immunoglobulin bearing, respectively. These figures correlate well with the known number of immunoglobulin-bearing splenocytes (44–51%) and demonstrate minimal nonspecific adherence.

To determine the immunological sensitivity of the microspheres, two different systems were tested. It was found that 100% of the chicken red blood cells was magnetically removed from suspension when ≥ 104 μg of microspheres was incubated with 1×10^6 chicken red blood cells. One milligram of staphylococcal protein A microspheres coupled with rabbit anti-human IgG effectively depleted all immunoglobulin-bearing human peripheral lymphocytes from a suspension containing $\leq 80 \times 10^6$ cells. These values demonstrate the capacity of staphylococcal protein A microspheres to separate a large number of cells rapidly and effectively *via* immunological means.

The ability of staphylococcal protein A microspheres to bind several different antiserums, which can confer a high degree of immunological specificity *in vitro*, was presented in this paper. Because magnetic microspheres can be used as drug carriers (1), it is proposed that with the addition of staphylococcal protein A to the microsphere matrix, tumor-specific antibody could be bound rapidly, resulting in a carrier capable of area-specific drug delivery and tumor-cell specificity. Thus, drug action could be limited solely to the desired cell population.

Because antibody coupling is rapid and easily performed, it is ideally suited for cell separation. No spacer group has to be coupled to the microspheres prior to antibody coupling. Moreover, no chemical coupling agents are used, which allows the reutilization of valuable antiserums. Since most IgG subclasses can bind staphylococcal protein A microspheres, this system may be valuable in rapid protein and enzyme purification. This system also may be useful as a mechanism for automated radioimmunoassays.

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In Vivo Kinetics of Magnetically Targeted Low-Dose Doxorubicin

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Abstract □ The *in vivo* kinetics of low-dose doxorubicin (0.05 mg/kg), entrapped in a carrier and magnetically targeted, were characterized in a rat tail model. Tissue concentrations of doxorubicin at a preselected target site and in various organs were followed over time. As late as 60 min postinjection, 3.7 μg/g of drug was found at the target site with no detectable drug levels found in any organ. In comparison, a 100-fold higher dose (5.0 mg/kg iv) of free doxorubicin yielded drug concentrations of 1.8 μg/g at the target site and 15.0 μg/kg in the pooled organs. Therefore, 1% of the free intravenous dose targeted magnetically yielded approximately twice the local doxorubicin concentration at a preselected target site with no detectable systemic distribution. Magnetic targeting of particulate drug carriers to localized disease sites is suggested as an efficient method of obtaining high local drug concentrations and may reduce many unwanted side effects from unrestricted systemic circulation.

Keyphrases □ Microspheres, magnetic—as drug carriers, doxorubicin, *in vivo* □ Carriers, drug—doxorubicin-bearing magnetic microspheres, *in vivo* □ Doxorubicin—*in vivo* fate of localized low dose, kinetics □ Delivery, drug—magnetic microspheres as drug carriers, doxorubicin, *in vivo* □ Microencapsulation—symposium, doxorubicin-bearing magnetic microspheres, *in vivo* fate of localized low dose

Current approaches to enhancing the tumoricidal activity of antineoplastic agents involve combining them with various carriers in the hope of favorably altering their systemic distribution. Early work by Chang (1), who encapsulated proteins and enzymes to develop artificial organs, suggested that drugs also may be encapsulated in carriers. The current targeting of drug carriers such as liposomes (2) or natural cells (3) depends on a moderate to marked enhancement of the endocytic activity (4) exhibited by some solid tumors. Other modalities for enhancing drug concentration at known tumor sites include regional perfusion, hyperthermia, and local installation and implantation of drugs. However, most of these modalities have met with limited clinical success (5).

A biophysical approach to targeting chemotherapeutic agents to known tumor sites was described recently (6). The system utilizes magnetically responsive albumin microspheres as the carrier for entrapped doxorubicin. The application of an extracorporeal magnetic field over a selected body area results in accumulation of the carrier containing the entrapped drug at the target site. The synthesis (7), magnetic responsiveness (8), and *in vivo* distribution (6) of the carrier at one time period postinjection were described previously. However, the correlation of carrier distribution with doxorubicin distribution over time was not examined. In this report, the levels of doxorubicin obtained at different time intervals at a preselected target site are characterized. In addition, a low dose (0.05

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Table I—*In Vivo* Distribution of ^{125}I-Labeled Magnetic Microspheres [a]

Time Magnetic Field Was Applied, min	Liver	Lungs	Spleen	Kidneys	Heart	Tail Segment 1	2	3	4
5	7	4	5	0	0	0	0	47	0
15	6	4	5	0	0	0	0	45	0
30	6	6	8	0	0	0	0	45	0
60	8	6	8	0	0	0	0	44	0
Control	13	6	10	0	0	0	0	0	0

[a] Values are expressed as counts per minute × 10^2 per gram of wet weight of tissue and represent an average of two animals. The control consisted of microsphere infusion without the application of a magnetic field. The level of detectability was 3×10^2 cpm/g. Values were rounded to the nearest 100, with zero indicating that counts were either not present or below the level of detectability. Tail Section 3 was chosen as the target area.

mg/kg) of magnetically targeted doxorubicin and a 100-fold higher free intravenous dose are examined with respect to the target and whole body distribution.

EXPERIMENTAL

Preparation of Microspheres—The synthesis and characterization of magnetic microspheres bearing doxorubicin hydrochloride[1] were described previously (7). The emulsion polymerization method was carried out as follows. An aqueous solution was prepared containing 250 mg of human serum albumin[2], 32 mg of bulk, purified, lactose-free doxorubicin hydrochloride, 72 mg of magnetite[3], and 0.028 mCi of ^{125}I-labeled bovine serum albumin[4] in 1 ml of distilled water. A 0.5-ml aliquot of this suspension was homogenized with 30 ml of cottonseed oil[5] by sonication[6] (100 w) for 1 min at 4°. The homogenate then was added dropwise to 100 ml of stirred (1600 rpm) cottonseed oil, which was preheated to 145°.

After 10 min of heating, the microspheres were washed free of the oil by centrifugation in anhydrous ether. After three washes, the ether was removed and the microspheres were stored in the dark at 4° until time of use. Prior to injection, the microspheres were resuspended in 0.1% polysorbate 80–saline[7] and washed once by centrifugation to remove any surface adherent drug. The microspheres contained 26.4 μg of doxorubicin/mg of microspheres and had a specific activity of 33,100 cpm/mg after suspension.

Animal Model—Female Sprague–Dawley rats, 300 g, were anesthetized with methoxyflurane, and the ventral caudal artery at the base of the tail was exposed for catheterization as described previously (6). The femoral vein was used for intravenous administration. The tail was demarcated into four equal sections, with Section 1 being proximal and Section 4 being the most distal. Tail Section 3 was the target site and was placed between the poles of a bipolar magnet[8]. The magnet exerted a field of 8000 Oe with a gradient of ±4 kOe within Section 3, and minimal or no magnetic fields were detected in adjacent segments.

A dose of 0.05 mg of doxorubicin/kg encapsulated in microspheres was infused in the ventral caudal artery in Section 1 at 0.6 ml/min by a constant-flow syringe pump[9]. The magnetic field at the target site was left in position for 5, 15, 30, or 60 min, after which the animal was immediately sacrificed by intracardiac injection of saturated potassium chloride. Control infusions consisted of 0.05 mg of free doxorubicin/kg *via* the caudal artery and 5.0 mg of free doxorubicin/kg iv, after which the animals were sacrificed at the cited time intervals in the manner described.

Tissue Analysis—Tissue extraction of doxorubicin was accomplished by a modification of a literature method (9). At the selected times, animals were dissected rapidly; the heart, liver, lungs, kidneys, spleen, and the four separate tail sections were frozen immediately at −60°. The excised organs and tail sections were examined for microsphere content by determining iodine 125 activity in a well-type γ-counter[10]. Two animals were used for each time point.

The organs were weighed and homogenized[11] in a 5% HCl solution in ethanol at 4° for 2 min. However, the tail skin sections were treated overnight in 0.5 *N* acetic acid at 4° to facilitate collagen solubilization and subsequent homogenization. Previous experiments showed that treatment of doxorubicin with 0.5 *N* acetic acid under these conditions did not significantly alter its fluorescence characteristics. The tissue homogenates were centrifuged[12] at 10,000×*g*, and the supernates were examined for total fluorescence using a spectrophotofluorometer[13].

Since the 0.05-mg/kg dose delivered in encapsulated form did not yield detectable drug concentrations in the individual organs, pooling of the organs was necessary.

In addition, quick-frozen sections of the tail skin segments were examined by fluorescence microscopy[14] according to the method of Egorin *et al.* (10). The characteristic orange-red fluorescence of doxorubicin in and surrounding small arterioles and capillaries of the rat tail skin was evaluated.

RESULTS AND DISCUSSION

Table I shows the *in vivo* distribution of ^{125}I-labeled microspheres at different times postinjection. Tail Section 3, the target area, contained a significantly higher concentration of microspheres than the surrounding tail segments. Moreover, in comparison to the liver and spleen, which are the normal sites for sequestration of particulate carriers, the target site had approximately a ninefold higher concentration of carrier. No discernible carrier redistribution was noted over the time periods studied. This finding is significant in that analysis of drug distribution was not complicated by this variable. The usual time required for 50% clearance of nonmagnetic microspheres varied from 2 to 8 hr, depending on the extent of matrix stabilization (11, 12).

The determination of doxorubicin content in the individual organs was not possible for the 0.05-mg/kg dose in either the free or encapsulated form because of the low levels attained after systemic distribution. However, the 5.0-mg/kg free dose showed individual organ values in agreement with values reported previously (13). Pooling of the individual organs of low-dose-treated animals still yielded barely detectable levels of doxorubicin but permitted a comparison between the 0.05- and 5-mg/kg doses.

Figure 1 shows the amount of doxorubicin found at 5 and 60 min postinjection in the 0.05-mg/kg carrier-delivered dose *versus* the free 5-mg/kg iv dose. Microsphere-delivered doxorubicin resulted in a target site concentration of 4.2 μg of doxorubicin/g of tissue at 5 min, which decreased to 3.7 μg/g at 60 min. In comparison, the 100-fold higher dose of 5 mg/kg resulted in a tissue concentration of 6.2 μg/g at the target site at 5 min, after which it rapidly decreased to only 1.8 μg/g at 60 min. Intermediate values between 5 and 60 min decreased in nonlinear fashion.

The rapid plasma disappearance of free doxorubicin is in agreement with current concepts of doxorubicin kinetics. Doxorubicin plasma decay curves follow a three-phase pattern (14). The half-life of the first phase is ~10 min and represents rapid tissue uptake. The other two phases represent redistribution and metabolism and vary from species to species (15).

Figure 1 also compares concentrations of free doxorubicin and magnetically localized low-dose doxorubicin in the pooled organs. Essentially nondetectable levels of doxorubicin were present in the pooled organs with the low carrier-delivered dose at both 5 and 60 min postinjection, whereas the free dose showed 15 and 8 μg/g at 5 and 60 min, respectively. With respect to correlating drug distribution with carrier distribution, the theoretically predicted doxorubicin concentration at the target site, based on iodine 125 counts of the carrier, is 3.5 μg of doxorubicin/g at 60

[1] Adriamycin, Adria Laboratories.
[2] Sigma Chemical Co.
[3] Ferrosoferric oxide (Fe_3O_4) in aqueous suspension (MO-I), Ferrofluidics Corp.
[4] Specific activity of 1.51 mCi/mg, New England Nuclear.
[5] Sargent–Welch.
[6] Branson sonifier model 185.
[7] Tween 80 (Sigma Chemical Co.) in 0.154 *N* NaCl.
[8] No. 70,752, Edmund Scientific.
[9] Model 341, Sage Instruments.
[10] Packard model 578.
[11] Sorvall Omni-Mixer.
[12] Sorvall RC-2B.
[13] Aminco SPF-125S (excitation at 470 nm, and emission at 586 nm).
[14] Leitz Ortholux microscope with KP490 excitation and K530 barrier filters.

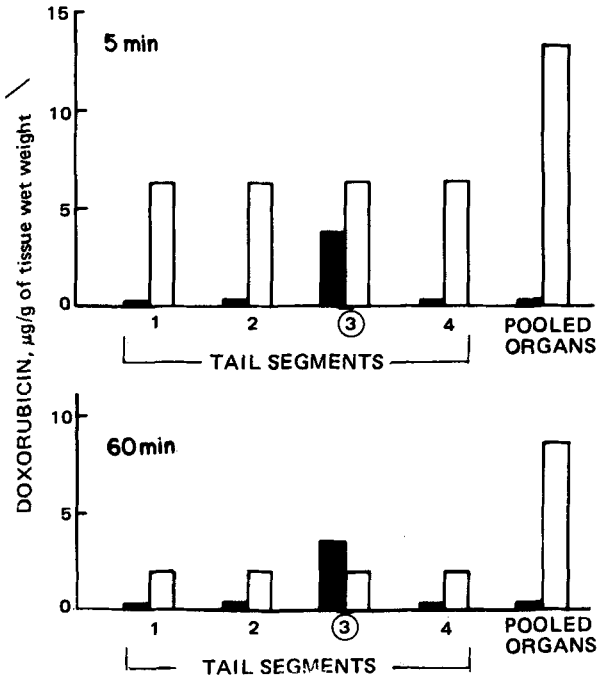

Figure 1—*Doxorubicin tissue concentration was determined spectrophotofluorometrically after infusion of either 5 mg of free doxorubicin/kg iv (□) or 0.05 mg of doxorubicin/kg encapsulated in magnetic microspheres via the caudal artery (■). Each value represents the average of two animals.*

min whereas the actual value found by spectrophotofluorometric methods was 3.7 μg/g.

The doxorubicin assay employed in these experiments was limited to determining the total tissue fluorescence. Adriamycinol, an active metabolite, accounts for a percentage of the total fluorescence. However, this metabolite, which is produced by the action of an ubiquitous intracellular aldo–keto oxidoreductase, is minimally active in rats in comparison to humans or monkeys (15). The magnetic sequestration of carrier-delivered doxorubicin from systemic circulation thus could be beneficial in eliminating many of the known acute and chronic toxic side effects. Although the data indicate a good correlation between carrier and drug distribution, the question of bioavailability of carrier-delivered doxorubicin has yet to be determined in a tumor model test system. However, analysis *in situ* by fluorescence microscopy of the microspheres 30 min postinjection at the target site suggested a radial diffusion of doxorubicin from the microspheres into the surrounding tissues. The rapid cellular uptake of doxorubicin that occurs as early as 0.5 min postinjection (10) should facilitate its diffusion into the cells once it is released from the microspheres.

In conclusion, magnetic targeting of chemotherapeutic agents to known sites of disease should be feasible with many currently available antitumor agents. As late as 60 min postinjection, 1% of a free intravenous dose of doxorubicin magnetically localized resulted in almost twice the local tissue concentration than was achieved by a 100-fold higher intravenous dose. The bioavailability of the localized drug is currently under investigation in a tumor model system.

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Preparation and Release Characteristics of Potassium Chloride Microcapsules

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Abstract □ The release characteristics of potassium chloride were studied when it was coated with a selection of polymers; from the results obtained, a suitable batch was microencapsulated using a gelatin–gum arabic coacervate system. The microencapsulated product offers better controlled release for this drug when compared to standard tablet and powder forms.

Keyphrases □ Potassium chloride—preparation and release characteristics of microcapsules □ Polymers—various polymers and waxes investigated for microencapsulation of potassium chloride □ Microencapsulation—symposium, preparation and release characteristics of potassium chloride microcapsules □ Drug release—potassium chloride microcapsules, preparation and release characteristics

The satisfactory control of drugs in tablet or powder form from protective membranes is often difficult to achieve (1, 2). The present work investigated the microencapsulation of a material that is usually given in a

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