eny females were refractory. The reciprocal cross Bx_1 was not compatible. The backcross Bx_2 (Ma/Po ♀ × Ma/Ma ♂ and Ma/Ma ♀ × Ma/Po ♂) gave refractory offspring. The crossing type including *A. malayensis* and *A. polynesiensis* is similar to that of *A. alcasidi* and *A. cooki*. This third series of crosses again indicates a maternal type of inheritance of susceptibility to brugian filariasis in the *A. scutellaris* complex of mosquitoes. It also indicates that the mode of inheritance of susceptibility to *B. pahangi* is similar to *B. malayi*. On the basis of these results we suppose that the inheritance of susceptibility of the *A. scutellaris* complex of mosquitoes to infection with the subperiodic *Wuchereria bancrofti* will be of the same mode.

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20. A generally incompatible cross Co/Co ♀ × Al/Al ♂ (Table 2) produced, in three of five replicates, offspring in the F_1 generation (1.0 to 6.4 percent). Seven females of this progeny (Co/Al) were dissected and all were susceptible to infection with *B. malayi*. Similarly, the cross Po/Po ♀ × Ma/Ma ♂ (see first asterisk in Table 3) gave a low percentage of F_1 and F_2 (Po/Ma) progeny. Eight females dissected for *B. pahangi* were all susceptible. Ten F_2 Po/Ma females dissected for *B. malayi* were also susceptible. These results provide additional evidence supporting the maternal type of inheritance.
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Ultrasensitive Stain for Proteins in Polyacrylamide Gels Shows Regional Variation in Cerebrospinal Fluid Proteins

Abstract. *A new silver stain for electrophoretically separated polypeptides can be rapidly and easily used and can detect as little as 0.01 nanogram of protein per square millimeter. When employed with two-dimensional electrophoresis, it should permit qualitative and quantitative characterization of protein distributions in body fluids and tissues. It has been used to demonstrate regional variations in cerebrospinal fluid proteins.*

Many biological studies require the detection and characterization of trace quantities of proteins. Developments in two-dimensional electrophoresis have made it possible to resolve thousands of proteins from complex biological mixtures (*1*). However, inability to detect proteins present in low concentration has limited the application of this method, particularly in clinical screening for pathological states, endocrinology, mammalian metabolism, developmental biology, and immunology.

The most common nonradioactive polypeptide detection methods employ intense organic stains such as Coomassie blue. These stains lack the sensitivity to detect proteins present in low or trace concentrations. Body fluids, such as cerebrospinal and amniotic fluids, are often difficult to obtain in quantity and frequently contain abundant proteins, which cause distortion of electrophoretic patterns when sufficient sample is analyzed to observe trace proteins.

This sensitivity problem has been overcome by the application of techniques employing a histologically derived silver stain to proteins in acrylamide gels. A hundredfold increase in sensitivity was achieved over Coomassie blue stain (*2*, *3*). These techniques had three main drawbacks: (i) they took 3 to 4 hours, (ii) they consumed large quantities of silver, and (iii) it was necessary to prepare several solutions just before use.

In this report we describe a new, photochemically derived silver stain. The method rquires three relatively stable solutions, takes less than 1 hour to perform, and uses 2 percent of the silver needed for the histological stain.

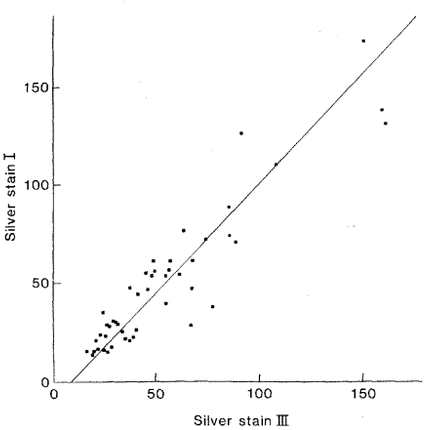

Fig. 1. Comparison of original histochemically derived stain (*Silver stain I*) with new photochemically derived stain (*Silver stain III*). This is a density versus density plot of all polypeptide spots within a small subregion of an *E. coli* lysate gel pattern. The slope was 1.08, the *Y* intercept −8.1, and the correlation coefficient .94. Gels were positioned next to a National Bureau of Standards calibrated photographic density standard and photographed with Tri-X 120-mm film (Kodak). These photographic images were then scanned at a resolution of 100 μm with an Optronics (Chelmsford, Mass.) 1000 HS scanning densitometer. Image densities were converted to optical density units by using the calibrated density standard. This conversion normalized gel images for the significant variations in photography and scanning densitometry. Measurements were made with a IP5000 image processor (DeAnza Systems Inc., San Jose, Calif.) and PDP 11/60 computer (Digital Equipment Corp., Maynard, Mass.); background was subtracted and identical measurement windows were used. The original gel pattern was produced by subjecting 10 g of *E. coli* lysate proteins to two-dimensional gel electrophoresis by the method of O'Farrell (*1*).

Proteins were separated by the two-dimensional electrophoretic method of O'Farrell (*1*). The second-dimension gels were 10 percent acrylamide, 16 by 12 cm by 0.8 mm thick. Proteins were fixed in a solution of 50 percent methanol and 12 percent acetic acid for a minimum of 20 minutes, and excess sodium lauryl sulfate was removed from the gels by three 200-ml, 10-minute rinses containing 10 percent ethanol and 5 percent acetic acid.

Gels were then soaked for 5 minutes in a 200-ml solution of 0.0034*M* potassium dichromate and 0.0032*N* nitric acid. They were washed four times, for 30 seconds in 200 ml of deionized water, and placed in 200 ml of 0.012*M* silver nitrate for 30 minutes. This was followed by rapid rinsing with two 300-ml portions of the image developer solution, which contained 0.28*M* sodium carbonate and 0.5 ml of commercial Formalin per liter. The gels were gently agitated in a third portion of this solution until the image had reached the desired intensity. Development was stopped by discarding the developer and adding 100 ml of 1 percent

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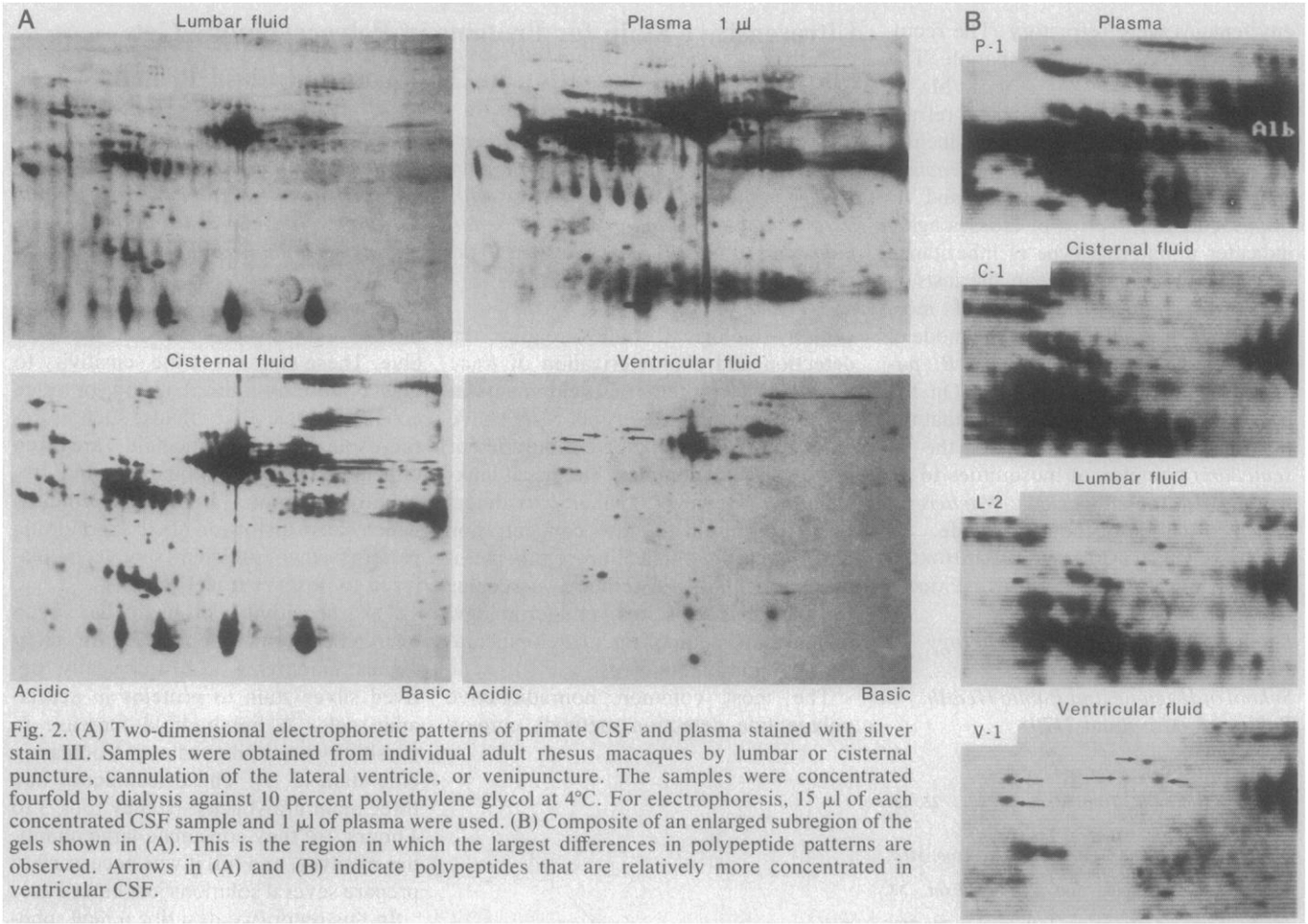

Fig. 2. (A) Two-dimensional electrophoretic patterns of primate CSF and plasma stained with silver stain III. Samples were obtained from individual adult rhesus macaques by lumbar or cisternal puncture, cannulation of the lateral ventricle, or venipuncture. The samples were concentrated fourfold by dialysis against 10 percent polyethylene glycol at 4°C. For electrophoresis, 15 μl of each concentrated CSF sample and 1 μl of plasma were used. (B) Composite of an enlarged subregion of the gels shown in (A). This is the region in which the largest differences in polypeptide patterns are observed. Arrows in (A) and (B) indicate polypeptides that are relatively more concentrated in ventricular CSF.

acetic acid. The gels were washed twice with 200 ml of water before storage and sealed in plastic bags. Maximum sensitivity was achieved when the gel was exposed to relatively intense uniform light during the first 5 minutes in silver nitrate. A fluorescent light source of uniform intensity gave the best results. We found that sensitivity was lost when gels were more than 1 mm thick.

The photochemical silver stain was found to be at least equal in sensitivity to the histologically derived silver stain (Fig. 1). Densities obtained with the two stains were proportional over a wide range of protein concentrations. Polypeptide "spot" counts on two-dimensional gels established that the photochemical silver stain was at least as efficient as the histological stain in detecting the presence of polypeptides in gels. Both stains showed the same specificity for polypeptides. Treatment of *Escherichia coli* or human cells lysates with proteinase K resulted in the disappearance of all discrete spots on the gels.

The utility of the stain is illustrated in Fig. 2, which shows two-dimensional electrophoretic patterns of primate cerebrospinal fluid (CSF) from various regions and of plasma. The CSF was obtained from six rhesus macaques, from the lateral ventricle, cisterna magna, and lumbar space. There were quantitative differences in the distribution of a number of proteins, including albumin, α_1-antitrypsin haptoglobin, immunoglobulin G (light and heavy chains), G_c-globulin, α_{2HS}-glycoprotein, α_1-antichymotrypsin, $\alpha_{1,\beta}$-glycoprotein, and transferrin, with lower concentrations in CSF from the lateral ventricle (*4*). Some CSF proteins not seen in plasma were also found to be present in lower concentration in lateral ventricular CSF. However, other CSF proteins, several of which are indicated by arrows in Fig. 2, were not diminished in lateral ventricular CSF, showing that individual protein variations occur in the subregions of CSF.

Hill *et al.* (*5*), using one-dimensional electrophoresis, demonstrated a gradient in CSF for albumin, prealbumin, and four globulin fractions; the concentrations of albumin and four globulin fractions were lower in ventricular CSF, which had a higher relative concentration of prealbumin. By use of immunological techniques, nine antigenic species have been demonstrated in CSF that are absent in serum (*6, 7*). Recently, a method for preparative separation of CSF-specific proteins by affinity chromatography and isoelectric focusing was described (*8*). Silver-stained two-dimensional electrophoretic gels permit the quantitative evaluation of more than 300 polypeptides in CSF (*4*) and the direct visualization of protein variations in CSF and other tissues and fluids.

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