Peroxisomal and Mitochondrial Defects in the Cerebro-Hepato-Renal Syndrome

Abstract. *The cerebro-hepato-renal syndrome is a rare familial malady with cerebral, renal, and skeletal abnormalities, severe hypotonia, cirrhosis, iron and lipid storage, and death within 6 months. Correlated electron microscopic, histochemical, and biochemical studies demonstrate defects in two oxidative organelles. Peroxisomes cannot be found in hepatocytes and renal proximal tubules. In hepatocytes and cortical astrocytes, mitochondria are distorted in their appearance and glycogen stores are increased. Oxygen consumption of brain and liver mitochondrial preparations with succinate and with substrates reducing nicotinamide adenine dinucleotide is markedly diminished, but the consumption is normal with ascorbate and tetramethylphenylenediamine, which suggests a defect in electron transport prior to the cytochromes. Histochemical studies of mitochondrial oxidation point to a defect between the succinate dehydrogenase flavoprotein and coenzyme Q, possibly in the region of nonheme iron protein.*

The cerebro-hepato-renal syndrome (Zellweger's disease) is a rare familial malady manifested by severe hypotonia, central nervous system abnormalities, hepatomegaly and cirrhosis, renal cortical cysts, and skeletal malformations (*1*). Afflicted infants have a characteristic facial appearance with hypertelorism, high forehead, and pursed lips. They do not thrive and usually die within 6 months. Increased tissue stores of iron (*2*) and sudanophilic lipid in white matter astrocytes (*3*) have been described in several patients, but the metabolic basis of the disorder is not understood. We studied biopsy and autopsy material from two patients, a 7-week-old boy and a 6-week-old girl, with the facial appearance and clinical signs that characterize this disorder. Correlated morphological, biochemical, and histochemical analyses of liver, brain, and kidney revealed structural and functional abnormalities in peroxisomes (*4*) and mitochondria, the two organelles principally concerned with cellular respiration.

Biopsies of liver (both patients), brain (7-week-old boy, referred to hereafter as the first patient), and kidney and muscle (6-week-old girl, referred to hereafter as the second patient) were immediately immersed in ice-cold aldehyde fixatives and processed for light and electron microscopic morphology and cytochemistry (*5*). Samples were also quick frozen in −70°C isopentane for cytochemical examination of oxidative enzymes (*6*); fresh biopsy specimens were used for biochemical studies of mitochondria and frozen autopsy tissue was analyzed for brain lipids.

Increased deposits of iron were demonstrated by the Prussian blue staining reaction in liver, spleen, skin, and brain. Excessive stores of acetone-extractable lipids that stained with oil red O were present in fibrous astrocytes and in renal tubules and glomeruli. The kidney contained scattered small cortical cysts and occasional dilated tubules. The liver was markedly fibrosed with bands of collagen disrupting the entire parenchyma. Hepatocytes were swollen with glycogen, and mitochondria were often extremely dense, attenuated, and reduced in number. Their cristae were twisted and irregular with dilation of their intracristate space (Fig. 1A). Although typical arrays of rough endoplasmic reticulum were present, the smooth endoplasmic reticulum was sparse. In cortical astrocytes, mitochondria were also unusually dense and often appeared to be degenerating.

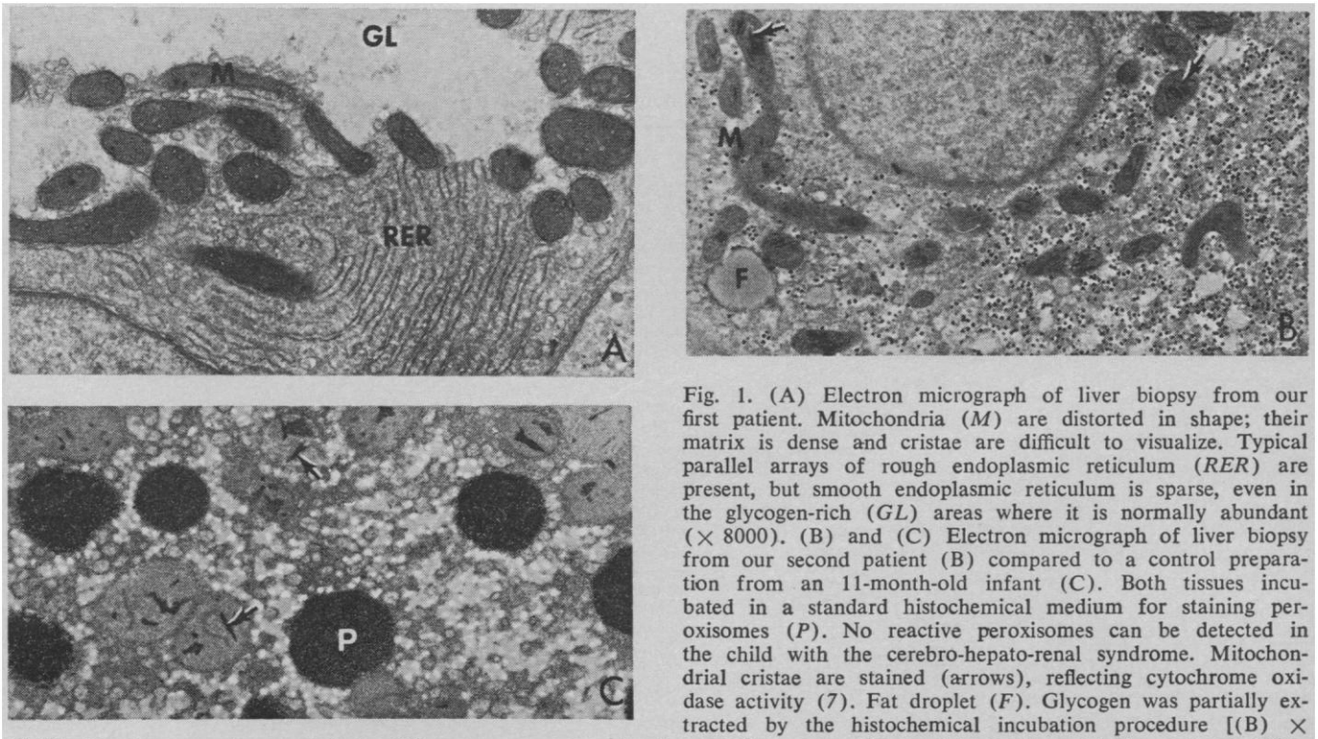

Fig. 1. (A) Electron micrograph of liver biopsy from our first patient. Mitochondria (*M*) are distorted in shape; their matrix is dense and cristae are difficult to visualize. Typical parallel arrays of rough endoplasmic reticulum (*RER*) are present, but smooth endoplasmic reticulum is sparse, even in the glycogen-rich (*GL*) areas where it is normally abundant (× 8000). (B) and (C) Electron micrograph of liver biopsy from our second patient (B) compared to a control preparation from an 11-month-old infant (C). Both tissues incubated in a standard histochemical medium for staining peroxisomes (*P*). No reactive peroxisomes can be detected in the child with the cerebro-hepato-renal syndrome. Mitochondrial cristae are stained (arrows), reflecting cytochrome oxidase activity (*7*). Fat droplet (*F*). Glycogen was partially extracted by the histochemical incubation procedure [(B) × 6000]. Reactive peroxisomes (*P*) in (C) are numerous and interspersed among mitochondria in the control specimen. Mitochondrial cristae reaction is also present (arrows) [(C) × 18,000].

No structures resembling peroxisomes could be found in hepatocytes or renal proximal tubules. This observation was further tested by incubating samples of glutaraldehyde-fixed liver and kidney in a standard cytochemical medium with diaminobenzidine for staining these organelles (*7*). This procedure demonstrates the peroxidatic activity of particulate catalase, a marker enzyme for peroxisomes (*4*). Peroxisomes were not ultrastructurally detectable in incubated tissues (Fig. 1B), although they are easily found in fetal and infant tissues prepared in this fashion (Fig. 1C). Particulate catalase could not be assessed in the frozen tissue available. Total catalase activity, however, was not reduced when compared to normal liver from a 7-month-old child. The relation between total and particulate (that is, peroxisomal) catalase in human liver is not known. In monkey liver most of the enzyme is found in the supernatant; in female rat liver almost all catalase is particulate (*8*).

Mitochondrial fractions were prepared from the brain biopsy of the first patient and the liver biopsy of the second patient according to the method of Moore and Jobsis (*9*), and respiration was monitored within 2 hours after the biopsy by the micromethod described by Holtzman and Moore (*9*). Comparison of oxygen consumption by the mitochondria from the patient and from a control (13-day-old rat) brain showed that, with succinate and with glutamate and malate, substrates reducing NAD (nicotinamide adenine dinucleotide), the rate of respiration in the cerebro-hepato-renal syndrome was diminished by 70 percent (Fig. 2). Previous studies have shown that mitochondrial fractions of normal human brain and of rat brain respire in a similar fashion (Fig. 2) (*10*). Addition of adenosine diphosphate to the patient's mitochondrial fractions failed to stimulate respiration, although this response was elicited in the control mitochondria. Oxygen consumption was similar in both preparations with ascorbate and tetramethylphenylenediamine, which transfer electrons directly to the cytochrome chain. The respiratory behavior of liver mitochondria from the biopsy of the second patient was almost identical. These data, together with the cytochemical staining of the mitochondria in the diaminobenzidine medium (Fig. 1B), suggest that the cytochrome portion of the electron transport chain is intact but that there is a defect in electron transport prior to the cytochromes.

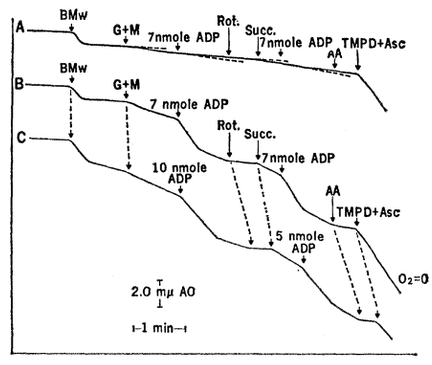

Fig. 2. Respiratory studies of brain mitochondrial preparations. Respiration was monitored with a 40-μl oxygen electrode system (*9*). (Trace A) Patient's mitochondria (patient No. 1); (trace B) control 13-day-old rat brain mitochondria studied at the same time as those shown in trace A; (trace C) control human brain mitochondria. The represented data were obtained with 0.8 to 1.0 mg of mitochondrial protein. *BMw*, Washed brain mitochondrial fraction; $G + M$, 1.5 μmole each of glutamate and malate; *ADP*, adenosine diphosphate; *Rot.*, 1 μg of rotenone; *Succ.*, 1.5 μmole of succinate; *AA*, 0.5 μg of antimycin A; *TMPD*, 2 nmole of tetramethylphenylenediamine; *Asc*, 1 mmole of ascorbate; and 2.0 mμ *AO*, 2.0 mμ atom equivalents of oxygen.

Histochemical studies demonstrated similar oxidative defects in the mitochondrial activities of brain, liver, and muscle. Cerebral tissue was compared to simultaneously assayed "control" brain biopsy specimens—a 4-month-old infant with a nonspecific astroglial proliferation and a 54-year-old woman with Alzheimer's disease. Liver was compared to normal biopsy tissue from a 7-month-old girl and muscle was compared to normal adult and to newborn biopsy material. In the cerebro-hepato-renal syndrome, succinate-tetrazolium reductase activity with menadione present as an intermediate electron acceptor (*6*) was virtually absent in brain and muscle, and was markedly reduced in liver. When phenazine methosulfate was substituted for menadione as the intermediate acceptor, the patient's tissues stained more nearly like the controls. Cytochrome oxidase activity, assayed histochemically only in muscle, was normal. Phenazine methosulfate accepts electrons directly from the succinate dehydrogenase flavoprotein and transfers them to the tetrazolium, whereas menadione is believed to pick up electrons later in the transport chain, probably at the level of coenzyme Q. The difference between the succinate reactions with the two intermediate acceptors and the presence of normal cytochrome oxidase activity complements the biochemical data and suggests that the defect is between the flavoprotein and coenzyme Q, possibly in the region of nonheme iron protein. Nonheme iron is present in the early portion of the chain where it functions to transfer electrons to coenzyme Q from such flavoprotein enzymes as reduced NAD dehydrogenase and succinate dehydrogenase (*11*). Whether the postulated block in the region of nonheme iron protein is related to the increased iron stores in this disease is not known.

Biochemical studies of brain lipids revealed the presence of abnormally large amounts of a neutral lipid (14 percent compared to a normal value of 0.5 percent) in the white matter of both biopsy tissue and that obtained post-mortem. It was identified as cholesterol ester by thin-layer and gas chromatography and undoubtedly is the oil red O–stained material found in the brain.

Peroxisomes have been identified in liver, kidney, protozoa, yeasts, and plants. Similar structures have been described in a variety of cells, including glia of spinal cord and dorsal root ganglia (*12*). It is difficult, if not impossible, to unequivocally demonstrate the absence of an organelle by morphologic studies. However, if any peroxisomes are present in the cerebro-hepato-renal syndrome, our observations indicate that they must be greatly reduced in number or altered in form and enzymatic activity. Studies of rodent liver and intestine suggest that peroxisomes are formed from, and may be connected to, smooth endoplasmic reticulum (*13*). The scarcity of smooth reticulum in these livers may therefore be related to the peroxisomal deficiency in the cerebro-hepato-renal syndrome.

Although the physiologic role of peroxisomes is not understood they appear to be involved in lipid metabolism (*14*). Serum triglycerides and cholesterol are reduced in a mutant strain of mice with a fragile peroxisomal catalase that displays increased peroxidatic activity (*15*). In germinating bean seedlings (*16*) and in *Tetrahymena* (*17*), peroxisomes have a key role in gluconeogenesis from fat. Whether the peroxisomal abnormalities are related to the lipid accumulations

in the cerebro-hepato-renal syndrome is not known.

A direct link between peroxisomes and mitochondria exists in plants (*18*), yeast (*19*), and protozoa (*17*). In these cells peroxisomes synthesize succinate which is further metabolized by mitochondria. This pathway has not been demonstrated in higher organisms, and the relation, if any, between the defects in these organelles in the cerebro-hepato-renal syndrome is not clear. The mitochondrial defect could represent lack of an element, presence of an abnormal constituent, or the effect of an inhibitor (*11*).

Volpe and Adams (*3*) have suggested that a neuronal migration defect is the fundamental one in the cerebro-hepato-renal syndrome. Our studies, demonstrating organelle pathology in brain, liver, kidney, and muscle point to an underlying subcellular defect in this inherited disorder.

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Velocity and Displacement Responses in Auditory-Nerve Fibers

Abstract. *With the help of nonsinusoidal acoustic stimuli, it is demonstrated that most fibers of the auditory nerve respond to both displacement and velocity of the basilar membrane. Except at very high stimulus levels, motion and displacement toward scala tympani produce excitation; motion and displacement toward scala vestibuli produce inhibition. The displacement and velocity responses interact. When both are excitatory or inhibitory, they reinforce each other; when they are of opposite nature, a partial cancellation occurs. The presence of both displacement and velocity responses in the single fibers suggests that outer and inner hair cells of the cochlea interact.*

Transduction of acoustic signals into neural potentials, which takes place in the cochlea of the mammalian auditory system, is effected by two populations of sensory cells—the inner and outer hair cells. There are about three times as many outer hair cells as there are inner hair cells, but about 90 percent of all afferent fibers of the auditory nerve end on the latter (*1*). This means that practically all single-unit recordings from the nerve concern fibers innervating the inner hair cells. The role of the more numerous outer hair cells remains obscure. There is no clear anatomical evidence of interconnections between outer and inner hair cells; however, some structural relationships suggest that one could exist. The number of fibers innervating the outer hair cells appears to be equal to the number of inner hair cells. These fibers course in close proximity of the inner hair cells and join the bundles of fibers innervating these cells. An interaction between the inner and outer hair cells is also suggested by experiments with ototoxic drugs, especially kanamycin. The drug affects the outer hair cells more strongly than the inner hair cells. In the parts of the cochlea where the former are destroyed but the latter appear intact, the sensitivity and frequency selectivity of single units in the auditory nerve are decreased (*2*). Since most of the recordings must refer to fibers ending on inner hair cells, the effect of missing outer hair cells is highly suggestive. Nevertheless, the possibility remains that relevant inner hair cells are also affected in these experiments without evident histological changes.

Experiments of Dallos and his coworkers (*3*) on cochlear microphonics suggest a way of testing the possibility of an inner-outer hair-cell interaction in normal mammalian ears. They found that, in such ears, the cochlear microphonics are approximately proportional to the displacement of the basilar membrane. After kanamycin treatment, the displacement microphonic disappears in the sections of the cochlea where the