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Merril C R, Goldman D, Sedman S A & Ebert M H. Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. *Science* 211:1437-8, 1981. [Lab. General and Comparative Biochem. and Lab. Clin. Sci., Natl. Inst. Mental Health, Bethesda, MD]

This paper describes the application of a silver stain with the capacity to detect as little as 0.01 ng of protein per square millimeter on polyacrylamide gels. When this silver stain is employed with high-resolution two-dimensional electrophoresis it permits qualitative and quantitative characterization of large numbers of proteins in body fluids and tissues. In this paper it was used to demonstrate regional protein variations in cerebrospinal fluid. [The *SCI*® indicates that this paper has been cited in more than 1,880 publications.]

Ultrasensitive Silver Stain for Proteins

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Shortly after the introduction of high-resolution two-dimensional protein electrophoresis (2DE) in 1975, I began to explore the potential of this method to provide information concerning diseases of unknown etiology affecting the central nervous system. I began these experiments with D. Goldman and M.H. Ebert at the National Institute of Mental Health, Bethesda. In our initial studies we were able to visualize about 50 cerebrospinal fluid (CSF) proteins on 2DE gels stained with Coomassie blue, at that time one of the most sensitive protein stains available.

Our inability to observe more proteins was caused in part by the large range of protein concentrations that exist in CSF and most body fluids. Many of the less abundant or trace proteins are present at concentrations that are less than 10^{-5} percent of the total protein in the fluid. Attempts to use larger volumes of CSF resulted in protein pattern distortions, caused by abundant proteins such as albumin. These results encouraged me to try to develop a more sensitive method for detecting proteins. I tried fluorescent stains and heavy metals which were known to interact with proteins. However, little progress was made until I remembered the capacity of silver nitrate to blacken skin, which I had observed while developing chemistry skills as a teenager. The first attempts to stain gels with silver nitrate failed, but fortunately R. Switzer, a neurohistologist, offered to help us adapt a histological silver stain. This stain provided a hundredfold increase over Coomassie blue staining.[1,2] However, the procedure took more than three hours, used large quantities of silver, and a number of the solutions had to be prepared just prior to use. To compound these problems, the price of silver was increasing at that time due in part to the Hunt brothers' perturbations of the market.

To reduce the amount of silver needed, I began an effort to gain an understanding of silver-based staining and imaging systems, including photography. These efforts led to the realization that the detection of proteins by silver on gels requires the reduction of ionic to metallic silver. The development of a positive image of a protein pattern on a gel requires that the areas of the gel containing proteins have a higher reducing potential than the surrounding regions.[3] After numerous experiments, with the assistance of S.A. Sedman, we were able to optimize the formulation of a stain that reduced the amount of silver by 98 percent and which took less than half the time to perform than our first silver stain. In our initial application of this stain we studied CSF taken from different regions of the primate central nervous system. These studies demonstrated that there are both qualitative and quantitative protein differences that appear to be region-specific.

Since 1981, I and a number of collaborators have been able to show that the colors which are often observed with silver staining are due to the diffractive scattering of light by the microscopic silver grains.[4] We also developed additional stains for special purposes and we have been able to reduce the background staining by using special gel cross-linking agents.[5] Applications of these methods have led to the observation of a number of protein alterations in disease states, ranging from schizophrenia to Creutzfeld-Jakob disease.[6] I was awarded the Public Health Service's Outstanding Service Medal for my contributions in these efforts. In addition, while no patents were filed for our first silver stain in 1979, the government encouraged the patenting and commercial licensing of the 1981 silver stain.

1. **Merril C R, Switzer R C & Van Keuren M L.** Trace polypeptides in cellular extracts and human body fluids detected by two-dimensional electrophoresis and a highly sensitive silver stain. *Proc. Nat. Acad. Sci. USA* 76:4335-9. 1979, (Cited 275 times.)
2. **Switzer R C, Merril C R & Shifrin S.** A highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels. *Anal. Biochem.* 98:231-7, 1979. (Cited 520 times.)
3. **Merril C R.** Development and mechanisms of silver stains for electrophoresis. *Acta Histochem. Cytochem.* 19:655-7, 1986.
4. **Merril C R, Bisher M E, Harrington M & Steven A C.** Coloration of silver-stained protein bands in polyacrylamide gels is caused by light scattering from silver grains of characteristic sizes. *Proc. Nat. Acad. Sci. USA* 85:453-7, 1988.
5. **Merril C R.** Silver staining of proteins and DNA. *Nature* 343:779-80, 1990.
6. **Harrington M G & Merril C R.** Cerebrospinal fluid protein analysis in diseases of the nervous system. *J. Chromatogr.* 429:345-58, 1988. Received October 28. 1992