Sucrose Gradient Centrifugation
Sedimentation Standards: A
Simplified Technique

To the Editor:

In the last few years, steroid receptor characterization methods, including sucrose gradient centrifugation to measure sedimentation properties, have gained in importance as a clinical diagnostic tool in such diseases as mammary and prostatic carcinoma. While studying the sedimentation characteristics of thymic estrogen receptor, we developed a technique for rapid visualization of the 4.8S region in our sucrose gradients by using rhodamine-labeled bovine albumin (rBA; M.A. Bioproducts, Bldg. 100, Biggs Ford Rd., Walkersville, MD 21793) as the sedimentation markers. After preparation of 50 to 200 g/L sucrose gradients as described previously (1–3), we added 0.1 mL of rBA (1 mg) to 0.8 mL of proestrus rat uterine cytosol (a 1 g/4 mL suspension), prepared by homogenization in buffer containing tris-(hydroxymethyl)aminomethane hydrochloride (pH 7.4, 10 mMol/L) and disodium ethylenediaminetetraacetate, 1 mMol/L, as described previously, and incubated with 6.5 μL of 100 mCi/mol [3H]estradiol. We then layered 0.2 mL on the top of each gradient.

Centrifugation was for 15 h at 202 000 × g; the gradients were then separated into 20 fractions. The location of the rBA standard was made visible under a long-wave ultraviolet light (Blak-Ray, Model 15; Ultra-Violet Products, Inc., San Gabriel, CA 91778). Each fraction was then treated for 10 min with dextran-coated charcoal to remove any nonspecific [3H]estradiol binding. Radioactivity in the fractions was measured with a scintillation counter.

Figure 1 shows a composite sucrose gradient profile. As can be seen, the 7S peak (fractions 11 through 15) in the profile run with rBA does not differ significantly in size from the 7S peak in the profile without rBA. The 4.8S peak (fractions 7 through 9) is, however, significantly increased in the presence of rBA. In the gradient profile to which rBA was added, the color was located in fractions 7 through 9.

We also studied the properties of the 7S standard, fluorescein-conjugated immunoglobulin G (M. A. Bioproducts). We found that the presence of this material in gradients significantly alters the apparent size of the 7S peak, probably as a result of quenching. While we do not recommend that fluorescein-conjugated immunoglobulin G be added to gradients containing sample, this substance as well as rBA may be run separately in a control gradient, to aid in locating the 4.8S and 7S regions.

References
5. Charles J. Grossman1 2
6. Patrick L. Uebel1
7. Leon J. Sholiton1
8. Paul Nathan2 4

1 Cincinnati VA Med. Center/151/
2 3200 Vine St.
3 Med. Res. Service
4 Cincinnati, OH 45220
5 Cincinnati, OH 45220
6 (address for correspondence)
7 UC Med. Center
8 Dept. of Physiology
9 Cincinnati, OH 45220
10 Dept. of Biology
11 Edgecliff College
12 Cincinnati, OH 45206
13 Shriners Burns Instit.
14 Cincinnati, OH 45219

Fig. 1. Composite gradient profile of the sedimentation characteristics of cytosol estrogen receptor
Control: Δ--Δ; rBA present: O—O, color localized in fractions 7–9. Each point is the mean of two observations.

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