which the chemical basis is similar to that in the Boehringer assay, indicated a small negative bias instead (*1*, *2*).

Ultimately, the real question is whether the greater sensitivity of the Boehringer assay would lead to falsely high values for serum owing to contamination with salivary enzyme, resulting in a wrong diagnosis. The incidence of such occurrences is being investigated.

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Sodium Dodecyl Sulfate/Polyacrylamide Gel Electrophoresis of Unconcentrated Cerebrospinal Fluid

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We applied a simplified sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE) method (*1*) to unconcentrated cerebrospinal fluid (CSF) and detected complex high-resolution protein patterns, even in samples with normal protein content, by Coomassie Blue (Serva Blue R) staining at 60 °C.

CSF, collected by lumbar puncture, was centrifuged (Eppendorf "Microfuge" 5414). Nine volumes (45 μL) of unconcentrated CSF was mixed with two volumes (10 μL) of glycerol and one volume (5 μL) of sample denaturing solution (per liter, 20 g of SDS in 625 mmol/L Tris HCl buffer, pH 6.8) and heated to 95 °C for 5 min. The SDS-denatured but non-reduced samples (10 μL = 7.5 μL CSF) were loaded into agarose wells on 40 to 200 g/L polyacrylamide gradient gels (75 × 75 × 3 mm) and electrophoresed in SDS buffer (per liter, 1 g of SDS, 25 mmol of Tris, and 192 mmol of glycine) at 50 mA per gel for 1 h until the bromphenol blue dye front reached the bottom of each gel (*1*). Protein bands were made visible by staining with Serva Blue R at 50 °C (*1*).

Figure 1 shows the SDS-PAGE protein patterns of unconcentrated CSF (7.5 μL), electrophoresed under non-reducing conditions, and made visible by staining. The relative staining intensity of each track was proportional to CSF total protein content (0.18–1.8 g/L; normal range 0.15–0.45 g/L). At least 14 bands were detected, including proteins corresponding in electrophoretic mobility to serum α_2-macroglobulin, IgG, transferrin, albumin, and apolipoprotein A-I. All CSF samples gave a double band in the serum transferrin region (Figure 1)—the fainter and faster component being of lower molecular mass (by ≈2000 Da). Some CSF also revealed a prominent band doublet of M_r 15 200 and 15 700, which was not detected in serum at the protein load analyzed. Other unidentified low-M_r (<20 000) and very high M_r (>200 000) components common to CSF and serum were detected. They probably include transthyretin, IgM, and the haptoglobins.

Routine methods for protein analysis of CSF require either (*a*) extensive concentration, which necessitates high sample volume and risks protein loss/modification; or (*b*) technically demanding detection methods such as silver staining (*3*). Our results reveal that complex, high-resolution electrophoretic patterns, revealing both protein content and distribution, can be obtained with as little as 7.5 μL of unconcentrated CSF of normal protein range.

SDS-PAGE has been claimed to demonstrate oligoclonal IgG banding patterns characteristic of demyelinating diseases (*4*, *5*) but "oligoclonal" is inappropriate, because the method separates according to molecular size (rather than charge or isoelectric point) and we believe it will be better suited for evaluating the permeability of the blood/brain barrier.

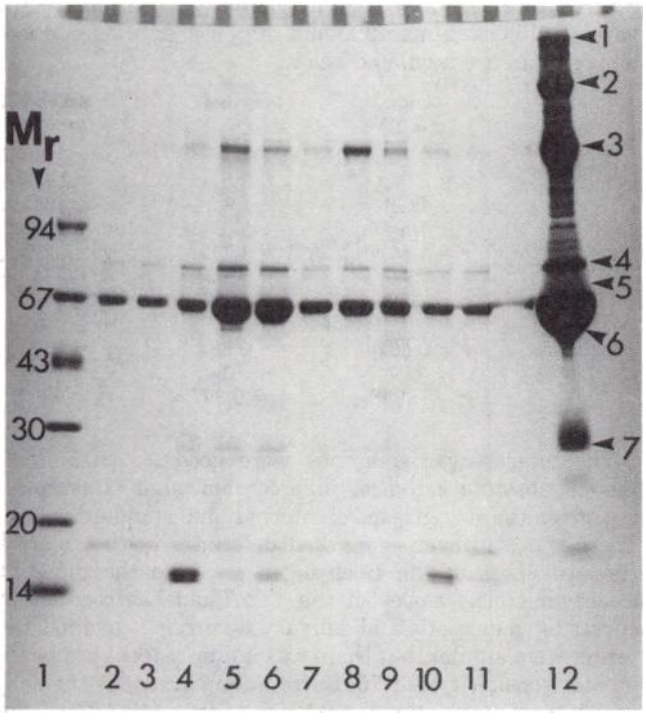

Fig. 1. SDS-PAGE of Pharmacia low-M_r marker proteins (*track 1*, 2.5 μL), unconcentrated CSF (*track 2–11*, 7.5 μL) and serum (*track 12*, 1 μL) electrophoresed under non-reducing conditions and stained with Serva Blue R

The CSF protein contents (as determined by Coomassie Blue protein dye-binding assay, ref. *2*) were 0.2, 0.18, 0.49, 1.8, 1.46, 0.34, 0.6, 0.47, 0.32, and 0.26 g/L (*tracks 2–11*, respectively). Identified serum proteins include: *1*, IgM; *2*, α_2-macroglobulin; *3*, IgG; *4*, transferrin; *5*, α_1-antitrypsin; *6*, albumin; and *7*, apolipoprotein A-I (ref. *1*). "M_r" indicates relative molecular mass × 10^{-3}

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