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Selective Targeting of Magnetic Albumin Microspheres to the Yoshida Sarcoma: Ultrastructural Evaluation of Microsphere Disposition*

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Abstract—*Magnetic albumin microspheres (1 μm average diameter) were selectively targeted to subcutaneous solid Yoshida sarcoma tumors (average size* 450 mm^2*) in Holtzman rats. This was accomplished by placing an external magnet adjacent to the tumor while the microspheres were infused. Microspheres contained ultra-fine particles of* Fe_3O_4 *and no drug (placebo). Placebo microspheres were used due to the previously demonstrated rapid tumoricidal effect of targeted low-dose doxorubicin microspheres. Animals were killed* 10 min, 60 min, 30 min, 24 hr *and* 72 hr *after microsphere administration and tumors were examined by transmission electron microscopy to determine the* in vivo *disposition of the magnetically targeted microspheres. Using placebo microspheres, we have demonstrated microspheres endocytosed in endothelial cells as early as* 10 min *after infusion. By* 30 min *microspheres can be seen in the extravascular compartment, sitting adjacent to tumor cells and occasionally in tumor cells. By* 24 hr *the majority of microspheres have been endocytosed by tumor cells. Microspheres were still observed within tumor cells as late as* 72 hr *after administration. The rapid extravasation and cellular uptake of magnetically focused microspheres explains the extremely rapid tumoricidal effect previously observed when doxorubicin-containing microspheres were targeted to the tumor.*

INTRODUCTION

MAGNETICALLY responsive albumin microspheres have been shown to be capable of being selectively localized *in vivo* with retention of bioactivity of the entrapped antineoplastic agent doxorubicin [1–3]. More recently, we have demonstrated a significant number of total remissions in animals bearing the Yoshida sarcoma by localizing a single dose of doxorubicin-containing magnetic microspheres to the tumor [4, 5]. Histologic sections of tumor obtained as early as three days after targeted treatment revealed massive tumor necrosis [4]. The present study was performed to determine the mechanism of biological processing of the localized microspheres within the tumor.

We have previously reported that microspheres targeted to the skin of normal rats have the ability to lodge between or become endocytosed by endothelial cells at the microvascular level [2]. These findings were obtained 30 min after microspheres were localized to the target site. However, a significant anatomical difference between the microvasculature of 'normal' tissue and tumors has been reported [6]. Consequently, we now present transmission electron microscopic data tracing the *in vivo* processing of the microspheres. Placebo spheres were used due to the extremely rapid necrosis observed when doxorubicin

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microspheres are localized to the tumor. The information gained from this study confirms the theory that magnetic targeting of drug-carrying microspheres creates an extravascular depot for sustained release of the entrapped drug. This effect is enhanced by the increase in the vasculature present in most tumors.

MATERIALS AND METHODS

Preparation of microspheres

Placebo microspheres were prepared as outlined previously [4, 5, 7]. Briefly, microspheres were prepared by mixing 125 mg of human serum albumin (Sigma Chemical) and 36 mg of magnetite (Fe_3O_4, Renaissance Technologies) in 0.5 ml distilled water. To this 30 ml of cottonseed oil (Sargent Welch) was added and the emulsion was homogenized by sonication for 1 min at 100 W using a Heat System (Model W185D) sonifier with a 1/4-inch titanium probe inside the recirculating attachment chamber. The resultant homogenate was added to 100 ml of constantly stirred cottonseed oil preheated to 120–125°C. After 10 min the suspension was cooled to 25°C and was washed four times with anhydrous diethyl ether by centrifugation. Resultant microspheres were stored in powdered form at 4°C.

Treatment of animals with placebo microspheres

Holtzman rats (180 g) were inoculated subcutaneouly with 3.5×10^8 cells of the ascitic form of the Yoshida sarcoma into the lateral aspect of the tail. Animals were used for experimental purposes 6–8 days after inoculation of the tumor. By this time solid tumor nodules of 50–60 mm length were present.

Animals were anesthetized with methoxyfluorane (Dow Chemical) and the ventral caudal artery was exposed at the base of the tail. A polyethylene catheter (Clay Adams PE10) was inserted caudally to a point 2 cm from the proximal margin of the tumor. Placebo microspheres (1 mg) suspended in 1 ml of 0.15 M saline containing 0.1% Tween 80 were infused by a constant-flow syringe pump (Sage Model 341) at 0.20 ml/min while the tumor was positioned between the poles of a 5500 Oe adjustable-gap permanent magnet (Edmund Scientific). Animals were allowed to remain in the presence of the magnetic field for 30 min, with the exception of one group of animals killed after 10 min exposure to the magnetic field. Remaining animals were killed at 30 min, 1 hr, 24 hr or 72 hr.

Processing tissue

Tumors taken from animals at the designated time intervals were fixed in 3% glutaraldehyde in 0.15 M sodium cacodylate buffer, pH 7.4. Specimens were dehydrated with ethanol and embedded in Epon and examined with a JEOL 100B transmission electron microscope as previously described [8].

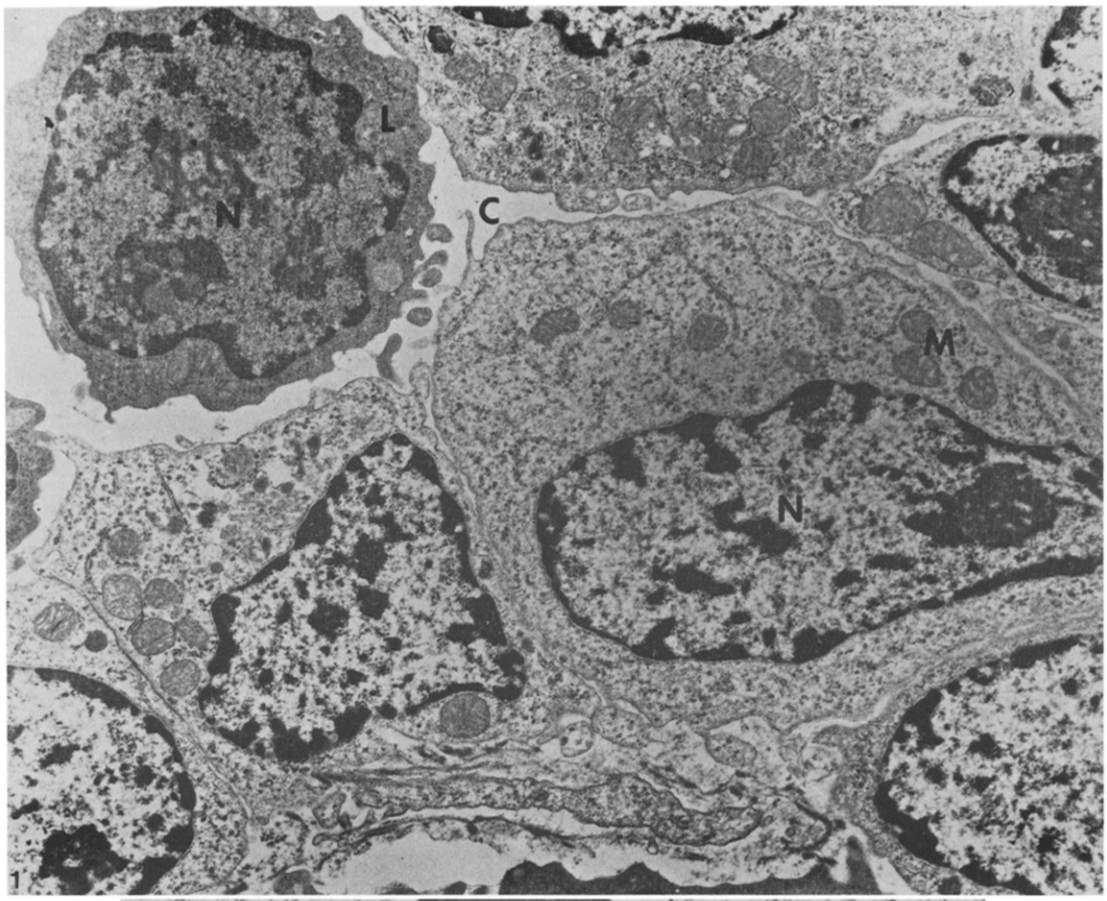
RESULTS

Electron microscopic sections examined from animals killed 10 min after placebo microsphere infusion demonstrated the presence of microspheres within endothelial cells lining capillaries as well as within capillary lumina (Fig. 2). As early as 30 min after targeting, microspheres were evident both in the extravascular compartment as well as within the cytoplasm of tumor cells (Figs 3, 4, 5). Similarly, microspheres were also seen intracellularly within tumor cells at 60 min (Fig. 6). Some microspheres were membrane bound, indicating a phagocytic mechanism for uptake, whereas others had no detectable membrane association. Microspheres were still detectable within tumor cells at both 24 and 72 hr (Figs 7 and 8), with some evidence of early microsphere degradation at 72 hr (Fig. 8).

DISCUSSION

Ultrastructural analysis of microsphere disposition supports experimental data demonstrating rapid sequestration of the drug carrier within the tumor. Microspheres are rapidly cleared from capillary beds as early as 10 min after infusion with subsequent extravasation of the carrier. The drug carrier most likely imparts its cytotoxic effects by two mechanisms. First, microspheres positioned extracellularly in the extravascular spaces are capable of releasing drug by radial diffusion. The released drug will have a concentration gradient decreasing over distance from the microsphere. However, since the microsphere will generally be surrounded by a number of tumor cells, many of these cells should be influenced by the released drug. In this fashion, multiple tumor cells may be destroyed by the presence of one microsphere creating a tumoricidal amplification system.

Secondly, drug-carrying microspheres can be internalized within the cytosol of tumor cells and the drug released from the carrier is capable of direct damage to the cell. Therefore, drug resistance based on the failure of the drug to be transported across a tumor cell membrane may be obviated by encapsulating the drug within magnetic microspheres. Moreover, once the minimum amount of drug released from the microspheres damages a given tumor cell it should become leaky, allowing remaining drug to



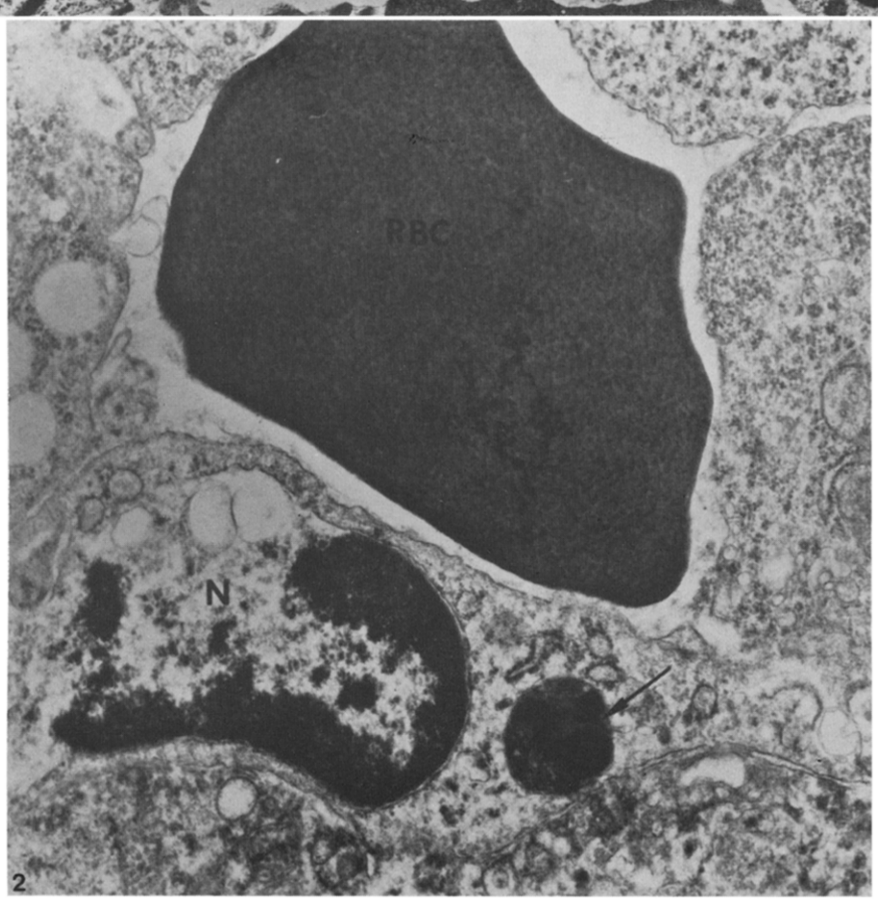


Fig. 1. Transmission electron photomicrograph of untreated tumor cells taken from an animal eight days after tumor inoculation (× 3600). Tumor cells contain abundant mitochondria (M) and rough endoplasmic reticulum. Cytoplasmic processes (C) are not infrequently seen. A lymphocyte (L) is present in the upper left portion of the micrograph. Its round convoluted nucleus (N) is contrasted with the more pleomorphic nuclei of the tumor cells.

Fig. 2. Transmission electron photomicrograph of tumor obtained from an animal killed 10 min *after placebo microsphere infusion and magnetic placement at the tumor site. A microsphere (arrow) can already be seen within the cytoplasm of an endothelial cell lining a capillary. A red blood cell (RBC) is present within the capillary lumen (× 4800).*

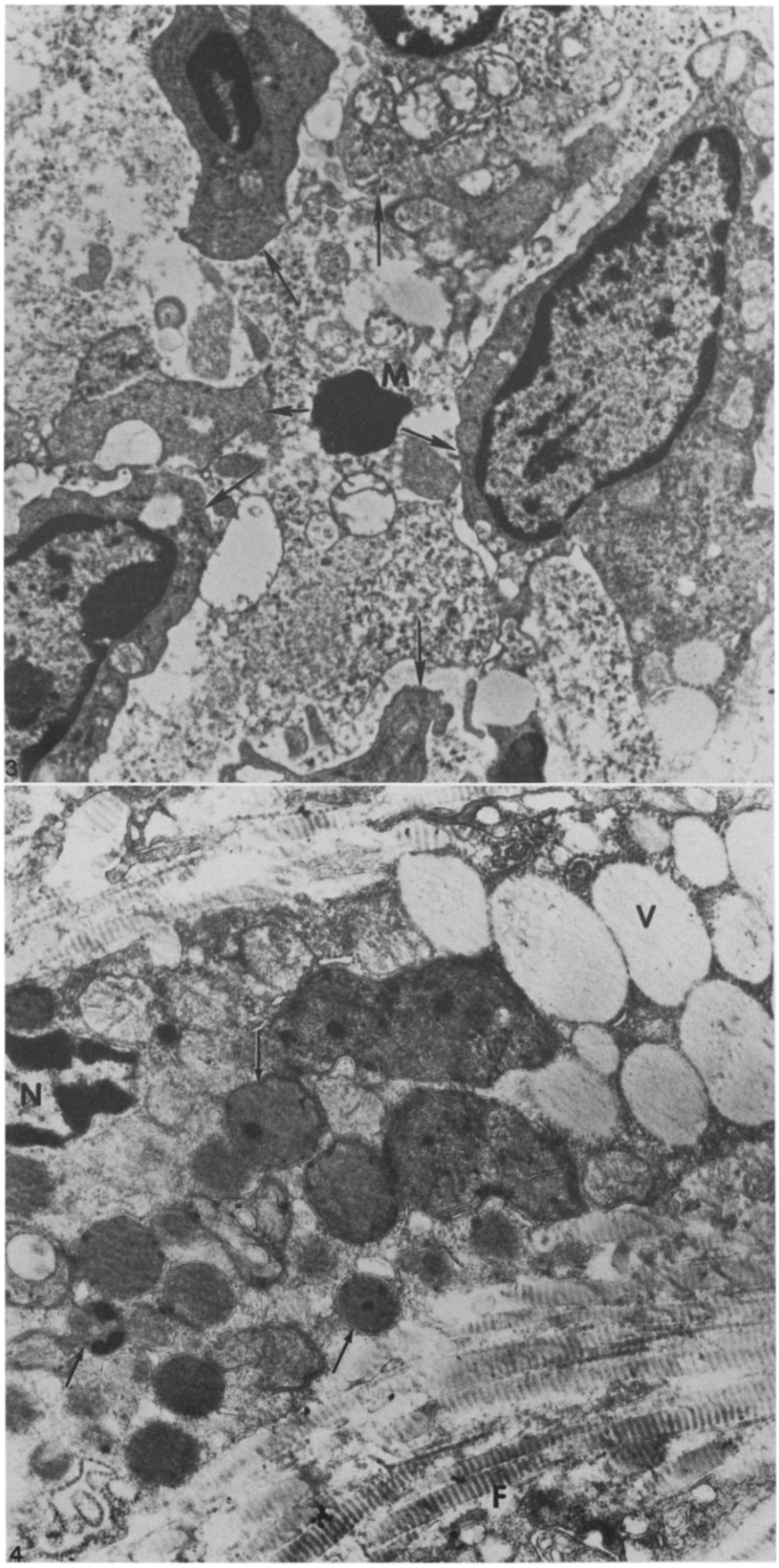


Fig. 3. After 30 min *microspheres can be seen outside the vascular compartment in the interstitial space. A microsphere (M) is seen extracellularly surrounded by tumor cells (arrows). Drug released from the microsphere could potentially damage the surrounding tumor cells* (× 2400).

Fig. 4. Transmission electron photomicrograph of a tumor cell which has invaded perivertebral muscle, obtained 30 min *after carrier infusion* (× 4800). *The nucleus of the tumor cell (N) can be seen at the left-hand portion of the micrograph. Collagen fibrils (F) demonstrating cross-banding are seen both in the upper and lower portions of the micrograph. Numerous microspheres (arrows) have been endocytosed by the tumor cell. Vacuoles (V) are also present.*

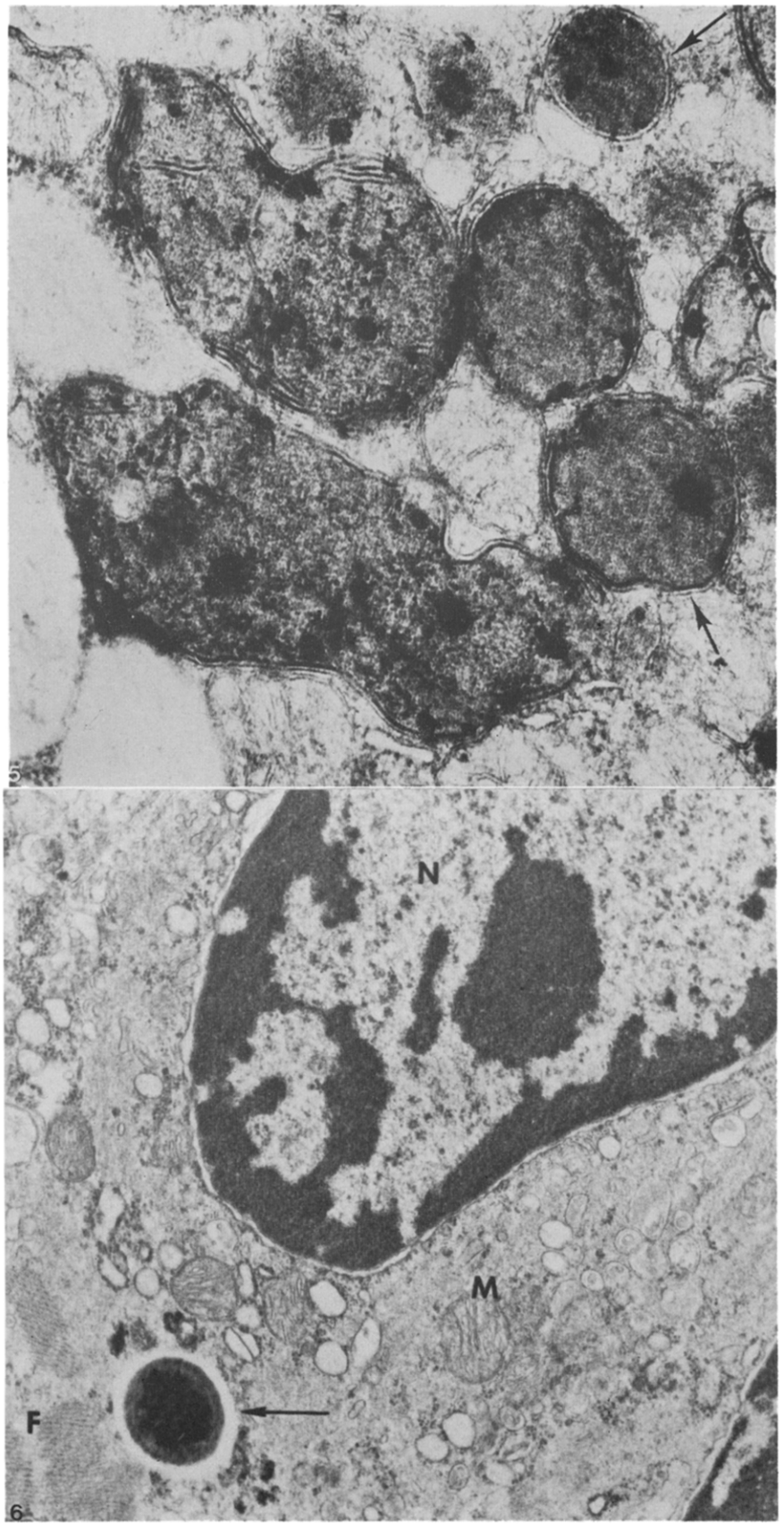


Fig. 5. A higher magnification (× 9000) of a portion of Fig. 4 illustrating the double membrane surrounding individual microspheres (arrows), suggesting their uptake by phagocytosis.

Fig. 6. Transmission electron micrograph of a tumor cell obtained from an animal killed 60 min *after carrier targeting. A microsphere (arrow) can be seen within the cytoplasm of the tumor cell adjacent to the nucleus (N). Again, numerous mitochondria (M) are present. Thick and thin filaments (F) can be seen, implicating this tumor to be of skeletal origin. Interestingly, a cleared zone is present surrounding the microsphere, the significance of which is unknown (× 9000).*

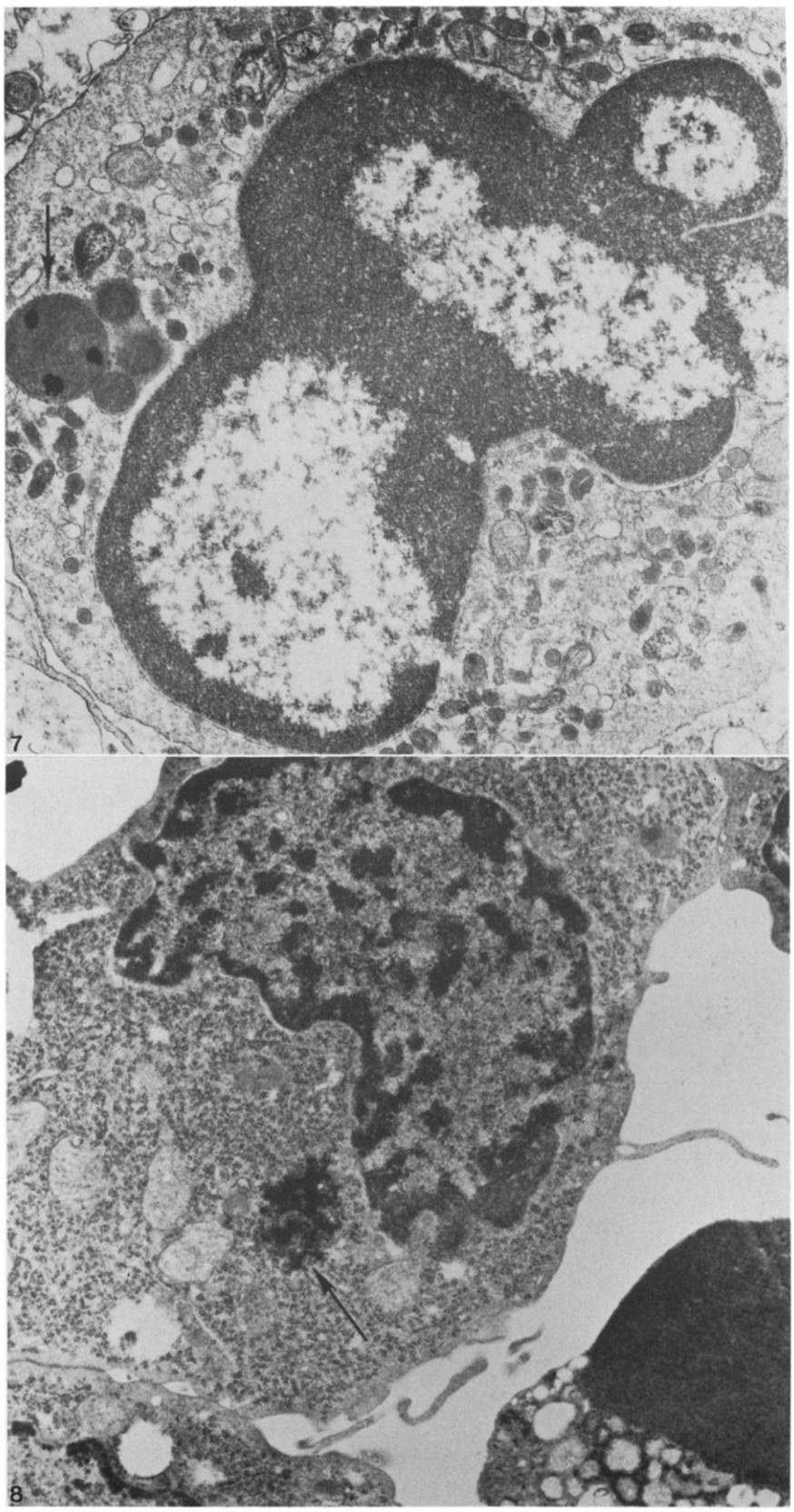


Fig. 7. As late as 72 hr *after administration, microspheres (arrow) can still be seen within the cytoplasm of tumor cells (×* 3600*).*

Fig. 8. Transmission electron photomicrograph (× 6000*) of a tumor cell taken from an animal killed* 72 hr *after targeting microspheres. A microsphere in the process of being degraded can be seen within the cytoplasm of the cell (arrow). Thus some microsphere breakdown is apparent as early as* 72 hr.

be released extracellularly into the tumor environment and thus allowing further tumoricidal activity of the drug. In other studies microspheres were detected at the initial tumor site as late as three weeks after administration as determined by [^{125}I] counts, though only 10–15% of the initial microspheres were present (unpublished data). These data suggest that although the microspheres are biologically degraded over time, a significant number remain within the tumor site to allow for sustained drug delivery.

Regardless of which mechanism of microsphere processing occurs within the tumor site, a significant local concentration of drug occurs which result in rapid tumor destruction [1, 4, 5]. This method for drug delivery allows for the potential use of highly toxic antineoplastic agents which are currently too toxic for systemic administration. It may also result in obtaining efficacy with specific antitumor agents against tumors with a transport-mediated resistance to the drug. Targeting antineoplastic agents by their incorporation within magnetic microspheres can establish high local concentrations of the entrapped agents with a concomitant reduction of systemic toxicity.

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