

### Trace Proteins in Biological Fluids III. Quantitation of $\gamma$ -Trace and Major Spinal Fluid Proteins Including $\beta$ -Trace.\*† (32525)

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Two proteins of  $\beta$ - and  $\gamma$ -mobility have been shown to occur in human cerebrospinal fluid (CSF) and other biological fluids(1,2). These globulins have been characterized by their electrophoretic mobility, the effect of enzymes and storage on their electrophoretic mobility(3), and their behavior on column chromatography(4). Both proteins exist in higher concentration in CSF than in serum, and together they account for almost 12% of the CSF total protein. They are not synthesized within the central nervous system(5). All attempts to demonstrate enzymatic activity or carrier function have so far been unsuccessful(3).

The present study was undertaken in order to quantitate both trace proteins ( $\gamma$ -trace and  $\beta$ -trace) in human CSF. In addition, the concentrations of both these proteins were compared to albumin,  $\gamma$ -globulin, and transferrin concentration in the CSF from some patients with diseases of the nervous system. This was done in order to determine whether  $\beta$ - and  $\gamma$ -trace globulin concentrations in CSF are influenced by similar factors to the ones leading to enhanced CSF protein concentrations in certain diseases. Unexpectedly,  $\beta$ -trace was found to be a major CSF component constituting as much as 10% of total proteins, and  $\gamma$ -trace concentration was 2-fold greater in infants than in adults.

*Materials and methods.* CSF was obtained from patients admitted to the Neurologic and Psychiatric Services of Bellevue Hospital, New York City. Patients with brain tumors and hydrocephalus were selected for these studies because they show a wide range of CSF protein concentration. Hydrocephalic infants (<1 year of age) were chosen in par-

ticular because it had been noted in initial studies that the  $\gamma$ -trace globulin content of their CSF was enhanced. A correlation with other CSF proteins seemed indicated.

The clinical diagnosis of patients with primary brain tumors and hydrocephalus was made histologically and neuroradiologically. Normal spinal fluid was taken from patients with no known structural disease of the central nervous system. It was not possible to obtain sufficient quantities of CSF from normal infants of comparable ages for a complete analysis. Fluids containing erythrocytes were discarded.

$\beta$ -trace and  $\gamma$ -trace proteins were isolated triethylaminoethyl cellulose column chromatography on diethylaminoethyl cellulose (DEAE) and Sephadex, as described previously(4,5).

Human serum albumin was obtained by further purification on starch block electrophoresis(6) of a commercially prepared Cohn fraction V. Human  $\gamma$ -globulin was provided by the American Red Cross as Cohn fraction II. Partially purified human transferrin (American Red Cross) was further purified by triethylaminoethyl cellulose column chromatography(7).

All antisera were prepared in rabbits by injection of the antigen and complete Freund's adjuvant in the footpad. The rabbits were reinjected 3 weeks later with antigen in incomplete Freund's adjuvant. One month later the animals received booster injections of the antigen intravenously and were bled. Where needed, antisera were absorbed until they produced only a single line in immunoelectrophoresis (Fig. 1) and double diffusion in agar with concentrated CSF.

Concentrations of trace proteins, albumin, transferrin, and  $\gamma$ -globulin were determined by the quantitative precipitin technique of Heidelberger and Kendall(8). Calibration curves of decomplexed antisera were prepared using purified solutions of antigen. All nitrogen determinations were carried out

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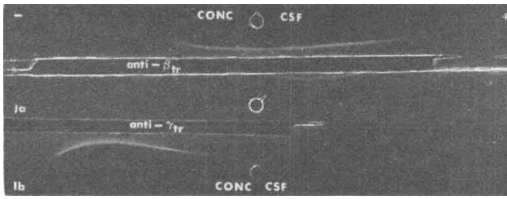


FIG. 1. Immunoelectrophoretic analysis of concentrated cerebrospinal fluid (CSF) (50 mg/ml) using specific antisera against  $\beta$ -trace (1a) and  $\gamma$ -trace (1b).

by the Markham micro-Kjeldahl method(9). The precipitates formed by CSF albumin, transferrin,  $\gamma$ -globulin and the respective antisera were analyzed colorimetrically by the Biuret reagent(9).  $\gamma$ - and  $\beta$ -trace precipitates were incubated 4 days at 4°C. The precipitates were dissolved in 0.1 N NaOH and analyzed by the Folin-Ciocalteu method(9). Total protein content was measured with the Biuret reagent(9).

**Results.** Calibration curves used for the quantitative determination of  $\gamma$ - and  $\beta$ -trace proteins are shown in Fig. 2. Both curves represent the interaction between the im-

munizing isolated antigen preparation and the corresponding specific antiserum. The precipitation curves gave no obvious indications of heterogeneity in the antigens used. All specific antisera used gave similar quantitative precipitin curves, and determinations were always made in the antibody excess zone.

The results of the quantitative determinations of CSF proteins can be seen in Table I. Total protein, albumin and  $\gamma$ -globulin levels in adults without neurological disease were similar to those found by Kabat *et al*(10) in a series of patients with presumed normal spinal fluids. The concentration of transferrin was approximately 6.5% of total proteins. This agrees with the results of Frick(11) who found that this protein represents 6.7% of total CSF proteins.

The  $\beta$ -trace concentration in CSF from normal adults was found to vary from 1 to 9 mg%. The average concentration, 4 mg%, represents approximately 10% of total CSF proteins. There was no statistically significant increase in concentration of  $\beta$ -trace globulin

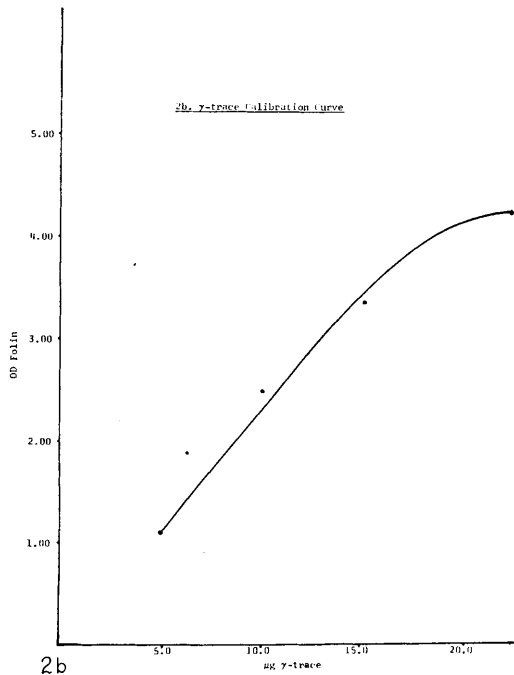
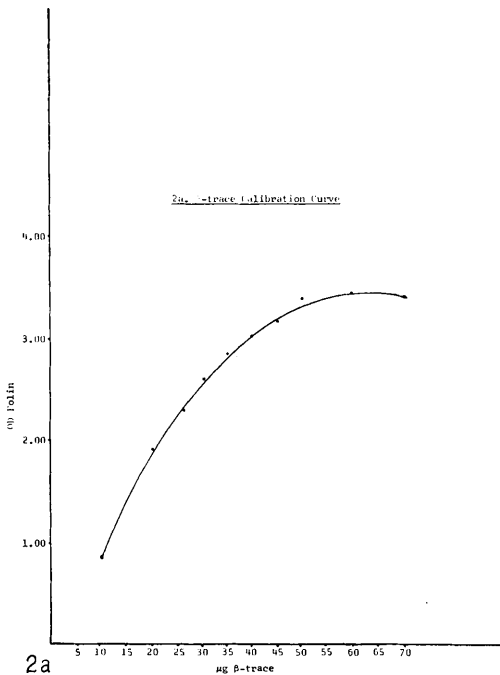


FIG. 2. Quantitative precipitin curves for  $\beta$ -trace (2a) and  $\gamma$ -trace (2b). For quantitation of  $\beta$ -trace, only the part of the curve below OD 2.75 (less than 35  $\mu\text{g } \beta$ -trace) was used. For quantitation of  $\gamma$ -trace, only the part of the curve below OD 3.50 (less than 15  $\mu\text{g } \gamma$ -trace) was used.

TABLE I. Albumin, Transferrin,  $\gamma$ -Globulin,  $\gamma$ - and  $\beta$ -Trace Globulin Levels in Cerebrospinal Fluids with Normal and Elevated Total Protein Concentrations.

CSF obtained from	No. of CSF	Total protein	Albumin	Transferrin	$\gamma$ -Globulin	$\beta$ -Trace	$\gamma$ -Trace
Normal adults	12	40.5 (15-55)*	13.8 (5.0-19.0)	2.6 (1.2-2.9)	4.0 (2.6-6.2)	4.0 (1.0-9.0)	0.83 (0.53-1.42)
Patients with CNS tumors	5	188.8 (155.1-237.3)	82.0 (40.0-132.0)	5.3 (4.0-6.0)	22.0 (20.4-23.6)	6.1 (3.9-10.0)	0.85 (0.85-1.15)
Infants with hydrocephalus	8	120.3 (30.1-364.7)	62.2 (6.0-233.0)	12.4 (0.9-31.0)	5.9 (2.0-19.0)	5.1 (1.0-8.4)	1.63 (0.76-2.36)

\* Range in parentheses.

with increase of the total protein, albumin, transferrin, and  $\gamma$ -globulin. In one adult patient with meningitis, a total protein of 308 mg% was found, while  $\beta$ -trace was only 4.8 mg%, or 1.6%.

In hydrocephalic infants with greatly varying total CSF protein contents, a similar range of  $\beta$ -trace concentration, average 4.7 mg%, was found as in normal adults. There was no apparent effect of age on the concentration of this protein, which was also seen in a few spinal fluid samples from infants without neurological disease.

The average  $\gamma$ -trace globulin concentration in CSF from normal adult individuals was 0.83 mg% or approximately 2% of the total protein. CSF from hydrocephalic infants showed a 2-fold increase in average  $\gamma$ -trace content as compared to CSF from adults. However, CSF from 8 normal infants studied was similar in this respect. Increases in total protein, albumin, transferrin and  $\gamma$ -globulin concentration were not accompanied by changes in the  $\gamma$ -trace content of the CSF. This was evident from the studies on CSF from adult patients with central nervous system tumors and in CSF from hydrocephalic infants with greatly varying total protein concentration. Isolated 2-3-fold increases in CSF  $\gamma$ -globulin content, such as were found in 3 patients with neurosyphilis, did not affect the  $\gamma$ -trace concentration. However, in CSF from 4 of 7 patients with multiple sclerosis, very low  $\gamma$ -trace concentrations were observed (<0.4 mg%).

Although differences in protein concentration and in albumin-to-globulin ratios exist between lumbar and ventricular fluids(12), no such differences were found for  $\beta$ - and  $\gamma$ -

trace proteins in 5 individuals.

*Discussion.* The immunochemically detectable amounts of trace proteins in human CSF have been determined to be 0.83 mg% of  $\gamma$ -trace and 4.0 mg% of  $\beta$ -trace. The concentration found for  $\gamma$ -trace is in agreement with data in the literature (0.97 mg% for normal adult)(13). Thus,  $\beta$ -trace can be considered as one of the major proteins in CSF. In infants  $\gamma$ -trace content is twice as high as in adults, so that the two trace proteins may represent as much as 14% of total CSF protein. These concentrations contrast with those in serum, where  $\gamma$ -trace is several-fold lower and  $\beta$ -trace has not been detected by double diffusion in agar(1). Immunoelectrophoretic patterns of concentrated normal human urine show a prominent  $\beta$ -trace and a small  $\gamma$ -trace arc(4), indicating that these proteins may represent a significant percentage of urine proteins as well. The use of the descriptive term "trace" is actually no longer applicable to  $\beta$ -trace since it is now clear that this protein represents a relatively large proportion of CSF proteins.

$\gamma$ -trace concentration was higher in CSF of infants with or without hydrocephalus than in adults. Since the biological properties of  $\gamma$ -trace are completely unknown, the significance of this higher concentration in infants cannot be evaluated at present. No effect of specific diseases on concentrations of the trace proteins was noted, except that in several cases of multiple sclerosis slightly lower values for  $\gamma$ -trace were found. This has also been observed previously(13).

Previous studies have shown that brain tissue is not a primary site of synthesis of trace proteins(5). The higher concentrations of these proteins in CSF than in serum may

be explained on the basis of their small molecular sizes as determined by their low sedimentation coefficients(5). As a result of their size, both proteins may be cleared rapidly from plasma, and found in higher concentration in other biological fluids. The efficiency by which the trace proteins are transferred from plasma to CSF under normal conditions can be seen from the fact that no increase in their CSF concentration occurs in the presence of markedly enhanced permeability to other serum proteins. In fact, the concentrations of both trace proteins were completely independent of the albumin, transferrin, and  $\gamma$ -globulin concentrations, and of total protein. In spite of marked elevation of serum proteins in the CSF,  $\beta$ -trace and  $\gamma$ -trace levels remained unaffected.

Recent experiments in our laboratory have shown that the clearance of albumin(14) and  $\gamma$ -globulin(15) from CSF is dependent on bulk absorption of CSF, so that all proteins may be removed at a rate independent of their size and concentration. It was also shown that the transfer of albumin(14) and of  $\gamma$ -globulin (15) from blood to CSF in the cat is only partially dependent on CSF formation by the choroid plexus, and that the concentration of proteins in CSF may thus be dependent on the permeability of the blood-brain-CSF barriers to the influx of these proteins from serum (14). It is, therefore, unnecessary to postulate the brain as a site of synthesis of  $\beta$ - and  $\gamma$ -trace, since proteins of low molecular weight can be expected to accumulate in CSF, if the permeability of the blood-brain-CSF barrier for protein is indeed determined by the size of the protein molecules.

*Summary.*  $\beta$ -trace,  $\gamma$ -trace, albumin, transferrin and  $\gamma$ -globulin protein concentrations were determined immunochemically in the cerebrospinal fluid (CSF) of patients with or without neurologic disease. Special reference was made to the relative concentration of the "trace" proteins with respect to total and individual CSF proteins. In normal CSF, the

concentration of  $\gamma$ -trace was approximately 0.83 mg% (2% of total proteins), and was 2 fold greater in infants than in adults. The concentration of  $\beta$ -trace was approximately 4 mg% (10% of total proteins). It was concluded that the  $\beta$ -trace is a major protein constituent of CSF. The absolute levels of both trace proteins were independent of the changes in total or individual proteins in CSF from patients with neurologic diseases studied. A mechanism which may account for the accumulation of these proteins in CSF is discussed.

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