

D-2-Hydroxyglutaric Aciduria With Cerebral, Vascular, and Muscular Abnormalities in a 14-Year-Old Boy

ABSTRACT

D-2-Hydroxyglutaric Aciduria is a rare metabolic disorder that can cause injury to the brain and other organs. This case report concerns a 14-year-old boy showing irritability and typical signs of pyloric stenosis early postnatally. From the age of 3 months he had epilepsy. He was mentally retarded, hypotonic with preserved reflexes, and dystonic. The features were dysmorphic with elongated head and high arched palate. Cardiomegaly with aortic insufficiency was diagnosed. Magnetic resonance imaging of the brain revealed atrophy, reduced periventricular white matter, and multiple bilateral aneurysms of the middle cerebral arteries. The boy died at the age of 14 years. Autopsy confirmed the white-matter reduction of the cerebral hemispheres as well as the arterial aneurysms of the middle cerebral arteries. Lesions of a few leptomeningeal and cerebral microvessels and of the renal and pulmonary arteries were also found. There were bilateral infarcts of the kidneys and signs of cardiomyopathy with noncompensated left ventricular failure. Signs of myopathy were evident. The clinical and postmortem findings imply a disseminated mesenchymal process. (*J Child Neurol* 2000;15:488–492).

2-Hydroxyglutaric acid is normally excreted in small amounts in the urine. It is an intermediate in metabolic pathways of relevance to the central nervous system. The excreted molecules appear as L and D isomers.¹ Excretion of excessive concentrations indicates an inborn metabolic disturbance inherited as an autosomal recessive trait. D-2-hydroxyglutaric aciduria is rarer than the L-isomer form with 25 and 40 cases, respectively, having been reported in the literature.²

Based on clinical, neuroimaging, and biochemical examinations, the spectrum of changes in D-2-hydroxyglutaric aciduria seemed to be diverse with one mild and one severe phenotype,³ creating doubt that the disorder might not be a single entity. However, recently it has become clear that the mild phenotype essentially shares the characteristics of the severe phenotype.² Neuropathologic examinations in D-2-hydroxyglutaric aciduria are limited to skeletal muscle with one study in a 4-month-old child⁴; a curiosity is a study in an 11-year-old dog.⁵

This report concerns clinical, biochemical, and extensive neuropathologic investigations in a boy with D-2-hydroxyglutaric aciduria who died at age 14½ years. In addition to cerebral atrophy with white-matter lesions, signs of myopathy of skeletal muscle and heart and arterial lesions of the brain, kidneys, and lungs were seen.

Case Report

This boy was born at term to nonconsanguineous parents with two older healthy children. Apgar scores were 8, 9, and 10, the birthweight was 3120 g,

length was 50 cm, and head circumference was 35 cm. Apneic spells and episodic vomiting during the first days of life led to a diagnosis of pyloric stenosis, and a pyloromyotomy was performed. At the age of 3 months, tonic seizures appeared. Neurologically, he was hypotonic with brisk reflexes and psychomotor development was delayed. The initial seizure treatment was carbamazepine, but valproate was added because of therapy resistancy. Electroencephalogram (EEG) showed a slow background with episodic epileptiform activity over the left temporal area. Metabolic screening at the ages of 10 months and 4½ years was normal. A computed tomographic (CT) scan revealed brain atrophy. At the age of 7 years the boy was referred to the university hospital because of signs of progressive encephalopathy with severe psychomotor delay. His epilepsy was well controlled on carbamazepine and valproate. He could not sit without support and gave only slight visual contact. On examination he showed a dysmorphic, elongated face, antimongoloid slanting of the eyes, carp mouth, prominent and cupped ears, and high narrow palate. Head circumference was 54 cm. He showed generalized hypotonia with brisk reflexes, legs more than arms, as well as left-leg dystonia. Left ventricular cardiac hypertrophy was verified. Hematologic investigation, including lactate, ammonia and chromosomes, and cerebrospinal fluid analyses were normal. Unfortunately, a urine test for organic acids was not performed. EEG showed generalized low-frequency rhythmic activity. On examination with visual evoked potentials there were normal cortical responses. Magnetic resonance imaging (MRI) showed general atrophy with periventricular white-matter reduction.

The boy deteriorated slowly and at age 14 years (Figure 1) he was re-investigated mainly because of severe psychomotor retardation and epilepsy with generalized tonic-clonic and absence seizures. Hematology, including serum creatine kinase, lactate, and ammonia, and cerebrospinal fluid analy-

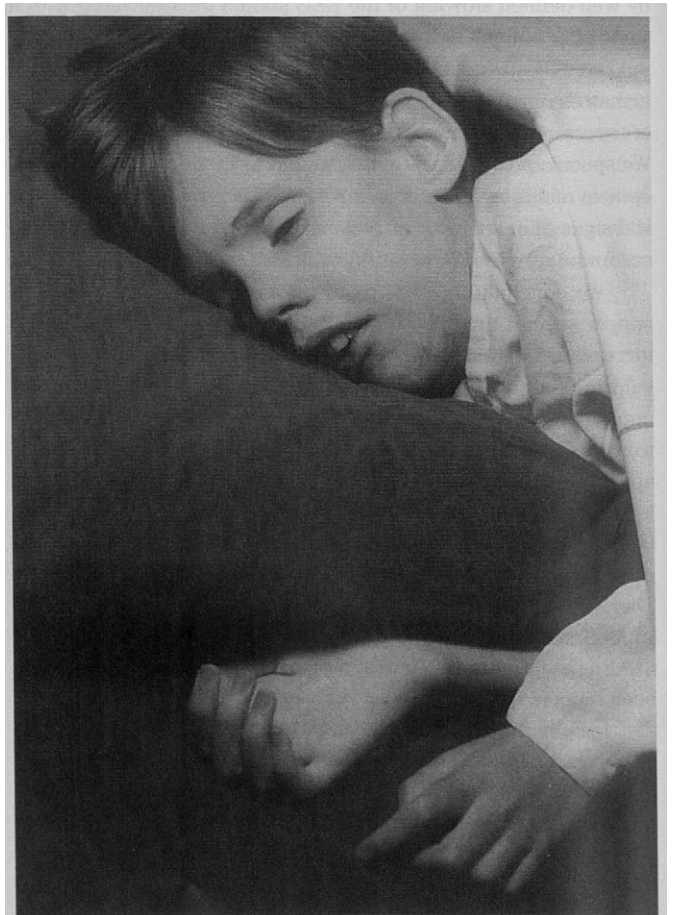


Figure 1. The boy at age 14½ years.

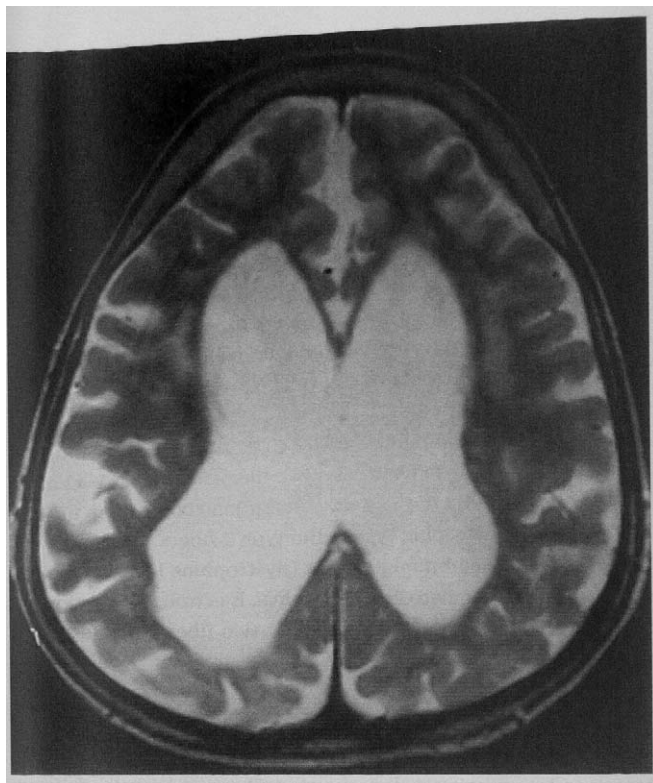


Figure 2. T_2 -weighted (TR: 3826, TE: 120) magnetic resonance image showing extensive reduction of the periventricular white matter. There are also abnormal signals in the remaining white matter as well as cortical atrophy. The corpus callosum is extremely thin.

ses were normal. The EEG was abnormal with slow background and bilateral episodic epileptiform activity. Electromyography and neurography showed signs of slight neuropathy. On MRI a general atrophy, occipital more than frontal, with reduced periventricular white matter was seen (Figures 2 and 3) as well as aneurysms of both middle cerebral arteries (Figure 3). A muscle biopsy was performed (see below).

Urinary organic acids were examined on three different occasions. The excretion of D-2-hydroxyglutarate was 3570 mmol/mol creatinine when the boy was treated with only valproate, 2000 mmol/mol creatinine on treatment with valproate and clobazam, and 1400 mmol/mol creatinine on monotherapy with clobazam. There was high urinary excretion of serine and glycine but also of threonine, alanine, and tyrosine. On fibroblast culture, D-2-hydroxyglutarate was not detected in any of the incubates.

Methods

Chemical Methods. Metabolic screening was performed at the ages of 10 months and 4½ years. The method used was not effective in revealing organic acids. However, when the boy was between age 14 and 14½ years, elevated concentrations of 2-hydroxyglutaric acid were found by combined chromatography and mass spectrometry. Further independent analyses identified it as the D isomer. The normal excretion range of this product is 1.3 to 19 mmol/mol creatinine.³ Fibroblasts in the growth phase from the boy and a control patient were incubated in minimum essential medium with the addition of 1.0 mmol/L of either 5-aminolevulinic acid, DL-2-aminoadipate, or 4-hydroxybutyrate. The medium was collected after 48 hours and analyzed by gas chromatography and mass spectrometry.

Morphologic Methods. Material for morphologic investigations included muscle biopsies at the age of 14 years and postmortem material from muscles, brain, spinal cord, and numerous extracerebral organs. Frozen material as well as formalin-fixed paraffin-embedded samples were available. Paraffin sections, 5 μ m thick, were stained with hematoxylin and eosin and

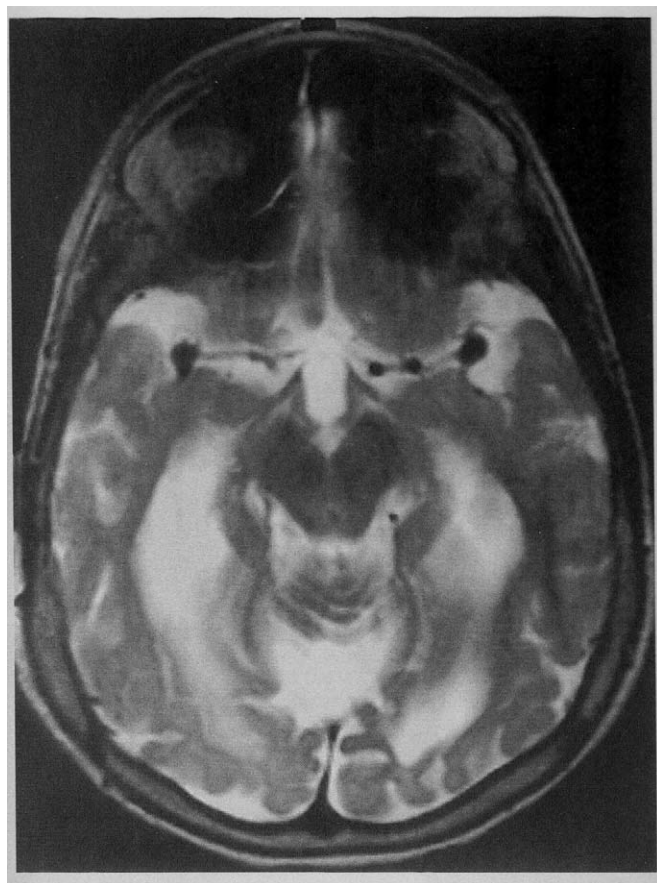


Figure 3. T_2 -weighted (TR: 3826, TE: 120) magnetic resonance image showing dilation of the temporal horns and reduction of the white matter. Signal void in bilateral aneurysms of the first bifurcation of the middle cerebral artery.

the van Gieson method. Samples from the brain and spinal cord were also stained by Luxol fast blue and cresyl violet.

Immunohistochemistry was applied on paraffin sections from the brain to elicit sites antigenic to glial fibrillary acidic protein (GFAP). To that end a primary antibody (DAKO polyclonal antibody Z334, 1:200) was used. Other immunohistochemical markers applied to the samples from basal cerebral arteries and cerebral microvessels included antisera to a smooth muscle actin (DAKO mouse antihuman smooth muscle actin, 1:200), collagen type IV (Chemicon mouse antihuman collagen IV, 1:300), and collagen type V (Southern Biotechnology goat antihuman collagen V, 1:300).

Cryostat sections from muscles were stained with hematoxylin and eosin, the van Gieson technique, Gomori trichrome stain, reduced nicotinamide adenine dinucleotide, and adenosine triphosphatase at pH 9.4 and 4.6.⁶ Immunohistochemistry for a number of plasma membrane markers was also performed using monoclonal antibodies to dystrophin I to III (NCL-DYS 1, 2, and 3), adhalin (NCL-50DAG), β -dystroglycan (NCL-43DAG), and merosin. All reagents were obtained from Novocastra Laboratories, UK. The expression of all the different antigens was visualized with the avidin-biotin peroxidase technique (Vectastain, Burlingame, CA) using ethyl carbazole or diaminobenzidine as chromogen.

Samples for electron microscopy were also obtained from the muscle biopsy taken at the age of 14 years. The tissue was fixed in phosphate-buffered glutaraldehyde, postfixed in osmium tetroxide, and epon embedded.

Pathologic Findings

Central Nervous System. The brain weight was 1300 g. The leptomeninges, including leptomeningeal vessels, were macroscopi-

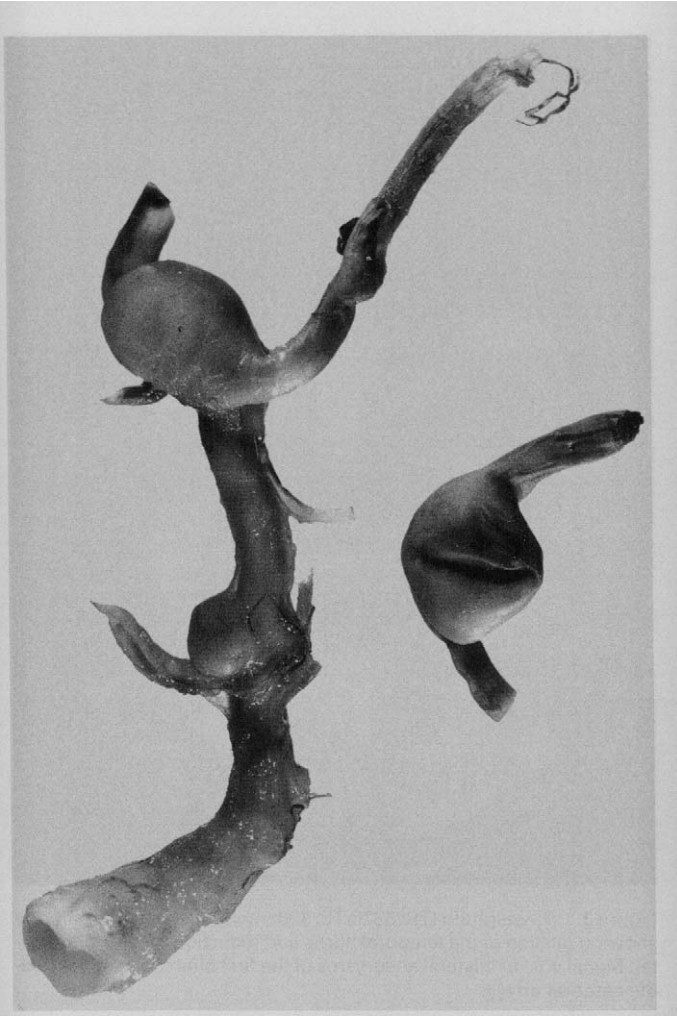


Figure 4. Postmortem specimens of the middle cerebral arteries. The left one has two saccular aneurysms located at bifurcations of the vessel. The right artery (small specimen) has one similar aneurysm.

cally normal. The middle cerebral arteries showed multiple saccular aneurysms (Figure 4) measuring up to 0.5 cm in diameter located on both sides and at the first bifurcation of the artery. In addition, a similar smaller aneurysm was located further distally at the left side. Microscopy showed characteristic lesions in the walls of the aneurysms with loss of smooth muscle cells, disorganization of remaining smooth muscle cells, splitting of the internal elastic lamina, and fibrosis with deposition of fibrillary collagens (Figure 5A). The microvessels in the cerebral parenchyma and leptomeninges showed occasional thickening of the walls of the arterioles. Such vessels had loss of smooth muscle cells and deposition of fibrillary collagens.

Macroscopically, the cerebral parenchyma (Figure 6) showed marked atrophy of the white matter with sparing of U-fibers in a few regions. The changes were symmetric in distribution. No lacunae or any other gross lesions were found. Microscopy with myelin staining showed marked loss of myelinated fibers and occasional regions with light astrocytic gliosis (Figure 5B and 5C). The cerebral cortex was normal macroscopically and microscopically with the exception of small regions with evidence of astrocytic gliosis.

The basal ganglia showed minor atrophy bilaterally. The cerebellum was slightly atrophic with widening of the sulci and narrowing of the convolutions. Microscopy revealed some loss of Purkinje cells and reduction of cerebellar white matter. Several cortical regions of the cerebellum showed increased numbers of Bergmann's glial cells and increased density of astrocytes of the molecular layer. The brain stem appeared normal. The ventricles (lateral and third) were enlarged in proportion to the atrophied white matter, the aqueduct was of normal size, and the fourth ventricle was slightly enlarged. The spinal cord had a normal configuration macroscopically. Microscopically, no obvious lesions were seen. The intradural part of the roots was normal. The extradural parts were not available.

Muscles. The biopsy from the right tibial anterior muscle taken 4 months before death showed unspecific lesions characterized by deposition of the extracellular matrix between muscle fibers and many atrophic type 1 and type 2 fibers. Immunostaining for various membrane proteins (dystrophins I to III, adhalin, β -dystroglycan, and merosin) was normal. Electron microscopy did not reveal any additional changes. The two fiber types formed a normal mosaic-like appearance and occurred at the expected frequency.

Autopsy muscle samples were removed from the quadriceps and gastrocnemius muscles. Both showed signs of myopathy characterized by occasional degenerating fibers, moderate fibrosis, large variation in fiber size, and atrophy, particularly of type 2 fibers (Figure 7A). Immunohistochemical investigation showed normal expression for dystrophins I to III, β -dystroglycan, adhalin, and merosin. There were no signs of denervation and no inflammatory cells were present.

Other Organs. At autopsy, several lesions were detected in extracerebral organs. The heart had a weight of 420 g and showed marked hypertrophy and dilation of the left side. At microscopy irregularities of muscle bundles separated by increased connective tissue were seen (Figure 7B). Signs of acute vascular congestion were present in the liver. Acute ischemic infarcts were seen in the kidneys. The renal arteries were grossly normal but microscopically smaller branches showed fibrosis of the media and intima. Similar vascular lesions were found in some pulmonary arteries. A marked pulmonary edema and evidence of bronchopneumonia and acute unilateral pleuritis were seen.

Discussion

The first case with D-2-hydroxyglutaric aciduria was described in 1980.⁷ Another seven cases were reported between 1994 and 1997.^{4,5,8-10} Recently, reports of nine more patients were collected through an international survey.³ The three patients reported by Wagner et al¹¹ are the same as patients 8, 14, and 15 in the survey. Van der Knaap et al² have now reported 25 patients; in that report our patient is briefly described as case 3. In only one study, on a 4-month-old child, was a pathologic investigation performed,⁴ and this was limited to a muscle biopsy. Histochemically, only excessive glycogen was found, but ultrastructural examination revealed subsarcolemmal cylindrical spirals and normal mitochondria.

Our case was diagnosed after the finding of massively increased concentrations of D-2-hydroxyglutaric acid in urine. Unfortunately, this finding was not made before the boy was 14 years

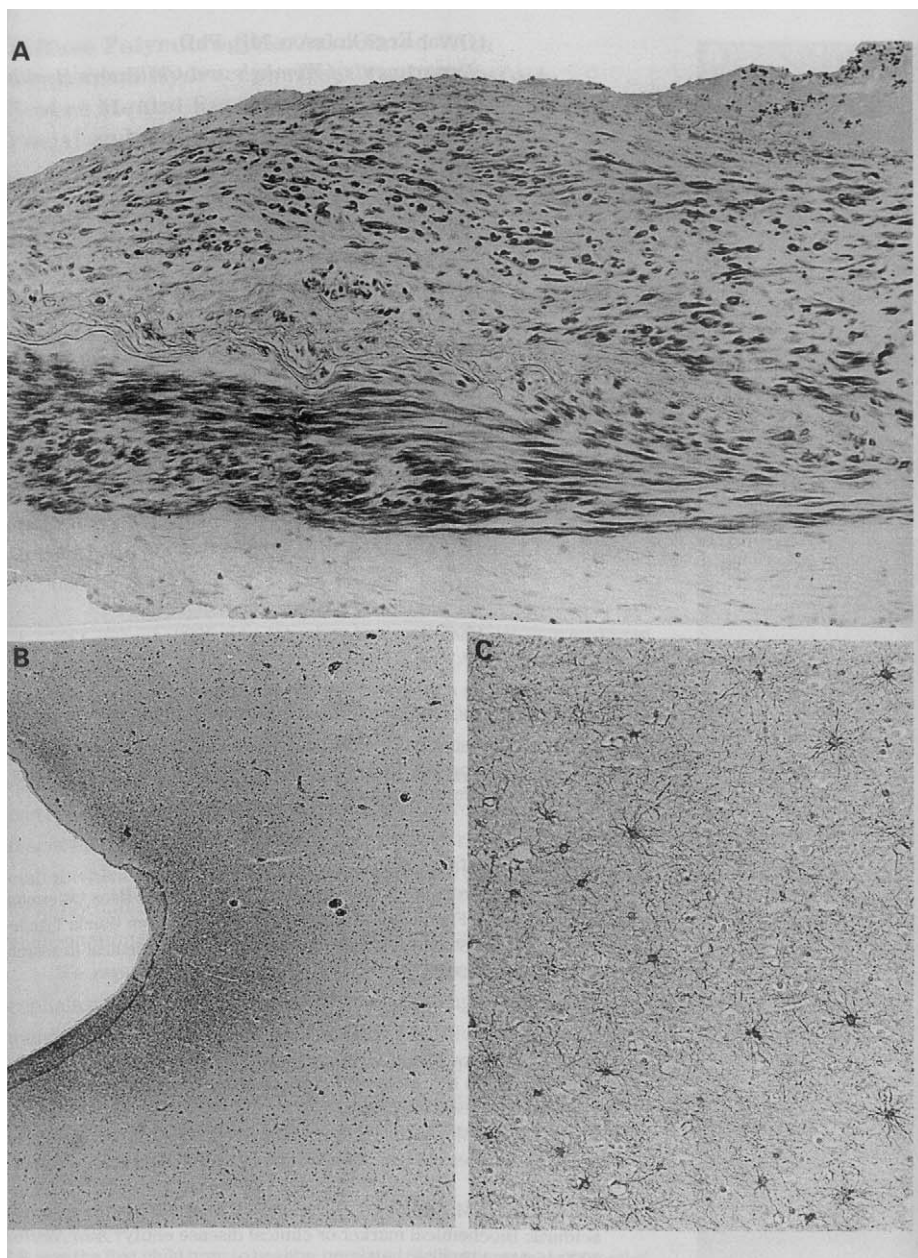


Figure 5. *A*, Longitudinal section through one of the aneurysms immunostained for smooth muscle actin. The smooth muscle cells in this part of the aneurysm are separated by an increased amount of extracellular matrix (light regions). The architecture of the wall is markedly abnormal. *B*, Section from the periventricular white matter of the cerebral hemisphere. The lateral ventricle is to the left. There is only a narrow zone of preserved white matter stained with Luxol fast blue. *C*, A few regions of the white matter show small collections of hyper-trophic astrocytes. Immunostained for GFAP, the cells appear star shaped.

old, and for several years he had been treated with valproate for his epilepsy. When this drug was withdrawn the urinary excretion of D-2-hydroxyglutaric acid decreased but was still very high.

In summary, the following symptoms and signs were found during the lifetime of our case: pyloric stenosis in the neonatal period leading to surgery, epilepsy and developmental delay from the third month, dysmorphic facial features, hypotonia with brisk reflexes and dystonia, visual defect, cardiomyopathy, skeletal muscle myopathy, arterial fibroplasia, aneurysms of the middle cerebral arteries, and general atrophy of the central nervous system with periventricular white-matter reduction. The postmortem investigation demonstrated marked white-matter atrophy of the brain with sparing of the cerebellar and spinal-cord white matter. The vascular changes with aneurysms of the middle cerebral arteries were confirmed as well as cardiomyopathy and myogenic myopathy of skeletal muscles. The patient also proved to have vascular lesions in the lungs and kidneys.

According to van der Knaap et al,² the most frequent findings in D-2-hydroxyglutaric aciduria are epilepsy, hypotonia, and psychomotor retardation. Additional findings, mainly occurring in the severe phenotype, are episodic vomiting, cardiomyopathy, and respiratory difficulties. The MRI results of the same study did not seem to be uniform, but cerebral white-matter abnormalities were the most common finding. The present case showed all these characteristics and in addition had multiple vascular abnormalities. One of the patients in the report by van der Knaap and colleagues² had multiple cerebral infarctions as revealed by CT scan. Interestingly, case 1 reported by Nyhan et al⁵ also underwent a pyloromyotomy during the first week of life.

This is the first report of a child with D-2-hydroxyglutaric aciduria in which results of a postmortem pathologic investigation have been presented. The clinical signs of the case, including affection of the brain; affection of the cardiac, pyloric, and skeletal muscles; as well as affection of the arteries, indicate a disseminated

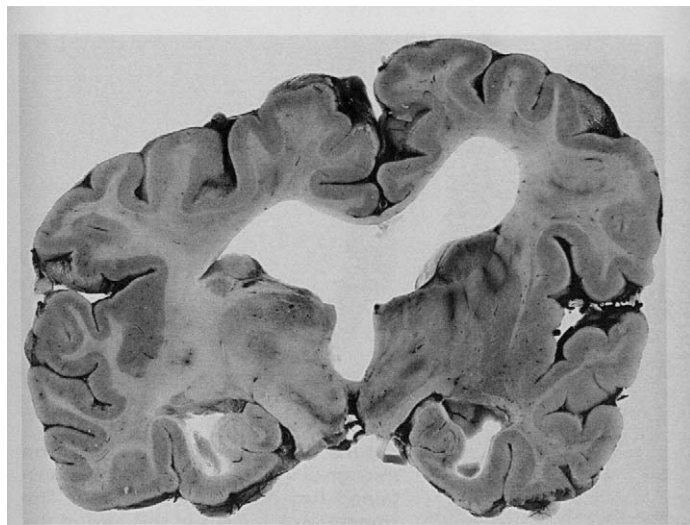


Figure 6. Parietal section of the brain. The cerebral parenchyma shows marked atrophy of the white matter, whereas the cerebral cortex is better preserved. Note the large ventricles and the thin corpus callosum.

mesenchymal process. The molecular defect underlying D-2-hydroxyglutaric aciduria, which is a well-defined metabolic disorder, is still not known.

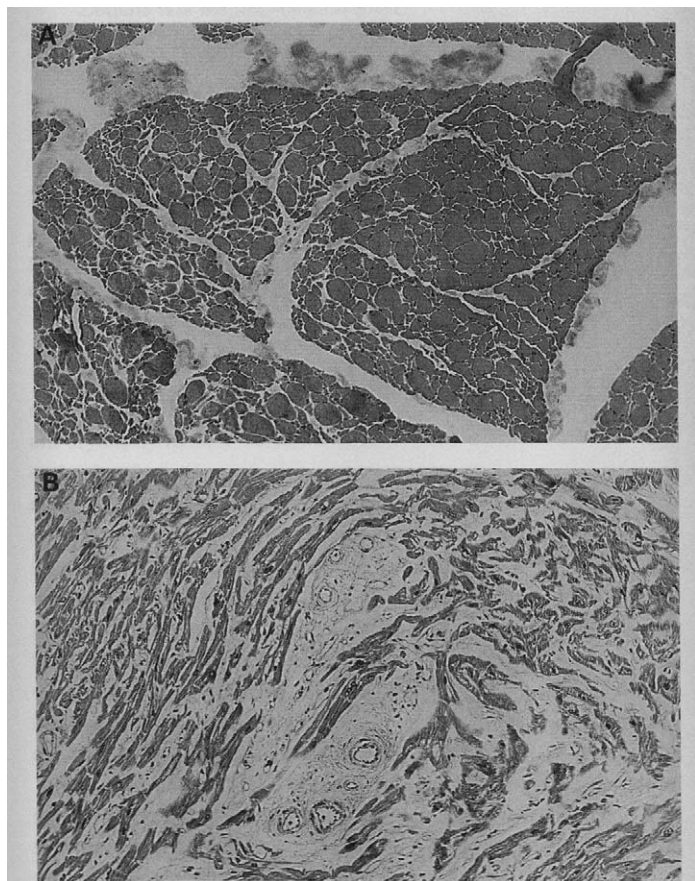


Figure 7. A, Cross section from the quadriceps muscle stained with hematoxylin and eosin. There is a large variation in fiber diameters and numerous atrophic fibers. B, Section from the myocardium stained with hematoxylin and eosin shows irregularities of muscle bundles separated by increased connective tissue (light areas).

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