the original artery, because a polyethylene tubing is easy to be used for an injection. One mg of the 1 μm microsphere was injected into the left renal artery. Two magnets were directly placed throughout the experiments at both sides of the left kidney which was exposed by a midline abdominal incision. Distribution of microspheres 10 and 60 min after administration was examined by the method described previously. The distribution of ^{125}I-albumin microspheres to various organs is represented as % of dose per g tissue or that in the whole tissue.

RESULTS

Magnetic Responsiveness of Magnetic Microspheres

The magnetic responsiveness of the microspheres suspended in 0.2 % (v/v) polysorbate 80 solution is shown by the sequential time sequence photographs in Fig. 2. It is obvious that the microspheres were sequentially localized on and around the disc-magnet. In that follow, it was tested in detail whether or not the microspheres were preferentially relocated in target sites by a magnetic field applied when the microspheres with highly magnetic responsiveness were intravenously injected to animals.

Tissue Distribution of Microspheres in Mice

Tissue distribution of radioactivity at 10 min after intravenous injection of magnetic microspheres is shown in Fig. 3. After injection of the 1 μm microspheres in mice without magnet (control), about 3.9 % of the administered dose (15.8 %/g tissue at the concentration) was found in the lung. When the particle size of the microspheres was enlargted from 1 to 3 μm in diameter, uptake of the 3 μm microsphere in the lungs increased from 3.9 % of dose (15.8 %/g tissue) to 10.7 % (51.7 %/g). When two magnets were applied to the lungs, the microsphere levels in the lungs increased about four-fold for the 1 μm microspheres and twice for the 3 μm microsphere compared with each control. After injection of the 3 μm microspheres, a peak lung level (104.0 %/g tissue) was measured, but the amount of the microspheres in the lungs was only 21.6 % of the dose.

When the 3 μm microspheres were injected in mice, tissue distribution of radioactivity at 60 min is as shown in Fig. 4, where there is also the distribution when two magnets were applied to the lungs only for the initial 10 min. The uptake

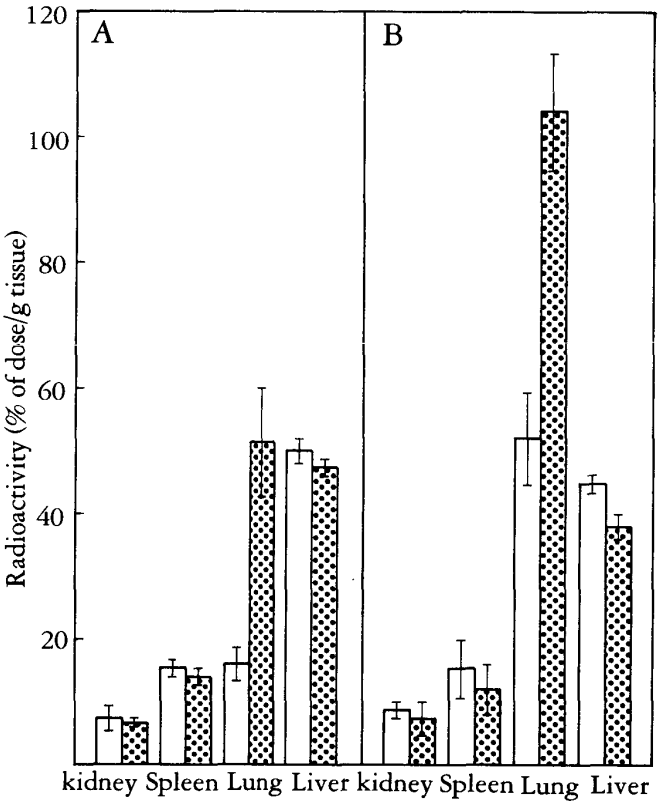

FIG. 3. *Tissue Distribution of Radioactivity at 10 min after Intravenous Injection of Magnetic Microspheres in Mice (1 and 3 μm in Diameter)*
A; 1 μm in average diameter, B; 3 μm in average diameter ▭ *; control (no magnet),* ▩ *; treatment with two magnets throughout the experiments.*
Results are expressed as the mean ± S.E. of 3—5 mice.

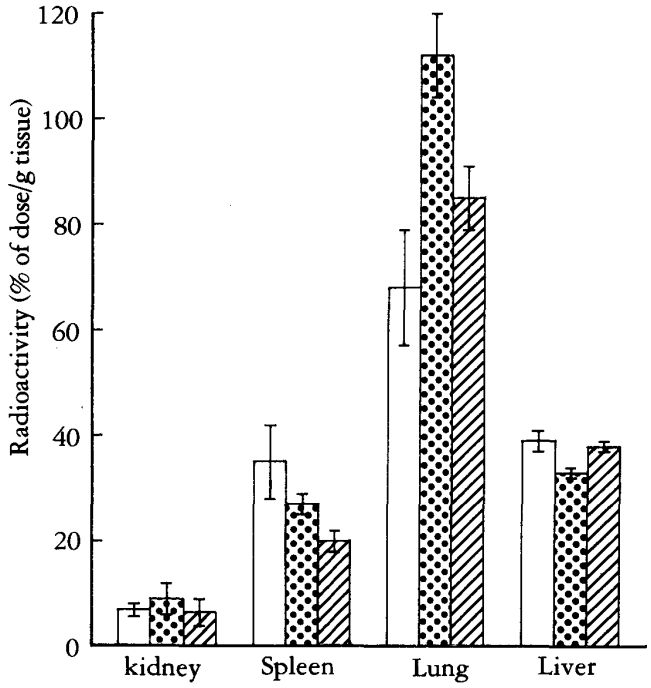

FIG. 4. *Tissue Distribution of Radioactivity at 60 min after Intravenous Injection of the 3-μm Magnetic Microsphere in Mice*
□; *control (no magnet),* ▩; *treatment with two magnets for 60 min,* ▨; *treatment with two magnets for the initial 10 min.*
Results are expressed as the mean ± S.E. of 3—5 mice.

of the microspheres in the lungs increased from 17.0 % of the dose (67.2 %/g tissue) to 28.2 % (111.3 %/g) and that in the spleen and liver decreased when two magnets were applied for 60 min. With application of two magnets for the first 10 min, there was an initial distribution of radioactivity to the lungs (Fig. 3) followed by rapid clearance from the lungs and localization in the liver (Fig. 4).

Tissue Distribution of Microspheres in Rats

Fig. 5 compares the tissue distribution of the 1-μm microspheres at 10 min following intravenous and intra-renal-arterial administration with or without application of two magnets to the left kidney. After intravenous injection, the microspheres did not concentrate in the kidney

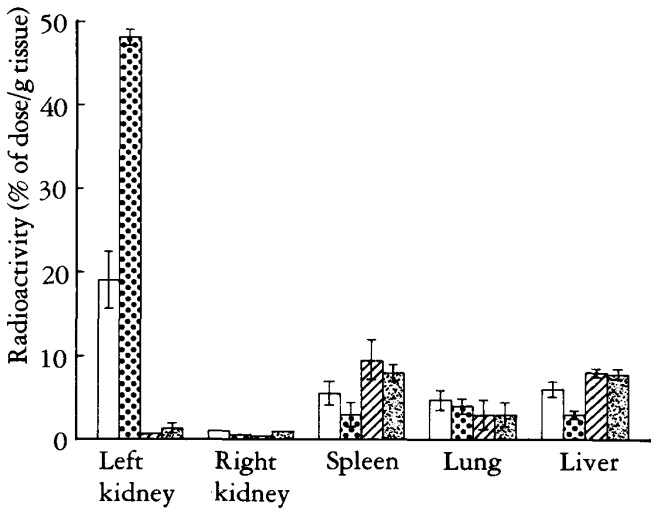

FIG. 5. *Tissue Distribution of Radioactivity at 10 min after Intravenous or Intraarterial Administration of the 1-μm Microspheres in Rats*
□; *intra-renal-artery (no magnet),*
▩; *intra-renal-artery (with magnet),*
▨; *Intratail vein (no magnet),*
▩; *Intratail vein (with magnet).*
Results are expressed as the mean ± S.E. of 3—5 rats.

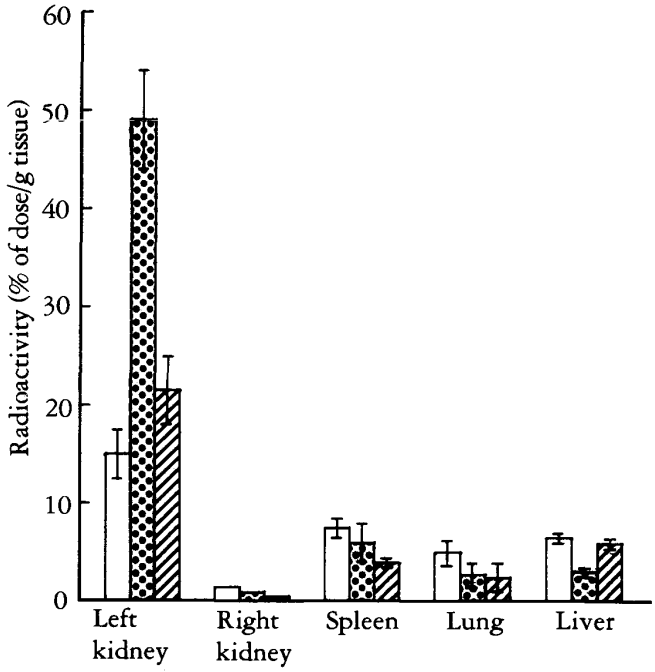

FIG. 6. *Tissue Distribution of Radioactivity at 60 min after Intra-renal-arterial Administration of 1-μm Magnetic Microspheres in Rats*
□; *control (no magnet),* ▩; *treatment with two magnets for 60 min,* ▨; *treatment with two magnets for the initial 10 min.*
Results are expressed as the mean ± S.E. of 3—5 rats.

and localized mainly in the liver regardless of application of two magnets to the kidney. The microsphere level in the kidney after intraarterial administration was higher than that after intravenous injection with and without magnets. Administration into the rat renal artery with magnets, on the other hand, concentrated the microspheres on the kidney at 10 min to 56.4 % of the dose (47.7 %/g tissue), and the value was about 2.5-fold higher than that in the control.

Fig. 6 compares the tissue distribution of the 1 μm microspheres at 60 min following intra-renal-arterial administration with application of two magnets to the left kidney for the initial 10 or 60 min. Microsphere level in the left kidney was found to be 56.4 % of the dose (47.7 %/g tissue) at 10 min after administration, when applying the two magnets.[1] In contrast to this value, the fraction of the dose remaining at the same organ at 60 min was much less when the magnet was removed 10 min after administration.

DISCUSSION

Recently, in several labolatories, it has come to be realized that magnetic microspheres with magnetic responsiveness may be used as target-selective homing devices in cancer chemotherapy.[14,15,17] Also, a considerable amount of information was presented regarding the fate of the microspheres and their contents after injection to rats.[14,21] It is, however, experimentally obscure whether magnetic guidance of the microspheres to the target sites such as the lung and kidney is possible. In addition, no information has been amassed regarding the effect of application time of the magnets to the target sites and removal from the sites on the behavior of the microspheres in blood. From a preliminary report[1] and these studies, it is evident that if the administration route of the microspheres is carefully selected and application time of magnets to the target sites is extended magnetic guidance of the microspheres to the sites such as the lung and kidney and the retention of the microspheres in those sites is possible (Fig. 5 and 6).

A small fraction of the intravenously administered dose distributed to the lungs is due to a simple filter effect of the pulmonary capillary beds, although vascular constriction may play some role in the trapping process. A large fraction is distributed mainly in the liver high in reticuloendothelial cell activity. This phenomenon of filtration in the lungs was clearly observed when the particle size of the microspheres changed from 1 μm in diameter to 3 μm. However, with the 1 or 3 μm microspheres injected in mice, there was a large distribution to the lungs by the application of the magnets, compared to no application of the magnets (Fig. 3). Moreover, distribution of the microspheres to the kidneys following the intra-renal-arterial administration due to a simple filtration of the superficial and juxtamedullary glomerular layers[18] was not negligibly but the microsphere level in the kidney with the magnets was higher than that without the magnets (Fig. 5).[1]

With the 3 μm microspheres injected in mice, retention in the lungs when applying the magnets was greater than that without application.[1] Those microsphere levels retained by an externally applied magnetic field in the lungs clearly decreased when the magnets were removed (Fig. 4). The pattern of distribution in the kidney when the magnets were removed was similar to that in the lung (Fig. 6). The decrease in the lungs or kidney seen between 10 and 60 min following administration of the microspheres may be due to washout within the lungs or kidney so that the microspheres initially trapped are allowed to recirculate in the bloodstream. The results obtained from the experiments of removing the magnets indicate that the application time of the magnets to the target sites is important for retention of microspheres in those sites.

A guarded selection of the administration route is also important for transporting microspheres to target sites. Kanke *et al.*[19] reported that no clearcut difference in distribution patterns was observed between intravenous and intraarterial administration of microspheres. Also

Sjöholm *et al.*[20] reported that the route of administration had no effect on the gross distribution pattern by studying the distribution of the polyacrylamide microparticles after intravenous and intraperitoneal injection in mice. The results shown in this paper, however, found a clearcut difference in the distribution pattern to be present between intravenous and intraarterial administration of microspheres in rats. Thus, the distribution of the microspheres *in vivo* after different routes of administration is really complicated, and further experiments and sufficient discussion would be needed.

The amount of microspheres retained at the target site (lung in mice) was 28.2 % of the dose at 60 min after intravenous injection of the 3-μm microspheres at a field strength of about 3000 Gauss. Failure to achieve greater retention of microspheres is most probably due to weak magnetic strength. When the strength of magnetic field is increased, this value might be improved considering that Widder *et al.*[14,21] used high magnetic field strength (8000 Oe) to retain 37—65 % microspheres into the tail after infusion through the ventral caudal artery. As mentioned above, many drug carriers which are capable of transporting drug molecules from the site of application directly to the site of action have been developed.[2,3] In the near future some carriers of them may be used in the clinical field, but the utility of some drug carriers is restricted because of the lack of specificity or selectivity. Controlled localization of drug carriers, however, has been difficult to achieve. Recently, Gregoriadis[22] documented that attempts have been made to rationalize liposome development by tailoring their structure to the particular biological milieu in which they are intended to act. In the case of magnetic albumin microspheres, it is certain that this drug carrier can be used as a target-selective homing device.

Studies are presently under way in our laboratory to examine the site specificity of the drug and its antitumor effect against experimental lung tumor after intravenous injection of magnetic albumin microsphere-entrapped antitumor agents.

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