The Use of Radioiodinated Latex Particles for In Vivo Studies of Phagocytosis*

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ABSTRACT. Polystyrene latex particles were radioiodinated, and combined radioisotope, histologic and electron microscopic studies traced the fate of these particles in vivo. Particular emphasis has been given to the role of charge and stability in determining distribution of the colloid. Non-phagocytic aspects of blood clearance and evidences for recirculation of phagocytized particles are discussed.

The functions of the reticuloendothelial system have been studied using a variety of test materials [1-4]. Although much information has been obtained, there have always been discrepancies among the results of workers using different test substances. The obvious conclusion is that the nature of the particles presented to the RES is one of the most important features governing response of the phagocytic system. Some of the variables which influence the choice of a test colloid include the physicochemical characteristics of the substance and the stability of the material in vitro and in vivo. The latter variable would appear, from our work, to be of considerable significance in governing the behavior of the colloid in the blood and in various organs containing phagocytic cells.

Polystyrene latex particles have been used by a number of workers to study phagocytosis, reticuloendothelial proliferation, and interaction of charged particles with the RES [3,5]. Latex particles are unique among colloidal materials used to test the RES in that their surface properties may be modified without changing the chemical structure of the particles, they may be manufactured in a wide range of sizes while retaining the same chemical properties, and they may be quantitatively coated with serum pro-

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teins. In addition, these particles are readily identified in electron microscope sections because of their size and electron density. They may be directly visualized by phase contrast and darkfield microscopy. In paraffin sections they may be stained with Oil Red 0 in propanol. By this method latex particles stain reddish, whereas fat droplets remain unstained.

Because of their particular physicochemical properties, monodisperse latex particles were used to investigate the effect of charge and stability on phagocytosis by cells of the RES.

Latex particles were obtained from Dr. J. Vanderhoff, Dow Chemical Company. These particles were prepared by the polymerization of styrene with relatively small amounts of butadiene. The polymerization of standard latex particles in the presence of butadiene was necessary because radioiodination must be accomplished through the butadiene double bond. Iodination was performed according to the procedure developed in this laboratory [6].

The amount of radioisotope incorporated into the structure of the particles is so small that the physicochemical properties, charge, stability, and homogeneity of size are not detectably altered. The level of radioactivity (125I or 131I) may be varied within certain limits according to experimental requirements, and particles are obtained which have no deleterious effect on the test animal and yet are readily detected in a gamma scintillation counter [6].

Latex particles are prepared by emulsion polymerization. The nature and amount of detergent used in this process are primarily responsible for the net surface charge of the particles. Charge may be simply and conveniently determined by using a moving boundary four-compartment micro-Tiselius cell developed in our laboratory [7]. Since it is the detergent that confers surface charge on the latex, it has been possible to reverse this charge by the use of different detergents [7,8]. All commercially available latex particles are negatively charged. Following iodination, the particles have an electrophoretic mobility toward the anode of 2.3 \times 10^{-4} \text{ cm/sec/V/cm} at 4^\circ \text{C} corresponding to a zeta potential of -46 \text{ mV}. Positive particles, prepared by exchange dialysis in benzalkonium chloride have a mobility toward the cathode of 1.47 \times 10^{-4} \text{ cm/sec/V/cm} (zeta potential +29.4 mV). The nonionic particles prepared in Tween 80 are slightly electronegative having an electrophoretic mobility toward the anode of 0.63 \times 10^{-4} \text{ cm/sec/V/cm} (zeta potential -12.6 mV). Stability is controlled by the amount of emulsifier used, and may be checked by observing the particles by dark field or phase contrast microscopy. Stable preparations are monodisperse. Particles of 2200-Å size were tested in vivo, using CF_{1} male mice for blood clearance, organ localization, histology, and electron microscopy studies.

Studies of reticuloendothelial function and of phagocytosis have given much attention to the rate at which injected test colloids are removed from the blood. In general, the shape of the clearance curve has been interpreted