procaine hydrochloride (2) but not in the official assay for proparacaine hydrochloride (3).

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Martin I. Blake [x]
David W. Bode
Harold J. Rhodes

Department of Pharmacy
University of Illinois
Chicago, IL 60612

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[x] To whom inquiries should be directed.

Albumin Microspheres as Vehicles for Achieving Specificity in Drug Delivery

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To the Editor:

There is a growing need in modern day drug therapy to develop methods of drug delivery that are highly cell or tissue specific. Cancer chemotherapy, for example, demands that drugs with pronounced systemic toxicities be delivered to target tissues in precise dosages and with minimal interaction with normal tissues. Past approaches to achieving specificity have centered around structural modification of the drug, alterations in route of administration or dose regimen, and efforts to physically position a drug delivery device, such as a polymeric implant, in the closest possible proximity to the target tissue.

The phagocytic cells of the reticuloendothelial system provide a physiological means of achieving cell and tissue specificity in drug delivery that has enormous potential. Radiologists utilize the phagocytic activity of these cells to achieve specificity in the delivery of radiolabeled albumin and sulfur colloids (1). Gregoriadis (2) entrapped anticancer agents within phospholipid vesicles, reported the phagocytic uptake of the vesicles by the liver in rats, and mentioned the possibility of directing drug-containing vesicles to other cell types by manipulation of the vesicle surface. Trouet *et al.* (3) reported reduced toxicity and increased effectiveness in the treatment of leukemia when DNA complexes of the anticancer agent daunomycin are pinocytized from solution. In an attempt to find a phagocytizable carrier capable of accommodating a wide variety of drugs, the anticancer agent mercaptopurine was entrapped within human albumin microspheres.

Table I—Incorporation of Mercaptopurine-8-^{14}C Hydrate in Human Albumin Microspheres

Total Drug Available, μg/mg Albumin	Drug Associated with Microspheres[a], μg/ml Albumin	Entrapment, %
4.00[b]	3.46	86.5
4.00	3.59	87.3
8.00[c]	1.57	—
10.5	1.32	—

[a] Average of two determinations. [b] Drug present in emulsion prior to denaturation (1 mg/ml in internal phase). [c] Drug added to previously prepared albumin microspheres (1 mg/ml).

Albumin microspheres provide a potentially useful means of delivering drugs to endocytic cells because they are physically and chemically stable, rapidly removed from the vascular system by phagocytosis, amenable to preparation in large batches, nonantigenic, metabolizable, and capable of accommodating a wide variety of drug molecules in a relatively nonspecific fashion. Human serum albumin microspheres containing the anticancer agent mercaptopurine were prepared by a modification of the method of Scheffel *et al.* (4). One milliliter of a 27% dimethylformamide–water solution containing 1 mg of mercaptopurine-8-^{14}C hydrate[1] and 250 mg of human serum albumin[2] was emulsified with 100 ml of cottonseed oil USP by repeated passage through a hand-operated homogenizer[3]. The emulsion was added with constant stirring to an additional 100 ml of oil that had been heated to 175°, held at that temperature for 10 min, and cooled. No degradation of mercaptopurine could be detected in dimethylformamide–water solutions kept at 175° in sealed ampuls for similar periods.

Two hundred milliliters of ether was then added and the suspension was filtered through a 0.5-μm filter[4]. The spheres were washed repeatedly with ether to remove residual oil and dried. The quantity of drug entrapped was determined by digesting the spheres in 0.5 *N* NaOH and counting on a liquid scintillation counter[5], using appropriate internal standards. Over 85% of the mercaptopurine present in the original emulsion was associated with the microspheres (Table I).

Since albumin would be expected to adsorb drug from solution, an experiment was performed to determine how much mercaptopurine could be adsorbed onto previously prepared drug-free microspheres. The microspheres were suspended in a 27% dimethylformamide–normal saline solution containing 1 mg mercaptopurine-8-^{14}C hydrate/ml (the same concentration present in the internal phase of the emulsion used to prepare drug-containing spheres), stirred well, centrifuged, and washed five times with a large excess of distilled water. About 40% as much drug was associated with the spheres as when drug was incorporated in the original emulsion (Table I).

[1] New England Nuclear.
[2] Sigma Chemical Co.
[3] C. W. Logeman Co.
[4] Solvent resistant, Millipore Corp.
[5] Packard Tri-Carb model 2009.

Table II—*In Vitro* Release of Mercaptopurine-8-^{14}C from Human Albumin Microspheres

Suspension Medium	Drug Released from Spheres, %	
	2 hr	24 hr
Buffer alone[a]	15.7	16.0
Buffer and dimethylformamide	17.6	21.0
Buffer, dimethylformamide, and unlabeled mercaptopurine (1 mg/ml)	39.8	42.8

[a] Isotonic, buffered saline, pH 7.4, containing 0.1% polysorbate 80.

The ability of drug-free spheres to adsorb mercaptopurine indicated that a substantial percentage of the drug entrapped from the emulsion might be present on the surface of the microspheres. A wash-off study was conducted to determine the loss of drug from microspheres prepared from a drug emulsion. The spheres (5 mg/ml) were suspended in isotonic, buffered saline solutions (pH 7.4) with and without unlabeled mercaptopurine. After centrifugation, the supernatant liquids were assayed for displaced radiolabeled drug (Table II). Only 16% of the drug was lost from the spheres into buffered saline in 24 hr, but about 40% could be exchanged for unlabeled mercaptopurine. The latter value is in accord with the percentage of drug that appeared to be surface associated in the adsorption study.

The quantity of drug entrapped in this system was limited by the low aqueous solubility of the drug. Preliminary experiments with the anticancer agent daunomycin hydrochloride indicated that a larger quantity of more water-soluble drugs can be entrapped without recourse to mixed solvent systems. Daunomycin hydrochloride was entrapped to the extent of 8 μg/mg albumin, about twice the mercaptopurine entrapment, and preliminary results indicate that this quantity can be increased considerably. Figure 1 shows a scanning electron micrograph of human albumin microspheres containing daunomycin hydrochloride. They range in size from about 0.2 to about 1.2 μm and are representative of the type of physical system discussed for mercaptopurine. They have not been sonicated, a process that produces a smaller, more uniform particle size.

Albumin microspheres containing chemotherapeutic agents may well be potentially useful in the treatment of cancer and fungal or bacterial infestations of the reticuloendothelial system (histoplasmosis and typhoid fever) and in the delivery of immunosuppressive agents. A particulate system containing an antimicrobial drug might, for example, effectively breach the barrier to drug transport provided by the macrophage in typhoid fever and give better access to the bacillus. Several reports indicated that the rate of clearance from the blood of human cancer patients of

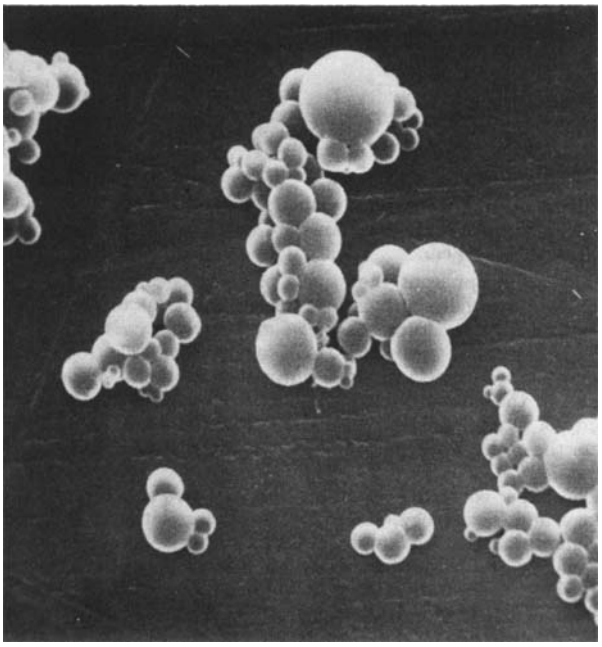

Figure 1—*Scanning electron micrograph (10,000×) of human serum albumin microspheres containing daunomycin hydrochloride (0.8 mg/100 mg albumin). Particles were dried from an ether suspension producing the observed aggregation.*

intravenously administered colloids, such as aggregated human albumin and lipid test emulsions, is proportional to the extent of neoplasia (5, 6).

It is possible that preferential uptake into such tissues as liver or bone marrow might be achieved and required total doses as well as systemic side effects of anticancer agents might be reduced. A study is in progress to determine the fate of intravenously administered microspheres containing radiolabeled mercaptopurine in mice and the kinetics of the release of mercaptopurine from the spheres *in vivo*.

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Paul A. Kramer

Department of Industrial and Physical Pharmacy
School of Pharmacy and Pharmacal Sciences
Purdue University
West Lafayette, IN 47907

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