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Striated adrenocortical cells in cerebro-hepato-renal (Zellweger) syndrome *

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Summary. Adrenal glands from eight patients with the cerebro-hepato-renal syndrome, a disease in which there are no morphologically demonstrable peroxisomes, were studied histologically; one of the eight was also examined ultrastructurally. Seven of the eight demonstrated striated adrenocortical cells in the inner portion of the adrenal cortex. Ultrastructural examination confirmed that the striated cells contained the lammellae and lamellar-lipid profiles of very long chain fatty acids-cholesterol esters that are characteristic of adreno-leukodystrophy. This morphologic observation further emphasizes the common pathogenetic features of the cerebro-hepato-renal (Zellweger) syndrome and adreno-leukodystrophy.

Key words: Cerebro-hepato-renal syndrome – Peroxisomes – Fatty Acids – Adreno-leukodystrophy – Adrenal Cortex – Zellweger – Schilder

Introduction

Striated, often ballooned, adrenocortical cells are recognized as a pathognomonic feature of adreno-leukodystrophy (ALD) (Powers and Schaumburg 1973). Striations reflect the storage of acetone-insoluble, very long chain (>C24) fatty acids, primarily esterified to cholesterol (Johnson et al. 1976; Igarashi et al. 1976). This adrenal lesion has been found in all types of ALD; juvenile and adult (Schaumburg et al. 1975), adrenomyeloneuropathic variant (Schaumburg et al. 1977), fetal (Powers et al. 1982), and even the distinctive neonatal form (Ulrich et al. 1978; Manz et al. 1980). Three recent

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lines of evidence have suggested a link between ALD and cerebro-hepato-renal (Zellweger) syndrome (CHRS) (Goldfischer 1982; Brown et al. 1982). The first relates to the observation that peroxisomes are absent in tissues from patients with CHRS (Goldfischer et al. 1973a, b); rat liver peroxisomes are responsible for the degradation of very long chain fatty acids (Kawamura 1981; Osmundsen 1982). Second, phenotypic similarities have been identified in neonatal ALD and CHRS (Brown et al. 1982). Third, cultured skin fibroblasts, plasma and selected tissues of patients with CHRS contain large amounts of very long chain fatty acids (Brown et al. 1982). Consequently, we have reevaluated adrenal sections from eight infants who died because of the CHRS. We wish to report the presence of striated cells and cytoplasmic inclusions that are morphologically identical to those of ALD.

Material and methods

Formalin-fixed, paraffin embedded sections of adrenal gland from eight infants with clinical and pathologic features of CHRS were examined with hematoxylin-eosin or Verhoff Van Gieson stains. Clinical and pathologic details of one of the cases have been previously reported (Patient No. 2 in Goldfischer et al. 1973). Four cases were generously provided by Dr. A.J. McAdams of the Department of Pathology at the University of Cincinatti and one case by Dr. A.E. Chudley of Children's Hospital, Winnipeg, Manitoba. The remaining case, from Johns Hopkins Hospital, was fixed in 3% buffered glutaraldehyde, post-fixed in 1% buffered osmium tetroxide, dehydrated in graded alcohols and embedded in araldite. One micron, semi-thin sections were stained with toluidine blue; thin sections were stained with uranyl acetate and lead citrate and examined in a Phillips 301 electron microscope. All cases exhibited clinical and pathologic signs of CHRS: facial dysmorphism, generalized hypotonia, psychomotor retardation, seizures, cortical renal cysts, hepatic fibrosis and central nervous system malformations.

Results

The adrenal sections usually did not contain medullary tissue. The gross architecture of the adrenal cortex was within normal limits and without evidence of atrophy. Striated cells, some of which were ballooned, were present in the reticularis-inner fasiculata of 7 of the 8 adrenals (Fig. 1). Autolysis prevented an unequivocal interpretation of the eighth case. There were two types of striated cells. The commonest, but still infrequent in comparison to juvenile, X-linked ALD, appeared to be adrenocortical parenchymal cells which occurred within normal trabeculae and were separated by sinusoidal spaces. The other, and more eosinophilic, cells had contracted cytoplasm often with an eccentric, small nucleus; they appeared to be located either within sinusoids or between parenchymal cells and sinusoidal lumina. Ultrastructural examination of one adrenal confirmed the presence of lamellae with 2.5 nm leaflets and lamellar-lipid profiles lying free in adrenocortical parenchymal cells (Fig. 2). Many non-parenchymal cells contained membrane-bound spicular inclusions as detailed in a prior communication (Powers et al. 1982).

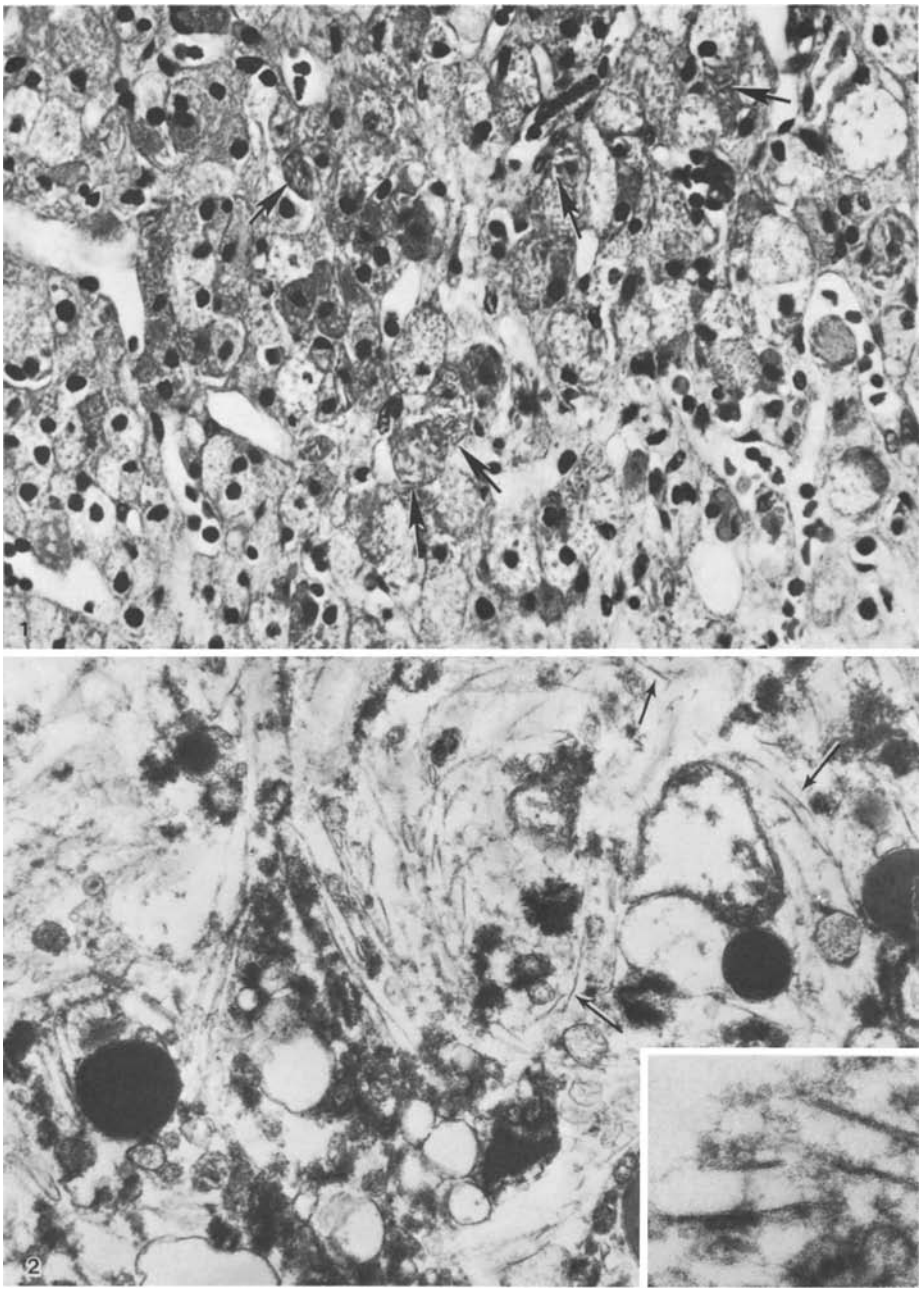

Fig. 1. Striated parenchymal cells and macrophages (*arrows*) are observed within the inner portion of adrenal cortex. H & E × 600

Fig. 2. A striated, partially autolyzed, parenchymal cell contains lamellae and lamellar-lipid profiles (*arrows*). *Inset:* Higher magnification of lamellae shows that they are identical to those previously detected in the juvenile, X-linked and neonatal forms of ALD (e.g. Figs. 13–15 in Powers et al. 1980; Figs. 1 and 2 in Schaumberg et al. 1974; Fig. 7 in Haas et al. 1982; Fig. 5 in Jaffe et al. 1982). Counterstained with uranyl acetate and lead citrate. (× 50,000, × 129,000)

Discussion

Striated adrenocortical cells, previously considered to be pathognomonic of adreno-leukodystrophy, have now been identified in the adrenal cortex of infants with CHRS. The striations reflect the presence of very long chain fatty acids that accumulate in these patients. The manifestations of CHRS, which appears to be inherited as an autosomal recessive disorder, are seen soon after birth and include profound hypotonia, a characteristic facial appearance with hypertelorism, high forehead and pursed lips, hepatic fibrosis and hemosiderosis, renal cortical cysts, CNS abnormalities including polymicrogyria and defective neuronal migration and minor skeletal abnormalities (Opitz et al. 1969; Volpe and Adams 1972). Patients with CHRS have an extraordinary defect in the biogenesis of peroxisomes; these ubiquitous organelles cannot be detected by cytochemistry and electron microscopy in hepatocytes and renal proximal tubular epithelium, where they are normally most abundant (Goldfischer et al. 1973a, b; Versmold et al. 1977; Pfeifer and Sandhage 1979; Müller-Höcker et al. 1981). In the rat, peroxisomes are responsible for the oxidation of very long chain fatty acids (Kawamura 1981; Osmundsen 1982). Our observations in the CHRS suggest that this also occurs in human tissues.

Unlike the very long chain fatty acids in ALD (Johnson et al. 1976) the brain lipids in one patient with CHRS (case No. 1 in Goldfischer et al. 1973a) were not birefringent and were totally extracted by acetone (ABJ, unpublished observation). Extraction studies have not been performed on adrenal lipids in CHRS and their solubility has not been determined.

In our patients with CHRS we could not determine whether the affected parenchymal adrenocortical cells were persistent fetal zone cells or emerging permanent zone cells. In view of the young age (usually less than 6 months) of these children at death, the marked involvement of the fetal zone in ALD and the apparent decrease in the number of striated parenchymal cells with increased postnatal survival, these cells are tentatively identified as persistent fetal zone cells. This impression must await morphologic confirmation of fetal zone involvement in CHRS. The more eosinophilic cells with contracted cytoplasm are probably macrophages, which are attempting to dispose of effete striated cells. Neonatal ALD adrenals similarly demonstrate small numbers of striated cells (Manz et al. 1980; Benke et al. 1981; Jaffe et al. 1982) when compared to the juvenile or adult cases (Powers and Schaumburg 1973). Striated cells in neonatal ALD adrenals also consist of both affected parenchymal cells and macrophages. The same striated macrophages have also been detected by one of us (JMP) in the adrenal of an hyperpipecolatemic infant (Gatfield et al. 1968), but they are very rare. Elevated concentrations of pipecolic acid have been reported in blood and urine of patients with CHRS (Danks et al. 1975).

Although the clinical and genetic aspects of the juvenile and neonatal forms of ALD differ, these two forms share major pathologic and chemical features. The increased concentrations of very long chain fatty acids that are present in blood and tissues suggest that deficiency or inhibition of a peroxisomal oxidase is a central pathogenetic factor in all forms of ALD.

Juvenile ALD is a X-linked disorder affecting young boys, usually 5–15 years of age, with progressive deterioration of the CNS and adrenocortical hypofunction. The distinction between juvenile ALD and CHRS is clear, but the situation in regard to CHRS and neonatal ALD is less well defined. In a detailed study of three patients with CHRS and five with neonatal ALD, elevated levels of very long chain fatty acids were detected in all cases; hypotonia, polymicrogyria, cortical heterotopia and severe hepatic fibrosis were also present in patients with both syndromes (Brown et al. 1982). However, the skeletal abnormalities, renal cysts and iron storage that are present in CHRS have not been described in neonatal ALD (Ulrich et al. 1978; Manz et al. 1980; Haas et al. 1982; Jaffe et al. 1982); adrenal atrophy is not a feature of CHRS. Children with neonatal ALD live much longer (up to five years) than those with CHRS and have far more abundant stores of very long chain fatty acids, with a widespread distribution, including Kupffer cells. Fatty acids storage in Kupffer cells has not been detected in CHRS. Partin and McAdams (1983), who believe that CHRS and neonatal ALD differ clinically and pathologically, have reported the absence of peroxisomes in two siblings with neonatal ALD. The present report establishes another common feature and emphasizes the need for continued investigation to aid in the nosologic and chemical delineation of these two diseases. It is now apparent that neither the inability to detect peroxisomes nor the presence of striated inclusions of very long chain fatty acids is sufficient to establish the diagnosis of either CHRS or neonatal ALD.

Infants with CHRS exhibit not only a peroxisomal deficiency, but also defective mitochondrial respiration (Goldfischer et al. 1973a, b; Versmold et al. 1977). In one patient with CHRS, studied histochemically and biochemically, succinate dehydrogenase was reduced, but alpha glycerophosphate dehydrogenase was normal (Goldfischer et al. 1973a, b). It is particulary noteworthy that enzyme histochemistry of adrenocortical cells in three young boys with the juvenile, sex-linked form of ALD demonstrated a consistent depression of mitochondrial alpha glycerophosphate dehydrogenase activity; succinate dehydrogenase was not affected (Powers et al. 1980). Interactions between peroxisomes and mitochondria have been detected in various phyla. Participation in fatty acid metabolism is a property of both organelles in plants, protozoa and vertebrates. Peroxisomes appear to shorten very long chain fatty acids prior to their oxidation by mitochondria (Osmundsen 1982). In plant cells and protozoa, succinate is synthesized by peroxisomes (glyoxysomes) and transferred to mitochondria, but this phenomenon has not been detected in vertebrate tissues (see Kindl and Lazarow 1982 for a review)[1]. Further enzymatic and morphological studies of peroxisomes and mitochondria in CHRS and ALD may contribute to a more precise characterization of these syndromes and also provide new information on metabolic links between these respiratory organelles.

[1] Recent studies have demonstrated two enzymes of the glyoxylate cycle, isocitrate lyase and malate synthetase, in peroxisomes of the chick yolk sac (Baumbach et al. 1980) and the toad bladder (Jones et al. 1982) indicating that succinate and malate may be supplied to mitochondria by peroxisomes in some vertebrate tissues

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