This Week's Citation Classic

Grabar P, Williams C A, Jr. & Courcon J. Méthode immuno-électrophorétique d'analyse de mélanges de substances antigéniques. (Method for immuno-electrophoretic analysis of mixtures of antigenic substances.) Biochim. Biophys. Acta **17:**67-74,1955. [Service de Chimie Microbienne, Institut Pasteur, Paris, France]

The paper describes a simple method which in a single operation enables the definition of complex mixtures of antigens or haptens by three independent criteria: the chemical or biochemical properties of the antigens (using various dyes or enzyme substrates), their electrophoretic mobility, and their antigenic specificity. [The *SCI*® indicates that this paper has been cited over 680 times since 1961.]

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"It is a particular pleasure for me to be classified as one of the most-cited authors during the years 1961-75. I think that, generally, articles on methods are more often cited than papers on experimental works if the described method can be and is easily applied in various domains. The method called 'immuno-electrophoretic analysis' is now designated 'immuno-electrophoresis' (which is grammatically incorrect), and in most recent publications in which this method is used the authors no longer cite my name, probably because they consider it a well-known method. Historically, I came to develop it when in 1951-52 I tried to establish a method for what is now known as electrofocusing, using paper-strips and a gradient of buffers. At that time no insoluble ampholites were available and my gradient was unstable. In order to stabilize this system I used agar-gels. In order to precipitate the electrophoretically separated proteins I tried to use specific antibodies, i.e., an immune-serum with which I covered the gel. Then, instead of such a covering, I disposed on the sides of the electrophoretic axis gels containing the immune-serum in which the antigens were diffusing and thus forming precipitin arcs. In the last period of these experiments I was helped by Curtis A. Williams, at that time a young student. A short preliminary paper was published in the beginning of 1953[1] which was followed by this in the same journal in 1955. But the complete and detailed description of the method and of some applications was published in a monograph in collaboration with Pierre Burtin and several others.[2] The French edition appeared in 1960 and was followed in a few years by English, German, Russian, Spanish, and Chinese translations. Very rapidly, the method came to be employed in many laboratories around the world, and we were then obliged to arrange many special short courses to teach it to the very large number of persons interested in its applications. Now it is applied as a diagnostic method in hospitals and in studies on immunology, microbiology, pathology, endocrinology, embryology, animal and plant physiology, etc.

"A dissertation for the degree of Doctor in Medical Sciences at the University of Rostock (East Germany) by M. Henker and G. Otto was entirely concerned with the bibliography on immuno-electrophoretic analysis from 1953 to 1970 and contained 5,275 classified citations. This shows that this method came to be used very rapidly in many studies.

"Several authors have proposed various modifications of the original method; the most interesting is the quantitative immuno-electrophoresis of Laurell."[3]

1. **Grabar P & Williams C A.** Méthode permettant l'étude conjuguée des propriétés électrophorétiques et immunochimiques d'un mélange de protéines. Application au sérum sanguin. (Method permitting the twin study of electrophoretic and immunochcmical properties of a mixture of proteins. Application to blood serum.) *Biochim. Biophys. Acta* **10**:193-4. 1953.
2. **Grabar P & Burtin P,** (eds.). Avec la collaboration de Chevance L G. *Analyse immuno-électrophorétique: ses applications aux liquids biolngiques humains.* (Immunoelectrophoretic analysis: its applications to human biological fluids.) Paris: Masson. 1960. 294 p.
3. **Laurell C B.** Quantitative estimation of protein by electrophoresis in agarose gel containing antibodies. *Analyt. Biochem* **15**:45-52. 1966.