

Ultraviolet Fluorescence Spectra of the Cerebrospinal Fluid in Tyrosinaemia

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Direct spectrofluorometric examination of the native CSF in tyrosinaemia showed characteristic fluorescence spectra. At an excitation wavelength of 280 μm a fluorescence maximum at 325 μm appears in addition to the fluorescence maximum depending on the total protein (tryptophan peak). At lower CSF tyrosine contents the fluorescence is at about 325 μm in the protein-free ultrafiltrate. In a case of tyrosinaemia in an infant with side-different convulsions, the spectrofluorometric examination of the CSF has led to the diagnosis.

Pathological tyrosinaemia is the main symptom of different diseases. The various forms differ in clinical symptoms, genetic aspects, pathomechanism and the pattern of excreted tyrosine metabolites [3, 6, 9]. Aetiology and pathogenesis of hereditary tyrosinaemia are incompletely understood and probably not due to a specific deficiency of p-hydroxyphenyl pyruvic acid oxidase [2]. A disturbance in tyrosine metabolism causes the tyrosine level to increase in the body fluids and "overflow"-aminoaciduria of tyrosine and its metabolites (tyrosyluria).

The acute fulminant form of tyrosinaemia is accompanied by severe liver injury. Early diagnosis is necessary because of the possibility of a dietetic influence on the significantly increased tyrosine level [4, 5].

Acute neurologic symptoms are unusual in tyrosinaemia. In a case with

ambiguous but characteristic clinical symptoms the increased tyrosine content was revealed by the spectrofluorometric examination of the native CSF.

METHOD

Spectrofluorometric measurements were carried out with a Beckman spectrofluorometer model SF 1078 (sensitivity 7, time constant 4, zero-suppression 0, speed 90, resp. 30 $\mu\text{m}/\text{min}$). Samples of about 1.5 ml CSF, in a layer of 10 mm were exposed for fluorescence excitation to light of wavelength of 260, 270, 280 and 290 μm . Measurements were taken at room temperature. In some cases the protein-free ultrafiltrate of the sample was measured under the same circumstances.

REPORT OF A CASE

Patient's mother. Ill 5 weeks before delivery with pruritus, insignificant proteinuria. 3 weeks before delivery, hospitaliza-

tion with subicterus (serum bilirubin, 1.73 mg/100 ml), urobilinuria, urobilinogenuria and slight increase of serum transaminase. Delivery of twins (a girl and a boy) 2 months before term.

Patient. J. A. A male baby of 1340 g birth weight, second twin.

First hospitalization with respiratory distress, pulmonic haemorrhage with thrombocytopenia (23 000/cu.mm on 3rd day). Antithrombotic serum factors in antihuman globulin consumption test traceable (mother negative). Serum bilirubin maximum on 4th day, 14.05 mg/100 ml (0.35 mg/100 ml directly reacting). Apnoeic episodes from 3rd to 6th day. Treatment with intravenous sodium bicarbonate, plasma fractions (PPSB, COHN I), vitamin K₁, ampicillin, oxacillin, polymyxin B, caffeine. Ascorbic acid, 50 mg daily, was

given from the 4th to 9th week; 15 mg vitamin D₂ in the 2nd week. Transfusion of erythrocytes (sediment) in the 6th week. After discharge the baby was thriving normally. A second prophylactic dose of vitamin D₂ was given in the 12th week.

Second hospitalization. Acute illness 2 days after reposition of an inguinal hernia, with vomiting of haematin, meteorism, and oedema of the back of hand and feet. At admission (age, 3 months; body weight, 3750 g) the child was soporous, icteric (6.48 mg/100 ml serum bilirubin) and had clonic convulsions on the right side. Lumbar puncture showed a water clear CSF with 7/3 cells (Fig. 1). Liver 2 cm, spleen 1 cm palpable. Normal blood sugar level. Blood counts, anaemia (Hb. 6.9 g/100 ml), leukocytopenia (3 400/cu.mm), normal thrombocyte count (210 000/cu.mm after Fonio).

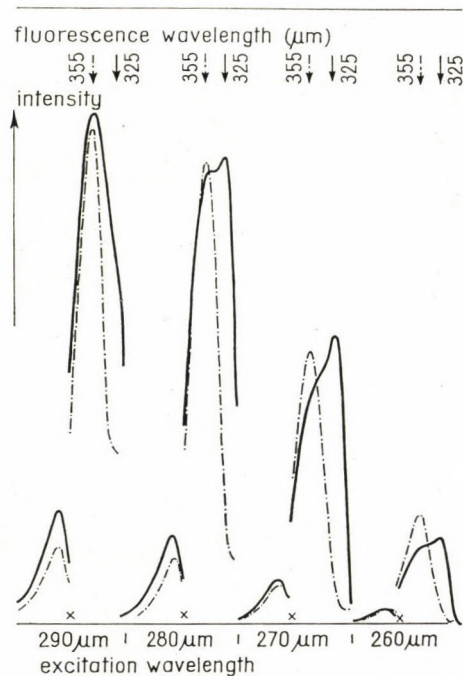


FIG. 1. Fluorescence (emission) spectra of native cerebrospinal fluid. Different excitation wavelengths. — patient with tyrosinaemia (tyrosine contents of CSF, 8938 $\mu\text{g}/100\text{ ml}$), - - - control case with encephalitis (protein content of CSF, 51 mg/100 ml), (x: artificial effect by changing of monochromators from ultraviolet to visible light; 390 nm)

Pathologic thromboplastin time (6%), hypoproteinaemia (4.0 g/100 ml). Increase of serum transaminases (GOT, 640; GPT, 820 Wroblewski-units). Australia (Au/SH)-antigen positive in patient's serum, negative in mother's and sister's.

Ophthalmoscopy revealed a small retinal haemorrhage of the left eye. X-rays revealed bronchopneumonia, osteoporosis and small-spotted destructions of the skull and other bones (radius, ribs).

Fluorescence of CSF at this time raised the suspicion of tyrosinaemia (Fig. 1).

Tyrosine estimation [14] in plasma, 99 $\mu\text{g/ml}$; in CSF, 8938 $\mu\text{g}/100\text{ ml}$. Further estimations showed an increase to 131 $\mu\text{g/ml}$ in plasma. Urinary tyrosine excretion amounted to 21.4 mg daily (methionine, 2.8 mg; glycine, 24.2 mg; threonine, 4.6 mg; serine, 2.7 mg; alanine, 13.7 mg; in 24-hour urine). The alpha nitroso beta-naphthol test for tyrosyluria gave a positive reaction.

Treatment with 100 mg ascorbic acid daily. The diet was woman's milk with a low fat content and with Ringer's solution and bovine serum. Convulsions, accentuated on the right side, appeared repeatedly during the first two days of hospitalization. After one week the EEG showed a diffuse cerebral functional disorder, no convulsive activity and no side-difference. After two weeks, clinical improvement ensued with a decrease of the serum transaminase and tyrosine levels (82.5 $\mu\text{g/ml}$ in plasma, 2210 $\mu\text{g}/100\text{ ml}$ in CSF [Fig. 3]).

Patient's sister (first twin). Birth weight, 1750 g. Normal findings apart from some anaemia with Hb, 9.6 g/100 ml. Plasma tyrosine, 20.3 $\mu\text{g/ml}$. No X-ray abnormalities. Plasma tyrosine of the parents: mother, 10.0 $\mu\text{g/ml}$; father, 10.2 $\mu\text{g/ml}$.

RESULTS

Fig. 1 shows the fluorescence spectra of the patient's CSF at admission, compared with those of a control case

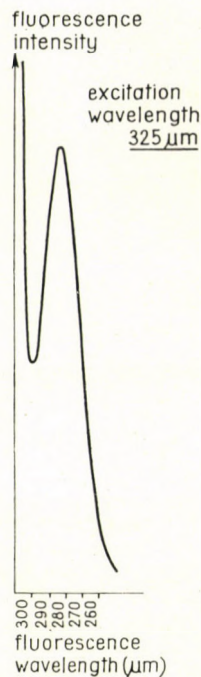


FIG. 2. Fluorescence (325 nm) excitation spectrum. Native cerebrospinal fluid in tyrosinaemia (tyrosine contents of CSF, 8938 $\mu\text{g}/100\text{ ml}$)

(patient with encephalitis) with nearly identical fluorescence intensity. Beside the fluorescence band at 355 μm (tryptophan $\hat{=}$ protein) an intensive fluorescence in the range of shorter wavelengths with a maximum at 325 μm was recognizable. The highest intensity of this fluorescence (325 μm) lay at an excitation wavelength of about 280 μm (Figs 1 and 2) whereas the maximum of the fluorescence at 355 μm in these cases lay at an excitation wavelength of 290 μm . The tyrosine content of the patient's CSF amounted to 8938 $\mu\text{g}/100\text{ ml}$. The protein content of the control CSF (Fig. 1) amounted to 51 mg/100 ml.

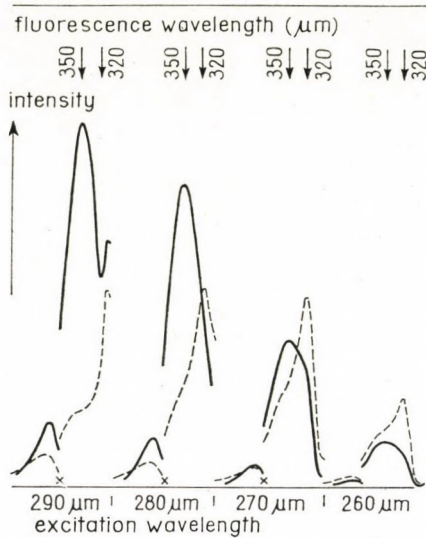


FIG. 3. Fluorescence (emission) spectra of cerebrospinal fluid. Different excitation wavelengths; identical conditions as in Fig. 1. — Native CSF in tyrosinaemia (tyrosine contents, 2210 $\mu\text{g}/100\text{ ml}$; protein contents, 49 mg/100 ml), - - - protein-free ultrafiltrate of the same CSF (tyrosine content, 1902 $\mu\text{g}/100\text{ ml}$) (\times : 390 μm , changing of monochromators; see Fig. 1)

After clinical improvement, the fluorescence spectrum of the patient's CSF showed no well-defined fluorescence peak at 325 μm (Fig. 3). At a lower registration speed, a distinct increase of fluorescence in the shorter wave range compared with a control case is to be seen (Fig. 4). In the protein-free ultrafiltrate of the patient's CSF, the fluorescence band at 320 μm was marked (Figs 3 and 4). The tyrosine content of the patient's CSF amounted to 2210 $\mu\text{g}/100\text{ ml}$, its protein content to 49 mg/100 ml. The average tyrosine content of the CSF of seven children with different non-meningitic acute neurologic diseases amounted to $266 \pm 90\ \mu\text{g}/100\text{ ml}$.

DISCUSSION

Absorption and fluorescence of protein solutions in the ultraviolet spectral range are based on their contents of the aromatic amino acids tryptophan, tyrosine and phenylalanine [8, 11, 14]. Studies of the fluorescence of CSF revealed a positive correlation between the fluorescence intensity at 355 μm and the protein content [7]. In tryptophan-containing proteins, the fluorescence spectrum is principally that of tryptophan [11]. In the absence of tryptophan, the emission

	protein	tyrosine / 100 ml	
- - -	49 mg	2210 μg	} patient
—	---	1902 μg	
- - -	18 mg	not est.	} control case
—	---	55,4 μg	

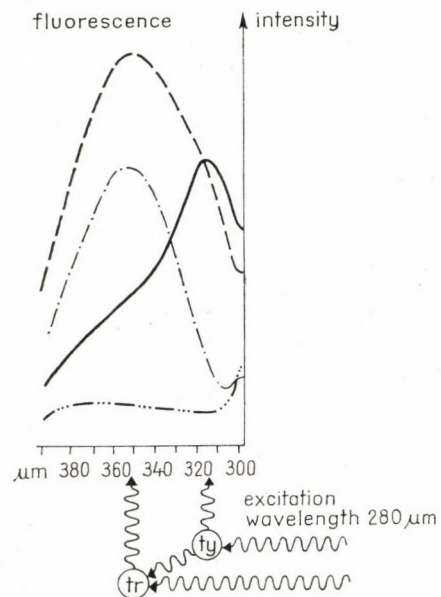


FIG. 4. Fluorescence emission spectra. Excitation wavelength 280 nm. Native and protein-free (ultrafiltrated) cerebrospinal fluid in tyrosinaemia and in a normal control case

(fluorescence) spectrum of proteins is typical of that of tyrosine, the only other amino acid with a significant native fluorescence [11]. Native phenylalanine shows a light fluorescence. In tryptophan containing proteins, the emission of tyrosine is diminished or absent, presumably due to either transfer of energy from the tyrosine to tryptophan, or a quenching action of tryptophan [11]. A marked fluorescence intensity of the ultrafiltrate at 320 μm in comparison with the negative CSF of the same specimen shows this effect (Fig. 3, excitation wavelength 270 μm). The different fluorescence emission peaks of the aromatic amino acids are, according to the literature, tryptophan 348–350 μm ; tyrosine, 303–313 μm [11, 14]. The difference between these and the values measured by us (355 μm , and 320–325 μm , respectively) seems to be explained by the modification of the fluorescence by the used type of fluorometer. Besides, an influence by tyrosine metabolites on tyrosine fluorescence may also come into question.

The clinical picture of our case corresponded to the acute form of tyrosinaemia. Until now, a more certain association of the disease with the known forms of tyrosinaemia could not be proved. The acute neurologic symptom of convulsions is unusual in tyrosinaemia [10]. In the case described by GENTZ et al. [4], this symptom occurred under metabolic strain after previous shortage. An excessive amino acid imbalance as a factor to release the convulsions is

also to be considered in our case. Finally, a last possibility is a cerebral haemorrhage following plasmatic coagulopathy at a normal thrombocyte count.

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