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The Preparation of Semipermeable Microcapsules Containing Antibody for Use in Radioimmunoassay

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One of the most critical stages in a radioimmunoassay is the separation of antibody bound from free ligand. A large number of methods are currently available for this separation stage [Ratcliffe (*1*)]. However, because of high separation efficiency and speed, solid-phase first antibody systems have grown in popularity [Chapman et al. (*2*)]. Microencapsulation of an antibody produces a reagent essentially equivalent to a solid-phase first antibody, but with a number of potential advantages related to the semipermeable nature of the surrounding membrane. Such advantages went unnoticed until Ashkar et al. (*3*) reported the use of microencapsulated antibody in a radioimmunoassay for free thyroxine. This publication was rapidly followed by a series of papers on microencapsulated antibodies in radioimmunoassays for digoxin [Halpern and Bordens (*4*)], free thyroxine [Halpern and Bordens (*5*)], and cortisol [Bordens and Halpern (*6*)]. Unfortunately, these authors provided little information on the methodology used for preparing

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antibody-containing microcapsules. In an attempt to find a reagent suitable for inclusion in radioimmunoassays, we have performed detailed evaluations of two methods for producing antibody-containing polyamide microcapsules. Initially, the method described by Wood and Whateley (*7*) was investigated. Thirty percent of antibody was recovered in microcapsules prepared in this way. However, a gross reduction in antibody-binding activity was observed. This reduction was probably related to the presence of the high concentration of hemoglobin (or contaminant therein) used as a "filler" in this method (*8*). Although these antibody-containing microcapsules worked well as a reagent for radioimmunoassay, the loss of such a large amount of antibody-binding activity was restrictive.

The second method was adapted from that of Grunwald and Chang (*9*), in which polyethyleneimine rather than hemoglobin was used as a "filler." Polyethyleneimine capsules retained 50% of antibody and, unlike hemoglobin-filled capsules, no gross loss of binding activity was observed. A major adaptation of this method was the use of an Ultra-Turrax homogenizer during the mixing stage of the microencapsulation procedure. By careful selection of homogenizer speed and Span-85 concentration, microcapsule size could be adjusted, and a method was selected that repeatedly produced capsules ranging in diameter from 5 to 40 μm, with a mean of 20 μm. Antibody-containing semipermeable capsules prepared in this way and stored at +4°C were stable for at least 3 months, with only a slight reduction of antibody titer. When used as a reagent in radioimmunoassays, the capsules were stable to repeat dispensing during the addition stage, sedimented only slowly during the incubation stage, and were robust enough to withstand the final centrifugation and washing steps.

We are currently using antibody microencapsulated by this method in a direct radioimmunoassay for the steroid, 17-hydroxy-progesterone (17-OHP). As well as providing a reagent that works well in the separation stage of the assay, microencapsulation of the antibody has eliminated the need for blocking the interfering binding of 17-OHP to a serum-binding protein. This simplification in the procedure has enabled us to use the assay in a large-scale screening program set up to identify congenital adrenal hyperplasia (a condition that causes a rise in circulating 17-OHP) in neonates from the West of Scotland.

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