The Cerebral Lesions in a Patient with Generalized Glutathione Deficiency and Pyroglutamic Aciduria (5-Oxoprolinuria)

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Summary. The clinical and pathologic features of a male patient with generalized glutathione deficiency and pyroglutamic aciduria are presented. The patient died at the age of 28 years. He was mentally retarded from infancy and developed progressive tremor, retardation of movement, and ataxia as from the age of 16.

Neuropathologic examination of the brain disclosed a selective atrophy of the granule cell layer of the cerebellum and focal lesions in the right frontoparietal cortex and bilaterally in the visual cortex and the thalamus. The type and distribution of the lesions resembled those seen after mercury intoxication. However, in our patient the damage was probably caused by the lack of protection by glutathione against oxidative damage in the brain. Possible treatment of this rare metabolic disorder might include external supply of an antioxidant, e.g., a thiol capable of penetrating the blood brain barrier.

Key words: Pyroglutamic aciduria – 5-Oxoprolinuria – Glutathione deficiency – Encephalopathy – Cerebellar atrophy The disorder is characterized by severe metabolic acidosis due to the accumulation of pyroglutamate in blood and other tissues and massive excretion of pyroglutamate in the urine. The primary defect proved to be a deficiency of the glutathione synthetase (Wellner et al. 1974; Marstein et al. 1976). The accumulation of pyroglutamate is a secondary event, caused by the lack of intracellular glutathione.

Glutathione has a nearly ubiquitous distribution in animal and plant tissues. A precise and complete picture of its biochemical functions has not yet been established. Apart from functioning as a coenzyme in certain enzymic reactions, glutathione is generally believed to be essential for the maintenance of a reducing environment by which functional SH-groups (thiol groups) may be kept in the reduced state (Hopkins et al. 1938; Jocelyn 1962), and for the protection of cell membranes and other constituents against the damaging effect of hydrogen peroxide and other oxidants (Mills and Randall 1957; Mills 1960; Cohen and Hochstein 1963; Christophersen 1968). Recently, glutathione has also been suggested to participate in the transport of amino acids across cell membranes (Orlowski and Meister 1970).

Our patient is the first case with generalized glutathione deficiency that has been subjected to postmortem examination. The clinical symptoms and the lesions in the brain resembled those of the Minamata disease (Takeuchi et al. 1962). A mercury intoxication, however, seems very unlikely in our patient. We suggest that the similarity is caused by the lack of protection by glutathione against oxidative damage in the brain.

Case Report

Our patient was a man who died at the age of 28 years. His parents, an elder brother, and all other relatives were healthy. After an uneventful pregnancy and delivery he was severely jaundiced for about 2 weeks. Later on, he had no episodes of jaundice nor any evidence of increased hemolysis. He showed psychomotoric retardation from the

Pyroglutamic aciduria is a rare metabolic disorder which was first described in 1970 (Jellum et al. 1970). Since then several cases have been reported from other countries (Hagenfeldt et al. 1974; Boivin et al. 1975, 1978; Spiegelberg et al. 1977; Porath et al. 1978; Mendelsohn et al. 1979). Numerous biochemical and clinical investigations have been performed on these patients (Eldjarn et al. 1973; Larsson et al. 1974, 1976; Marstein et al. 1976). Pyroglutamic aciduria is probably inherited as an autosomal recessive (Larsson et al. 1976).

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onset. Between 8 and 14 years of age he stayed in a school for mentally retarded children, where he got special training for speech disturbances which were difficult to classify. From the age of 16, the course was steadily downhill with progressive deterioration of the motor functions, retardation of movement, intention tremor, and rigidity. The tendon reflexes became exaggerated and the plantar responses were extensor. The muscular strength was normal.

The pyroglutamic aciduria was recognized when the patient was 19 years old. He had suffered from frequent vomitings and sporadic hematemeses for about 1 year before admission to the hospital. A diaphragmatic hernia with esophagitis was disclosed and treated surgically. In spite of the vomitings a postoperative severe metabolic acidosis developed and became life-threatening. Subsequent biochemical investigations revealed the inborn error of metabolism. Since then he was constantly treated with sodium bicarbonate by mouth (10-20 g daily) to control his metabolic acidosis, and by oral potassium salts to replace loss of potassium ions in the urine. Despite this treatment, he showed an increasing mental deterioration and his movements became gradually more clumsy and incoordinated. During last 4-5 years of his life he developed attacks of generalized convulsions and received antiepileptic therapy. Three years before death he was confined to a wheel-chair and profoundly demented.

Some weeks before death the patient was admitted to The National Hospital to get brain and muscle biopsies performed for biochemical and histological examinations. No structural changes were found in the biopsies from the right frontal lobe and tibialis anterior muscle by routine and ultrastructural examination, and ultrastructural examination of erythrocytes did not reveal any abnormalities.

The patient was transmitted from the hospital to a nursing home. About 3 weeks after the craniotomy, on June 24, 1978, he was found dead in bed without any previous worsening of his condition.

Biochemical Findings

A detailed description of the biochemical investigations will be published elsewhere. The essence of the findings is as follows: All autopsied organs (liver, lung, kidney, skeletal muscle, and brain) had a low activity of glutathione synthetase (2-8%) of normal) and a high content of the secondary product, pyroglutamic acid. Particulary high contents of pyroglutamate were found in the kidneys and the brain. Twelve areas of the left half of the brain were cut out; all had reduced glutathione levels with a particulary low content in the cerebellar cortex (0.01 µmol/g as compared to controls 1.58 ± 0.56



Fig. 1. The right half of the brain. Diffuse cerebellar atrophy with widening of the fourth ventricle

 μ mol/g). Neutron activation analyses were used to determine the level of mercury and other trace metals in the frontal and cerebellar cortex. The concentration of mercury in the frontal cortex and cerebellum



Fig. 2. The right half of the brain with the operative defect (arrow) and the cystic cavities (arrow head) anterior and posterior to the central sulcus



Fig. 3. Part of the cerebellar cortex with diffuse loss of granule cells. H.E. $\times 95$



Fig. 4. Part of the visual cortex with laminar loss of nerve cells and astrocytosis. Holzer $\times 95$

was 40 and 294 μ g/g wet tissues weight, respectively, which are within the range reported from normal subjects. The concentration of other trace metals were also normal.

Autopsy Findings

The body was of average height with a slightly poor state of nutrition. The immediate cause of death appeared to be a leg vein thrombosis with left-side pulmonary embolism and an early segmental infarction, combined with terminal aspiration of gastric contents into the tracheo-bronchial tree. An acute right-side pyelonephritis might also have contributed to death. The general autopsy revealed no gross or histological changes in any organ that could be ascribed to generalized glutathione deficiency or metal intoxication.

The brain weighed 1,130 g. The left half was immediately frozen. Later on, it was partly thawed to get specimens from different areas for biochemical and neuropathological investigations. The right half of the brain was fixed in formalin.

The meninges were unremarkable, except for minimal changes corresponding to the craniotomy in the right frontal region. The cerebral arteries were patent and normal. The main change was a generalized cerebellar atrophy (Fig. 1). In addition to the operative defect, there were two cystic cavities anterior and posterior to the central sulcus of the right hemisphere (Fig. 2), which looked like ordinary old infarctions. There were no macroscopical cortical lesions in the left hemisphere but a small cystic cavity in the thalamus. The fourth ventricle was remarkably enlarged, the lateral ventricles were only slightly dilated.

Histological Features

The generalized cerebellar atrophy was caused by a diffuse loss of granule cells, while the Purkinje cells were generally spared (Fig. 3).

The dentate nuclei were normal. The lesions in the right central region and the cystic cavity in the left thalamus looked like ordinary old infarctions. Examination of the visual cortex disclosed focal loss of neurons with astrocytosis mainly involving the middle layers (Fig. 4). The meningeal and intracerebral vessels were normal. No abnormalities were found in the hippocampal formations, the striatum, the pallidum, or in the brain stem.

Discussion

Twelve cases of generalized glutathione deficiency have been reported up to now (Larsson 1980). Our patient is the first adult and the first patient that was subjected to postmortem examination. Only three of the other patients (aged 6 months to 10 years) have shown neurological symptoms. The remaining patients so far have had normal psychomotor development. They are all infants or small children.

All patients have been given bicarbonate therapy to control the metabolic acidosis. Our patient was 19 years old when acidosis correction therapy was first started. The observed brain damage cannot be attributed to the acidosis, because acidosis per se does not give any pathological changes in the brain, and because the patient showed progressive deterioration in spite of the bicarbonate therapy.

The low incidence of neurological symptoms in previously reported cases may indicate that brain damage will occur only when the level of glutathione falls below a critical value. It is of interest in this respect that our patient during several years had a particulary low erythrocyte glutathione content. One should also bear in mind that most of the reported cases are still infants or small children that may develop neurological symptoms at later stages.

The changes found in our case, viz., the cerebellar atrophy of the granular layer, the patchy loss of neurons in a laminar pattern in the visual cortex, and the focal lesions in the central region and the thalami, resembled those of the Minamata disease (Takeuchi et al. 1962). However, to our knowledge our patient had never been exposed to any mercury intoxication. Neutron activation analyses revealed normal level of mercury (Olszewski et al. 1974). However, patients with generalized glutathione deficiency may be more susceptible to heavy metals than normal individuals because of the lack of glutathione, which is believed to play an important role in metal detoxication. Alternatively, the changes could be caused by the lack of protection against oxidative damage by other agents due to the glutathione deficiency.

The cerebellar atrophy in our case was confined to the granule cell layer, while the Purkinje cells were generally spared. The cerebellar lesion had probably occurred after the neonatal period since the Purkinje cells were neither disaligned nor dislocated, and the Purkinje dendrites were normal (Friede 1975). The dentate, olivary, and pontine nuclei were also normal.

The reason for this selective vulnerability in glutathione deficiency is unknown. The nerve cell loss of the visual cortex might be due to a similar vulnerability, or it could be of a vascular origin. Since the hippocampal formations, the pallidum, and the Purkinje cells were normal, it is unlikely that the lesions were caused by a posticteric encephalopathy (Friede 1975), or anoxic damage due to the epileptic seizures. Leg vein thrombosis and pulmonary embolism are generally rare in young patients (Linder et al. 1967). The presence of cerebral infarcts, leg vein thrombosis and pulmonary emboli may therefore raise the question if the glutathione deficiency also may predispose for thromboembolic episodes.

The neuropathological findings raise the question as to how the brain damage could have been avoided. A direct stimulation of the glutathione production appears to be difficult in view of the lack of glutathione synthetase in the patient. One possibility might be to supplement the diet with a thiol, such as N-acetylpenicillamine, which penetrates the blood brain barrier and may thus protect the sensitive thiol groups and prevent oxidative damage. Treatment with other antioxidants, such as vitamin E, (Boxer et al. 1979) might also be worth trying in future cases.

Acknowledgements. We want to thank Britt Salbu, Chemical Institute, University of Oslo, for determination of the levels of trace metals in the brain.

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Received June 19, 1980/Accepted July 31, 1980