This Week's Citation Classic®

Reisfeld R A & Small P A. Electrophoretic heterogeneity of polypeptide chains of specific antibodies. *Science* 152:1253-5, 1966.
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This paper indicates that heavy and light polypeptide chains isolated from different specific antibodies to haptens and immunoglobulin (IgG) of normal rabbits can be resolved into distinct, multiple components by acrylamide gel electrophoresis in the presence of urea. Although both types of polypeptide chains could be resolved into multiple components, this method failed to distinguish antibodies of different specificity and normal rabbit IgG. [The *SCI*® indicates that this paper has been cited in over 420 publications.]

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This paper is an extension of work done with Parker A. Small and Sheldon Dray that indicated that the electrophoretic heterogeneity observed among light and heavy polypeptide chains of rabbit immunoglobulin (IgG) was unrelated to the genotype of these molecules under the control of different allelic genes of at least two genetic loci.[1,2] Although we failed to show any apparent relation between antibody specificity and electrophoretic heterogeneity, we were able for the first time to resolve rabbit IgG heavy polypeptide chains into distinct electrophoretic subgroups.

I believe that this article became a *Citation Classic* mainly because it contains a modified acrylamide gel electrophoresis procedure allowing the use of 4-percent gels in the presence of 10 M urea. This procedure became quite popular since it facilitated separation based on both electrophoretic charge and molecular mass of extensively reduced and alkylated protein and glycoprotein molecules that were sufficiently unfolded to allow for optimal resolution. This method of electrophoretic analysis came to be widely accepted because we clearly demonstrated in the paper that the electrophoretic resolution obtained was not due to artifacts. Thus, it was possible to cut out some of the components and then subject them again to gel electrophoresis under identical conditions, thereby obtaining single components with essentially the mobility observed in the original pattern. We also demonstrated that the banding observed was not due to polymerization of the chains since mass heterogeneity of similar preparations of rabbit IgG polypeptide chains was not observed by high-speed sedimentation equilibrium analysis.[2] A few months later we showed that the banding patterns could not be attributed simply to differences in alkylation since other extensively reduced but not alkylated IgG heavy chain preparations showed quite similar banding patterns.[3]

The usefulness of this method was further demonstrated in later work that showed that extensively deionized 10 M urea solutions with a conductance of 3-5 μmho did not cause carbamylation of ε-amino groups of lysine since amino acid analysis of IgG light polypeptide chains isolated from such gels revealed neither a change in lysine content nor the appearance of homocitrulline.[4] This particular paper also illustrated quite clearly the range of the applications of the method first described in the *Science* paper.

[For a recent review citing this article, see reference 5.]

1. **Small P A, Reisfeld R A & Dray S.** Peptide differences of rabbit γG-immunoglobulin light chains controlled by allelic genes. *J. Mol. Biol.* 11:713-21, 1965. (Cited 90 times.)
2. ———. Peptide maps of rabbit γG-immunoglobulin heavy chains controlled by allelic genes. *J. Mol. Biol.* 16:328-33, 1966.
3. **Terry W D, Small P A & Reisfeld R A.** Electrophoretic heterogeneity of the polypeptide chains of human G-myeloma proteins. *Science* 152:1628-30, 1966.
4. **Reisfeld R A, Inman J K, Mage R G & Appella E.** Chemical and immunochemical characterization of electrophoretic subfractions of rabbit γG-immunoglobulin light chains with b4 allotypic specificity. *Biochemistry—USA* 7:14-24, 1968.
5. **Mestecky J & Kilian M.** Immunoglobulin A (IgA). *Meth. Enzymology* 116:37-75, 1985.

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