

Preparation of Radioactive Iodinated Serum Albumin.* (19747)

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The preparation of proteins tagged with Iodine-131 has been described by many workers(1-4). In order to use these preparations for dilution experiments, exhaustive dialysis is necessary to free the tagged protein from free radioactive iodide.

A more rapid method for this preparation has been developed which eliminates the lengthy dialysis step. Ion exchange resins have been used elsewhere in the de-salting of solutions. The studies reported here indicate that the Amberlite resins IR-100H and IR-4B may be used to advantage for the preparation of radioactive iodinated serum albumin solutions free from inorganic iodide.

Preparation. In a 125 ml Erlenmeyer flask, 20 ml of salt-free human serum albumin (25% solution as supplied by the American Red Cross) were mixed with 5 ml of sodium carbonate solution containing 25 g Na_2CO_3 per 100 ml. The iodinating mixture was prepared in a dropping funnel by adding 1.2 of 0.002 M sodium iodide solution and 1.2 ml of 0.02 M sodium nitrite solution to 1.0 ml of a solution of carrier-free Iodine-131 in sodium bisulfite, as supplied by Oak Ridge, with an activity of approximately 1 millicurie per ml. The solution was stirred and brought to a pH below 4 (indicator paper) by the addition of 3 drops of 0.5 N hydrochloric acid. After a few minutes, the solution was neutralized with 2 drops of 0.25 N sodium hydroxide solution. The reagent was diluted with 16.5 ml of water, stirred, and added dropwise to the alkaline albumin solution with gentle stirring over a period of 5 minutes. The funnel was rinsed with 5 ml of water, which were added to the reaction mixture. After 10 minutes of further stirring, the protein solution was poured onto a column of Amberlite IR-4B (analytical

grade, previously backwashed with distilled water for 30 minutes). The effluent was then passed through a second column containing backwashed analytical grade Amberlite IR-100H. The first 20 ml of the effluent was made isotonic by the addition of 1.3 ml of 15% sodium chloride solution and filtered through a sterile Seitz-Manteufel filter into a sterile tube. The tube was stoppered with a sterile rubber dam and stored in the refrigerator until needed. The total time elapsed in the preparation was usually about 2 hours.

Efficiency of column-desalting. To determine the amount of inorganic iodide not removed by the column, the following experiment was routinely performed. One ml of the preparation was deproteinized with 1 ml of 10% sodium tungstate solution and 8 ml of N/12 sulfuric acid. The filtrate was counted, with an end-window type Geiger tube, as was a 1:10 dilution of the protein solution. The following results are typical:

1:10 albumin solution: $25,893 \pm 2\%$ cpm

Deproteinized solution $323 \pm 2\%$ cpm

It is apparent that virtually all of the non-protein-bound iodine has been removed by the deionization procedure.

Summary. A rapid procedure for the preparation of radioactive iodinated human serum albumin suitable for injection for dilution studies has been described. This technic used ion exchange resins instead of dialysis for the removal of free inorganic iodide.

1. Crispell, K. R., Porter, B., and Nieset, R. T., *J. Clin. Invest.*, 1950, v29, 513.

2. Fine, J., and Seligman, A. M., *J. Clin. Invest.*, 1944, v23, 720.

3. Shulman, N. R., and Tagnon, H. J., *J. Biol. Chem.*, 1950, v186, 69.

4. Warren, S., and Dixon, F. J., *Am. J. Med. Sci.*, 1948, v216, 136.

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