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Lipid Microencapsulation of Hemoglobin

C. ANTHONY HUNT* AND RONALD R. BURNETTE

Department of Pharmaceutical Chemistry, University of California, San Francisco, CA

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The idea of microencapsulating a solution of hemoglobin (Hb) to make a prototypal artificial red blood cell (RBC) has been around for decades. T.M.S. Chang discussed the possibility in the late 50s and early 60s. The ideal properties and characteristics of a transfusable suspension of microencapsulated Hb have been specified. Progress has been limited by three major obstacles: (i) selecting an acceptable microencapsulation material; (ii) developing a microencapsulation process that yields the desired size range, but avoids denaturation of Hb; and (iii) encapsulating sufficient Hb while maintaining an acceptable final viscosity. We have made considerable progress in all three fronts. The resulting microcapsules are called Neohemocytes.

Neohemocytes (Nhc) are artificial RBC prototypes and consist of small amounts of Hb and other solutes microencapsulated in a biodegradable, biocompatible membrane. The size range, 0.1–1.0 μm, is small enough to allow free passage through capillaries. Neohemocytes have features in common, at one production stage or another, with complex emulsions, classical microcapsules, and liposomes. Our procedure is derived from one developed by Szoka and coworkers (*1*) for preparation of unilamellar phospholipid vesicles. There are three basic stages: (i) formation of an O/W/O emulsion consisting of a mixed, volatile nonaqueous solvent (O) and the aqueous Hb solution (W); (ii) partial vaporization of

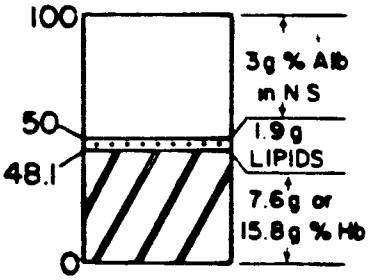
*Author to whom all correspondence and reprint requests should be addressed.

the nonaqueous phase and phase inversion to form the membrane network; and (iii) complete removal of solvents and unencapsulated Hb. The lipids that will form the membrane are included initially in the nonaqueous phase. In this report the lipids consist of a mixture of phosphatidylcholine, α-tocopherol, phosphatidic acid, and cholesterol in the molar ratio 4:0.1:2:5. The initial aqueous phase is a 17–20 g% solution of Hb (met-Hb must be <5%) plus 2,3-DPG at a DPG:Hb molar ratio of 1:5; buffer salts are also included.

Properties of a transfusable solution of Nhc are listed in Fig. 1. Currently the suspending solution is 3 g% BSA in sterile, pH 7.4, normal saline. The relative volume fractions of membrane and encapsulated Hb solution vary from batch to batch, but other properties are relatively constant. For a suspension having an apparent hematocrit of 0.5, the membranes account for 1.9 to 6% of the suspension volume and 3.8 to 12% of the displaced Nhc volume (assuming a lipid density of 1 g/mL). The concentration of the encapsulated Hb averages 15.8 g%, giving a Hb content for the suspension of 7.6 g/dL. The O_2-binding properties of the Nhc and the original starting, stroma-free Hb (SFH) are also listed in Fig. 1. Because of the added 2,3-DPG, the P_{50} for Nhc is increased to about 26, whereas that of the starting SFH is only 13. The Hill Number ("*n*") is unaffected by the encapsulation procedure. The procedure consistently causes about a 100% increase in met-Hb levels (e.g., from 0.4 to 0.8% or from 2 to 4%, depending on starting values). Met-Hb levels of 5% or more are incompatible with the current procedure.

We have completed several transfusions in rats using Nhc suspension having an apparent hematocrit of 0.25 (3.8 g/dL of Hb), where >97% of the blood volume was replaced. Controls were identically transfused with an unencapsulated 3.8 g/dL solution of Hb. The average survival time for controls was about 4 h. Rats (n = 4) exchange transfused with Nhc survived at least 18 h, and half were long-term survivors. All (n = 4) rats that were 50% exchange transfused with Nhc survived without signs of acute toxicity.

The overall vascular clearance of Nhc is the sum of three processes: clearance resulting from irreversible binding to tissues followed by



	Neo-hemocytes	SFH
P_{50} :	26	13
"n" :	2.1	2.1
met-Hb :	4% (.8)	2% (.4)

Fig. 1. Properties of a transfusable solution of Nhc.

breakdown, Cl_B; clearance caused by rapid or early destruction or breakdown, Cl_D; and clearance from uptake by the RES, Cl_{RES}. For typical liposomes, emulsions, and microcapsules, Cl_{RES} is the dominant process, a situation that may be unacceptable for Nhc if it results in reticuloendothelial blockage. Our objective is to have Nhc that interact minimally with tissues and are relatively invisible to the RES. Cl_{RES} of Nhc decreases with decreasing mean diameter, increasing dose, and appropriate surface modifications, consistent with results reported for liposomes [Abra and Hunt (2)]. At 24 h following complete exchange transfusions in rats, the circulating Nhc level has declined to approximately 50% of the post-transfusion value.

The data clearly indicate that Neohemocytes are artificial RBC prototypes both in vitro and in vivo. Further optimization of properties is in progress.

ACKNOWLEDGMENT

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