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A simplified procedure for the preparation of Sephadex G-200 for the separation of rat serum IgG and IgM

To establish the roles of serum antibodies (IgM and IgG) in the immune response it is often necessary to separate different immunoglobulin classes[1]. Gel chromatography has been used successfully to separate IgM and IgG on the basis of differences in molecular size[2,3] for a wide variety of immunological studies[4], and for this reason it is the method of choice. In eluting antibodies from rat serum using gel chromatography we have noted a quick and simplified method of preparing Sephadex G-200 for its application to the column.

Preparative method

Sephadex G-200 (fine)* was added to isotonic unbuffered saline and boiled for 2–3 min to evaporate gases and allow the gel to swell. This procedure is considerably quicker than swelling a minimum of 5 h on a boiling water bath or 3 days at room temperature as the manufacturer recommends[5].

Results and discussion

Using the above technique complete separation of rat IgM and IgG antibodies (Hemolysin) was obtained with Sephadex G-200. The IgM associated with the first absorption peak and the IgG with the second. Further proof that complete separation was obtained came from sucrose density gradient ultracentrifugation of the fractions[6].

There are several advantages to this procedure. The Sephadex gel can be swollen and ready for packing in 3 min. Good separations can be obtained with small sample sizes (as low as 0.05 cc rat serum) with no detectable loss of antibody activity using the K9/60 Sephadex laboratory column (diameter 0.9 cm, length 60 cm, bed volume 38 ml), obtained from Pharmacia. Recovery rates of antibody activity were high (up to 100 % in some samples). The same column can be used up to two months with no detectable change of the elution behavior of the serum antibodies.

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