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Ey P L, Prowse S J & Jenkin C R. Isolation of pure IgG_1, IgG_{2a} and IgG_{2b} immunoglobulins from mouse serum using protein A-Sepharose. *Immunochemistry* 15:429-36, 1978.
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This paper describes a simple and rapid method for isolating pure mouse IgG_1, IgG_{2a}, and IgG_{2b} immunoglobulins in nearly 100 percent yield. The immunoglobulins bind at pH 8.0 to protein A-Sepharose and can be eluted sequentially in functionally active form using buffers of decreasing pH. [The *SCI*® indicates that this paper has been cited in over 1,665 publications.]

Purification of Mouse IgG Isotypes

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This paper resulted from a study by Charles R. Jenkin and me on the development of immunity in mice to a natural helminth parasite, *Nematospiroides dubius*. Multiple infection with this nematode induces an extraordinary, specific increase in IgG_1, and we wished to isolate the different immunoglobulin isotypes from sera in order to study the functional properties of the parasite-specific antibodies.

Stephen J. Prowse (then a PhD student) and I began separately to adsorb the IgG immunoglobulins from mouse sera onto protein A-Sepharose, recovering the IgG by elution in dilute acid.[1] As seems often to be the case, serendipity played a significant role in our subsequent discovery. Steve had several times adsorbed aliquots of infected serum at room temperature, washing the protein A-Sepharose column with phosphate-buffered saline (PBS) prior to acid elution of the bound immunoglobulins. I worked with the same column, but used it in the cold room and washed it with 0.15 M phosphate buffer rather than PBS. In talking to Steve about our results, I noticed that unlike my adsorptions, which were characterized by a sharp effluent peak with virtually no trailing edge, he regularly obtained a prominent second effluent peak during his washing step with PBS.

I have always been very stringent in methodology, and, with a long-standing interest in the physicochemical properties of proteins, I was sufficiently motivated to investigate the basis for this phenomenon. Our initial assumption that it was a temperature effect proved incorrect—pH was the crucial factor: Steve's PBS was pH 7.2, whereas my buffer was pH 8.0. I immediately began a series of adsorptions at different pH's, following this up with elutions using continuous pH gradients and, later, with stepwise elutions (increasing acidity). From the start I had appreciated that the interaction between the adsorbed IgG and the immobilized staphylococcal protein A was very sensitive to fluctuations in pH in the range 7.0 to 8.0. However, the elution experiments revealed large differences in the yield of IgG protein at different acidities, and we realized that the conditions may have been resolving the different IgG isotypes. This was confirmed by immunochemical analyses.

At the time there was a need in many laboratories for a method to isolate antibodies belonging to different IgG subclasses in a biologically active state. Malcolm R. MacKenzie[2] in Melbourne had attempted to resolve mouse IgG isotypes by differential elution from protein A-Sepharose using sodium isothiocyanate gradients. This had given unsatisfactory resolution, but the effects of denaturation, induced by the chaotropic properties of the eluent, also concerned me. I had been especially interested in his work, and at the time I was alert for any information on affinity chromatography of immunoglobulins. In this sense we were blessed by a combination of circumstances that led us to notice, appreciate, and then explore a minor detail in an experiment that was in all other ways merely a preparative exercise. Moreover, were it not for the enormous amount of IgG_1 in the helminth-infected sera, we may have failed to detect any difference in our elution patterns.

The paper is frequently cited because it described a simple but efficient method for separating antibodies belonging to different IgG subclasses. Because the conditions used to elute the different immunoglobulins from the protein A-Sepharose were extremely mild compared with previous procedures, the technique provided investigators with a means of isolating different types of functionally intact IgG antibodies from the sera of immunized or infected mice. Although our data were confined to mouse immunoglobulins, we suggested that the method should be applicable to immunoglobulins from other species. Subsequent work by others proved this to be largely true; the method, or modified versions of it, therefore became widely adopted.[3] Its utility lies in the fact that it allows sera to be fractionated for analytical or preparative purposes: antibodies in the different IgG fractions can be easily quantitated and they can be used for functional studies both *in vitro*[4,5] or *in vivo*.[6] The increasing use of monoclonal antibodies during the past decade led many laboratories to use our technique to purify antibodies from hybridoma culture supernatants or ascitic fluid.[7]

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2. **MacKenzie M R, Warner N L & Mitchell G F.** The binding of murine immunoglobulins to staphylococcal protein A. *J. Immunology* 120:1493-6, 1978. (Cited 75 times.)
3. **Langone J J.** Applications of immobilized protein A in immunochemical techniques. *J. Immunol. Method.* 55:277-96, 1982. (Cited 40 times.)
4. **Ralph P, Nakoinz I, Diamond B & Yelton D.** All classes of murine IgG antibody mediate macrophage phagocytosis and lysis of erythrocytes. *J. Immunology* 125:1885-8, 1980. (Cited 50 times.)
5. **Sarvas H O, Seppälä I J T, Tähtinen T, Péterfy F & Mäkelä O.** Mouse IgG antibodies have subclass associated affinity differences. *Mol. Immunol.* 20:239-46, 1983. (Cited 15 times.)
6. **Phipps R P, Mitchell G F, Mandel T E & Tew J G.** Antibody isotypes mediating antigen retention in passively immunized mice. *Immunology* 40:459-66, 1980. (Cited 10 times.)
7. **Villemez C L, Russell M A & Carlo P L.** Mouse IgG_1 heterogeneity: variable binding of monoclonal IgG_1 antibodies to protein A-Sepharose. *Mol. Immunol.* 21:993-8, 1984. (Cited 10 times.)

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