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Quantitation of High-Molecular Proteins in Cerebrospinal Fluid

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The finally adopted technical conditions for the routine quantitation of α_2-macroglobulin, immunoglobulin A, G and M in 2 to 3 ml normal cerebrospinal fluid by *Laurell*'s electroimmuno assay are described. Using a two step concentration procedure fibrinogen and β-lipoprotein have been detected in normal cerebrospinal fluid.

Es werden die technischen Bedingungen beschrieben, die sich für die Bestimmung des α_2-Makroglobulins und der Immunglobuline A, G und M in normalem Liquor cerebrospinalis bewährt haben.
Mit der elektroimmunologischen Technik nach *Laurell* ist es möglich, diese vier Proteine in 2–3 ml Liquor cerebrospinalis quantitativ zu bestimmen. Auch Fibrinogen und β-Lipoprotein konnten mit dieser Methode nach zweistufiger Konzentrierung in normalem Liquor cerebrospinalis nachgewiesen werden.

Quantitation of single proteins in cerebrospinal fluid is hampered by their low levels as compared to serum. Since serum filtration is the dominant process in the formation of cerebrospinal fluid, the levels of the high-molecular proteins (which, according to present knowledge, have the greatest diagnostic significance) are several hundred to thousand times lower than in serum. Any method for single protein quantitation should be sensitive enough to allow measurements in small amounts of cerebrospinal fluid, as far as possible without preconcentration. *Laurell*'s electroimmuno assay fulfils this criterion for numerous proteins (2,3) but some technical adaptations are necessary for the reproducibel quantitation of the high-molecular proteins. The present work describes the adapted method for the routine quantitation of α_2-macroglobulin and the three major immunoglobulin classes A, G and M.

Material and Methods

The technique applied was essentially that described by C.-B. *Laurell* (1) and B. *Weeke* (4).

Quantitation plates

As recommended by G. *Kostner* and A. *Holasek* (5) 1% polyethylene glycol 6000 (Merck A.G., D-6100 Darmstadt) was added to 24,4 mmol/l barbital buffer pH 8.6, ionic strength 0,02 prior to 1% agarose (Litex, DK-2600 Glostrup). Antisera from Behring-Werke, D-6550 Marburg were added at 50° C to final concentrations between 0,2% and 0,8% (Tab. 1). Agarose layers were 1.5 mm thick and the wells had diameters of 2.5 mm to receive 4 µl of sample.

Sample preparation

IgA and α_2-macroglobulin were quantitated in normal cerebrospinal fluid without any pretreatment. IgG was carbamylated at 45° C for 30 min by adding an equal amount of 1.25 mol/l KCNO. Thereafter the sample was diluted up to forty-fold with distilled water, depending on the protein content (1000 mg/l = 1 : 10; 4000 mg/l 1 : 40). Samples with protein levels below 300 mg/l were not diluted.
For quantitation of IgM, 2.5 to 3.0 ml cerebrospinal fluid were concentrated about 10–20 fold in a collodion bag with 50% Dextran as described earlier (6), then carbamylated as above. The sample was further concentrated 3–4 fold by a Sephadex G 25 microtechnique (7) after the carbamylation step.
Using this two step concentration procedure fibrinogen and β-lipoprotein can also be detected in normal cerebrospinal fluid. The concentration factor for the carbamylated proteins IgM and fibrinogen was calculated from the IgG levels before and after concentration. The total protein ratio cannot be used for this purpose, because small-molecular proteins are lost during the first collodion bag concentration step. Reference serum from Behring-Werke was used throughout and treated like cerebrospinal fluid after appropriate dilution with barbital buffer.

Electrophoretic conditions

The electrophoresis apparatus of Dansk Laboratorieudstyr A/S (3 Ryesgade, DK-2200 Copenhagen) and the power supply of Vitatron (Dieren, Netherlands) were used. A constant voltage of about 100 V was chosen, ensuring field strengths between 2 and 3 V/cm within the gel layer. The running temperatures were kept constant at 7–8° C below room temperature by the Cryostate MC 3 from Colora (D-7073 Lorch) and up to seven separation chambers were connected in series without loss of cooling efficiency. Electrode and gel buffers were identical. Whatman chromatography paper No. 1 was used for the wicks. Runs were performed for 16–22 hours overnight and the rockets could be evaluated only two hours later after staining.

Normal values

Adult patients thought to have a normal cerebrospinal fluid protein compostion were carefully selected mainly from our psychiatry ward. None of them had symptoms of a neurologic or internal disease, as judged by clinical criteria and laboratory findings.

Tab. 1. Normal values of proteins in cerebrospinal fluid

Protein	$\bar{x}$ [mg/l]	s [mg/l]	Normal range [mg/l]	N	Antiserum concentration	Charge Nr.
α_2-Macroglobulin	2.0	0.7	0.5 – 3.5	90	0,2%	1511
Immunoglobulin G	12.3	6.4	3.0 – 30.0	90	0.25%	2459
Immunoglobulin A	1.3	0.6	0.3 – 3.0	54	0.8%	2388,2833
Immunoglobulin M	0.6	0.3	0.1 – 1.0	22	0.5%	2214
					1.2%	2470

Cerebrospinal fluids with the following routine laboratory values were considered as normal: Cell count below 3 cells per mm^3; protein level according to O. H. *Lowry* et al (8) below 450mg/l and according to *Kafka* (9) below 1,0 Unit; "Globulin"-fraction not above 0,1 Unit according to *Kafka*; colloidal mastix reaction: 1,1,1,1,1,1,1,1,1,1.
The normal values of four proteins are listed in Table 1.

Discussion

The sensitivity of *Laurell*'s electroimmuno assay allows the quantitation of numerous proteins in normal cerebrospinal fluid despite their low concentrations (2, 3). The clinical relevance of these protein levels remains to be evaluated. So far the immunoglobulin G level has the greatest diagnostic significance. Since even normal levels of IgG, IgA and IgM can now be quantitated in about 3ml cerebrospinal fluid, the differential behaviour of these major immunoglobulin classes under pathological conditions can be studied.

The α_2-macroglobulin level is a sensitive criterion for the condition of the blood-cerebrospinal fluid-barrier, and an elevated immunoglobulin level without a parallel α_2M increase indicates an antibody production within the central nervous system (10). From a theoretical viewpoint other large proteins like fibrinogen and β-lipoprotein, now detectable in normal cerebrospinal fluid, could also serve for barrier evaluation, but their levels are several thousand times lower as compared to serum and can only be quantitated after concentrating normal cerebrospinal fluid up to 100 fold, a procedure too tedious for routine purposes.
E. *Schuller* has recommended various technical modifications of *Laurell*'s technique for singel cerebrospinal fluid proteins (11, 12). The advantages of these modifications do not seem great enough to abandon the simplicity of the basic technical scheme as elaborated by the Scandinavian workers. In fact, the only essential variables are antiserum concentration and the necessity to reduce the isoelectric points of some proteins by carbamylation or other procedures.
Several normal range values of IgG published correspond to our values (13, 14, 15, 16, 17) while some are higher (2, 18, 19). For IgA the values of H. W. *Delank* (16) and E. *Bock* (2) are in the same range, while those of H. *Bauer* and A. *Gottesleben* (14) and H. *Link* and R. *Müller* (17) are above ours. The normal α_2M-value of E. *Bock* (2) is somewhat lower than that found by us. To our knowledge the normal content of IgM in cerebrospinal fluid has not been published. In comparing the values of different laboratories one has to be aware, however, that the choice of antiserum and reference does influence the absolute quantitative values. If the method is to be used for diagnostic purposes, the normal range should be controlled applying the finally adapted technical conditions.

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