Neonatal Adrenoleukodystrophy

Clinical, Pathologic, and Biochemical Delineation of a Syndrome Affecting Both Males and Females

R. JAFFE, MB, BCh, P. CRUMRINE, MD, Y. HASHIDA, MD, and H. W. MOSER, MD

From the Departments of Pathology and Pediatrics, University of Pittsburgh School of Medicine, Children's Hospital of Pittsburgh, and the J. F. Kennedy Institute, Baltimore, Maryland

We describe the detailed clinical, pathologic, and biochemical features of brother and sister with the neonatal onset form of adrenoleukodystrophy, together with evidence of the biochemical defect. When compared with reports of previous cases, it becomes clear that this is a newly described clinical entity with remarkable uniformity of signs and very different from the usual childhood form. Some pathologic features are shared, including the morphologic abnormality of the adrenal in both neonatal and childhood forms, but deposition of abnormally metabolized lipids is more systemic and widespread in the neonatal form. The biochemistry of the disease is presented in both children and parents. Plasma values of long-chain fatty acid C26:0 are 0.328 ± 0.18 μg/ml in a control population and 0.381 ± 0.312 μg/ml in the father and mother. Values for C26:0 in the plasma of childhood adrenoleukodystrophy are 1.62 ± 0.87 μg/ml and in our two cases, 2.79 μg/ml in the male, 1.83 μg/ml in the female. The basic biochemical defect appears to be a diminished capacity to oxidize these fatty acids leading to accumulation in cholesterol esters. Fatty acid oxidation to CO_2 by cultured skin fibroblasts was 51% of control value for stearic acid, 5% for lignoceric acid in the male, and 39% of control value for stearic acid, 5% for lignoceric acid in the female. The genetics of this disease is different; whereas childhood adrenoleukodystrophy is X-linked, the neonatal onset form affects males and females equally and is most probably autosomally recessive in inheritance. (Am J Pathol 1982, 108:100–111)

ADRENOLEUKODYSTROPHY (ALD) is a metabolic disorder in which unbranched very long-chain fatty acids of 24–30 carbon atoms accumulate in a variety of human tissues.[1,2] Some of the fatty acids are derived from the diet; much is probably endogenously produced.[3] Recent studies have shown the basic biochemical defect to involve a diminished capacity to oxidize these fatty acids,[4] leading to accumulation of the fatty acids in cholesterol esters extractable from lesions in the white matter of the brain and adrenals.[5] The demonstration of excess hexacosanoate in cultured fibroblasts[6,7] and plasma[8] promises to be a useful diagnostic procedure.

The clinical disorder can present in a number of forms. Classic adrenoleukodystrophy affects young boys toward the end of the first decade with progressive deterioration of motor and intellectual function, deteriorating vision, and often adrenocortical hypofunction.[1] This clinical pattern was first described by Siemerling and Creutzfeldt in 1923, and the eponym survives in the European literature,[9] as does the term "sex-linked Schilder's disease."[10] This clinical form has clearly been shown to be X-linked.[5,11] Adrenomyeloneuropathy is seen later, in young adults, with peripheral neuropathy, sphincter disturbance, spastic paraparesis, and often adrenal insufficiency.[12] The two clinical forms have occurred

Supported in part by Grants HD 10981 and NS 10885, 13513 from the U.S. Public Health Service and by Grant 6-271 from the National Foundation—March of Dimes.

Case 1 was presented at the Pediatric Pathology Club Annual Meeting, March 1978, Atlanta, Georgia, and Case 2 at the American Association of Neuropathologists, June 1981, Vancouver, British Columbia.

Accepted for publication March 2, 1982.

Address reprint requests to Ron Jaffe, MD, Children's Hospital of Pittsburgh, 125 DeSoto Street, Pittsburgh, PA 15213.

0002-9440/82/0709-0100$01.10 © American Association of Pathologists

within the same families, and female carriers of adrenomyeloneuropathy may have progressive paraparesis.[5] Selective favoring of the mutant allele may account for clinical symptoms in females carrying the X-linked defect.[11]

More recently a neonatal-onset form of the disease has been described.[13-16] Children with this form of the disease have profound hypotonia and severe seizure disorders, but biochemically evident adrenocortical hypofunction has been described in only one of the six cases thus far reported. We present two further neonatal onset cases in a brother and sister and describe the clinical, pathologic, and biochemical features of this newly described syndrome. Concordance of features in the described cases is high, making this a clearly recognizable clinical-pathologic entity, different from the usual childhood form in clinical and possibly genetic respects, but similar in major pathologic and biochemical findings.

Clinical Cases

Case 1

J.S. was the term product of an uncomplicated pregnancy to a 26-year-old primigravida. The labor was 11 hours in duration, and the delivery was vaginal under a pudendal block. The 5-minute Apgar score was 7. Birth weight was 2.95 kg. The initial examination revealed an infant who was floppy, had a shrill cry, and was macrocephalic with a head circumference of 38 cm (98th percentile). The child was transferred to Children's Hospital of Pittsburgh (CHP) at 2½ days of age for further evaluation. Additional findings on this admission (CHP) included a café-au-lait spot 1.5 × .75 cm on the abdomen, a liver 3 cm below the costal margin, micrognathia and right cryptorchidism. There was no Moro, suck, tonic neck, stepping or placing reflex elicited. A skull X-ray revealed no abnormalities. A generalized tonic seizure was observed on admission. There was no previous family history of seizures and neurologic disease. Because of concern about an intrauterine infection, TORCH titers were obtained from the infant and mother. The results of the mother's serologic test was positive for toxoplasmosis with IgM of 1:28; the infant's were negative at 1:16 dilution. The results of other antibody studies for cytomegalovirus, herpes, rubella, and syphilis were negative. Serum lactate and pyruvate were normal, as was a urine assay for inborn errors of metabolism. Nerve conduction velocities were obtained at 8 and 27 days of age. On both occasions the velocities were decreased below normal with a left peroneal reading of 11 and 14.2 meters per second (mps) and a left posterior tibial nerve reading of 11.9 and 20.5 mps. Distal peroneal latency readings were 4.4 and 3.4 msec and readings for the posterior tibial were 9.7 and 4.7 msec.

Seizures developed at 2 days of age and were refractory to standard anticonvulsant medications, including phenobarbital, phenytoin, primidone, diazepam, and pyridoxine. Electroencephalograms (EEGs) were persistently abnormal, showing focal and multifocal spike discharges. The infant was discharged at 7 weeks of age on phenobarbital, primidone, and carbamazepine. At the time of discharge, he remained profoundly hypotonic and showed no developmental progress. Seizures persisted following discharge from the hospital. At about 3–4 months of age he developed infantile spasms and had a burst-suppression pattern on the EEG; clonazepam was added to the anticonvulsant regimen.

Development was minimal. He grew along the 50th percentile for height and weight, reaching 10.5 kg and 80 cm at 14 months of age. His head circumference at 52 cm was still above the 98th percentile. His only acquisitions were rolling from supine to prone and smiling; these were achieved at 7 and 9 months, respectively, and were lost shortly thereafter. He never acquired any degree of head control and remained hypotonic throughout his life. Responses to auditory and visual stimuli were minimal; funduscopic examination with indirect ophthalmoscopy revealed small, pale disks. A follow-up computerized axial tomogram (CT) was normal at 15 months.

Seizures persisted with no evidence of developmental progress and no further growth. He was found dead in bed at home at 17 months.

Case 2

R.S. was the sister of J.S. The mother was pregnant at the time that the diagnosis of ALD was made at autopsy on J.S. She declined amniocentesis. The pregnancy, labor, and delivery were uncomplicated. Apgar scores at birth were 8 and 9 at 1 and 5 minutes, respectively. Mild hypotonia and a shrill cry were noted on the newborn examination. Following the development of seizures at 22 hours of age, she was transferred to Children's Hospital of Pittsburgh. There, at 30 hours of age, her vital signs were stable; the height and weight were at the 98th and 35th percentile, respectively, and the head circumference was slightly above the 98th percentile at 36.5 cm. The general physical examination showed a cataract and an enlarged liver. Neurologic examination revealed diffuse hypotonia, poor Moro, suck, and grasp reflexes, and diminished deep tendon reflexes. Several

generalized tonic seizures were observed during the initial examination.

Laboratory studies, including complete blood count, electrolytes, calcium, glucose, venous blood gases, and serum amino acids were normal. The cerebrospinal fluid (CSF) protein was 130 mg%, and CSF lactate and pyruvate levels were elevated, with normal serum values. The electroencephalogram showed focal sharp waves over the bitemporal areas. Nerve conduction velocities were mildly decreased. A CT scan showed a mild decrease of white matter.

Seizures were poorly responsive to anticonvulsants and were decreased, but not controlled, at therapeutic levels of mysoline, phenobarbital, tegretol, and pyridoxine. Lysosomal enzymes from a skin biopsy showed mildly decreased levels for arylsulfatase A and B, sphingomyelinase, hexosaminidase A, and total hexosaminidase, compared with controls. This was not felt to be significant. Glucuronidase was five times lower than the control values. Assays for glucuronidase on the mother and father were normal. Lysosomal enzymes obtained from both parents were normal. A fasting cortisol level was obtained and was reported as low.

Seizures continued to be a problem for this infant following hospital discharge at 3 weeks of age. Clonazepam and phenytoin were added to the anticonvulsant regimen; carbamazepine was discontinued because of elevation in hepatic enzymes. Developmental progress was minimal during the first few months of life except for some head movement. The infant did not develop responses to auditory or visual stimuli and had progression of the hypotonia from birth.

At 4 months of age she was readmitted to CHP for evaluation of adrenal function. Cortisol levels following ACTH stimulation were normal. Repeat nerve conduction velocities and CT scan were also normal. An EEG showed multifocal spike-wave complexes with clinically recorded seizures.

The child was subsequently admitted to CHP at 5½ and 11 months of age for increased seizures and pneumonia, respectively. Physical measurements, weight, height, and head circumference were in the 95th percentile. There was progressive hepatomegaly from birth. At 5½ months there was an increase in several of the hepatic enzymes, with an SGOT of 53, SGPT of 95, LDH of 218, and GGPT of 682. The child made little to no developmental progress except for some lateral head movement and a social smile, which disappeared after 12 months. She remained profoundly hypotonic and unresponsive to auditory or visual stimuli until her death at home at 24 months of age. Her length and head circumference (90 cm, 49 cm) were still above the 95th percentile, although her weight was at this point only 12 kg (50th percentile).

Materials and Methods

Histochemical Studies

Frozen sections of both fixed and unfixed tissues from both cases were examined under polarized light for accumulations of needlelike lipid crystals resistant to acetone and ethanol extraction but soluble in chloroform.[17] Frozen tissues were stained for lipid with the use of oil red O. Lipochromes (ceroid) were identified with periodic acid–Schiff (PAS) stains after diastase digestion, Sudan black B, Ziehl-Neelsen acid-fast, and the demonstration of bright autofluorescence of unstained sections. Luxol fast blue and Woelke stains were used for myelin and the Bielschowsky stain for axons. Immunohistochemical demonstration of pituitary hormones was achieved with the use of an unlabeled peroxidase–antiperoxidase (PAP) procedure.[18] Anti-ACTH was obtained from INC Corp., Stillwater, Maine; anti-LH from Calbiochem-Behring Corp., LaJolla, Calif; and anti-human growth hormone from Wellcome Reagents, Beckenham, England. Specificity controls consisted of adjacent sections stained identically but with omission of the primary antibody, and use of antisera absorbed with purified antigen. Case controls were from age-matched autopsy subjects without evidence of adrenal disease. Diaminobenzidine (Polysciences, Worthington, Pa) was used as the chromogen.

Electron Microscopy

Tissues from Case 1 were retrieved from formalin once the diagnosis was made, diced and refixed in 2% glutaraldehyde. Tissues from Case 2 were fixed primarily in 2% glutaraldehyde, embedded in Epon-araldite, and examined on a Philips II electron microscope.

Fibroblast Cultures and Fatty Acid Determiniations

Fibroblasts were obtained from skin biopsy specimens, in Case 1, as part of the diagnostic workup of a possible metabolic disorder, and in Case 2, for confirmation of XX genotype. Both parents underwent skin biopsy. Details of the procedure for fatty acid determination have been published.[7] Briefly, fibroblasts were harvested 3–4 days after confluence, trypsinized, suspended in water, and disrupted by sonication. An aliquot was taken for protein analysis, and total lipids were extracted according to the method of Folch-Pi et al.[19] Fatty acid methyl

esters, cholesterol, and its derivatives were extracted with hexane. Residue from the extract was applied to a thin-layer chromatography plate and developed with hexane–ether 7:3. Methyl esters were eluted with hexane after spraying of the plate with bromothymol blue and drying. The area used corresponded to the standard methyl ester (16:0 and 27:0), and the hexane extract was dissolved in 100 μl of hexane; 2.0 μl was injected into a Hewlett-Packard 5880A splitless injection port onto a 12-meter SP-2100 fused silica gel capillary column with an inside diameter of 0.2 mm. Injection port temperature was 250 C; detector temperature was 300 C. The oven initial temperature was 150 C; after 2 minutes it was raised to 285 C at 10 degrees per minute and maintained there for 5 minutes. Peaks were identified by co-chromatography with authentic standards in at least three systems; the C26 fatty acid was also identified by combined mass spectrometry (Dr. Catherine Fenselau). Retention times for C20, C22, C23, C24, C25, and C26 fatty acids were 8.89, 9.62, 10.38, 11.17, 11.75, and 12.54 minutes, respectively, and were constant to within ± 0.02 minute. The peaks were measured with a Hewlett-Packard 5880 A Level 4 integrator and expressed as percentage of total fatty acids with chain length of 14 carbons or more. Standard deviations of the differences between duplicate determinations were 11–13%.

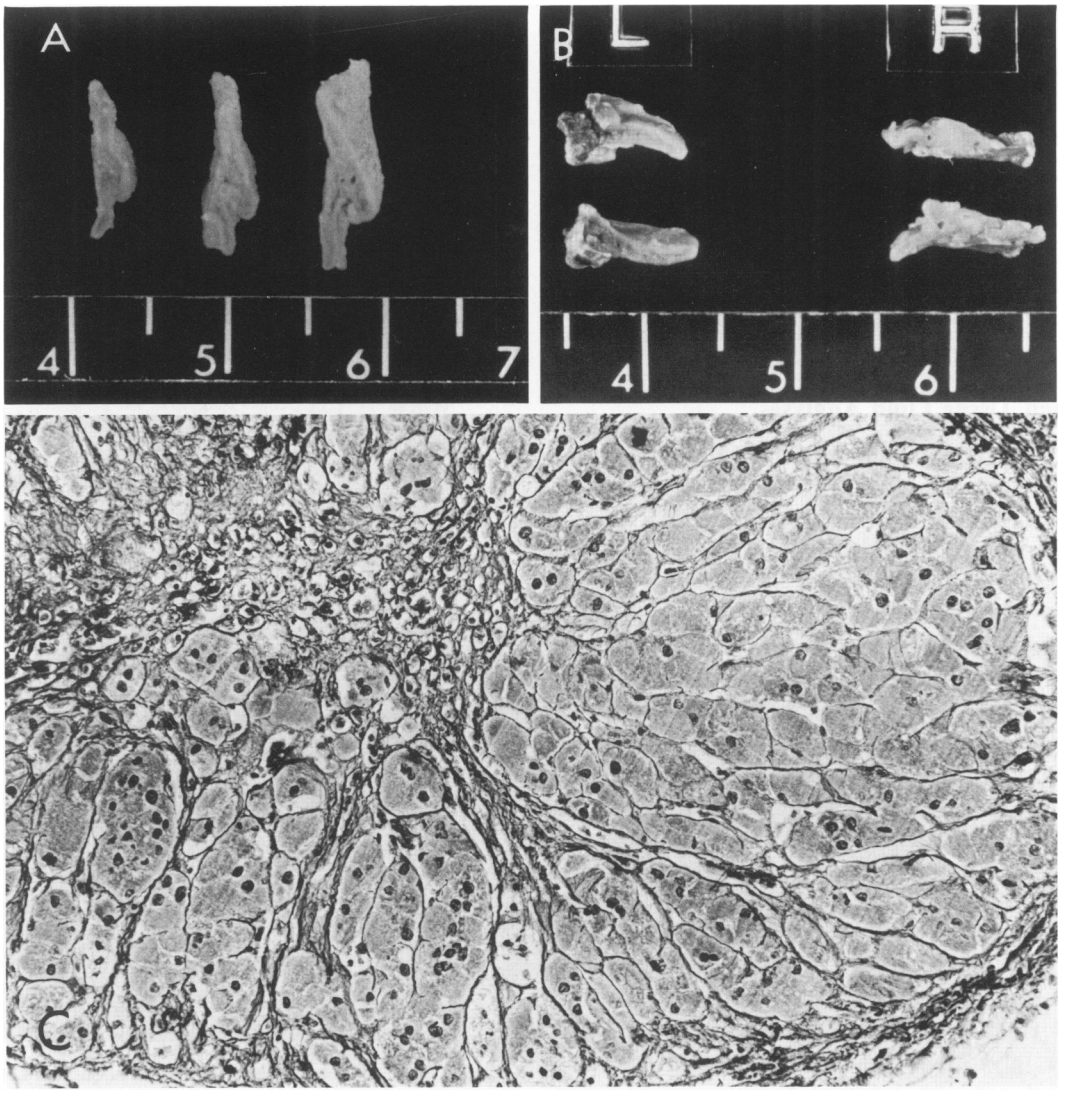

Figure 1A and **B** – Adrenal from Cases 1 and 2. The adrenal glands are tiny, measuring little more than 1 cm in their greatest dimension.
C – The adrenal cortex is occupied by vague nodules of large cells having homogeneous glassy cytoplasm. (Wilder's reticulin, ×64)

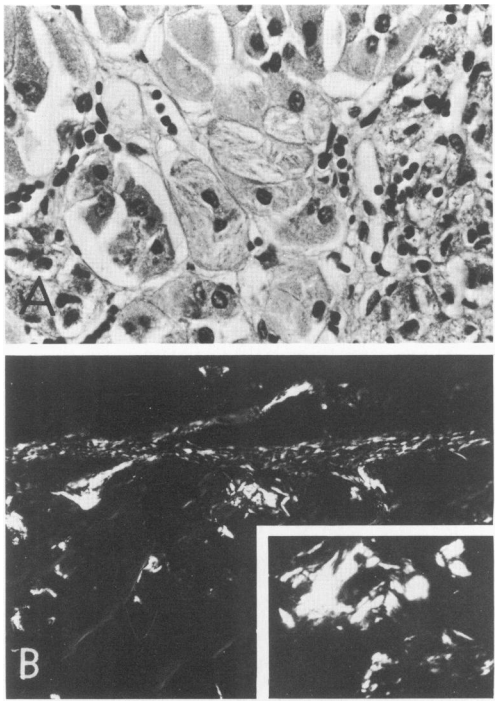

Figure 2A—Occasional cells show well-marked cytoplasmic striations where lipid material has been removed by processing through xylene. (H&E, ×300) **B**—Frozen sections of the adrenal seen through crossed polarizing lenses shows the presence of refractile accumulation of lipid crystals in the cortical cells. (×200) **Inset**—The crystalline nature of the birefringent material is apparent. (×340)

Plasma and Tissue Lipid Analyses

Total lipid extracts and fatty acid determinations were performed as above. The following procedure was used to obtain major lipid fractions. An aliquot of the washed total lipid extract was taken to dryness under nitrogen and subjected to alkaline methanolysis by the addition of 1 ml 0.21 N methanolic NaOH and 2 ml chloroform for 1 hour at room temperature. This treatment released fatty acids from triglycerides and glycerophospholipids; cholesterol esters were partially split, and sphingolipids remained intact. The extract was then subjected to Folch partition[19]; lipids in the washed lower phase were applied to a 3.0 × 0.5-cm Unisil column and eluted with 7 ml each of hexane/benzene 6:4, chloroform, acetone/methanol 9:1, and methanol. The first fraction contained fatty acid methyl esters and unhydrolyzed cholesterol esters. The acetone/methanol fraction contained mainly glycolipids; the methanol fraction, mainly sphingomyelin. Cholesterol ester, glycolipid, and sphingomyelin fractions were hydrolyzed with 1.5 ml 1 N methanolic HCl at 75 C overnight. The cholesterol ester fraction was dissolved in 50 μl benzene before methanolysis. The methyl esters were analyzed by gas–liquid chromatography as described above.

Enzymatic assays were performed as described.[4] Briefly, fatty acids labeled with 1-^{14}C in the one position were incubated at 37 C for 1 hour at pH 7 in the presence of $MgCl_2$, adenosine triphosphate, nicotinamide adenine dinucleotide, flavin adenine dinucleotide, coenzyme A-SH, and alpha cylodextrin, and measurements were made of the radioactive CO_2 produced.

Results

Pathologic Findings

Chromosome analysis on Case 2 showed a 46 XX normal female karyotype. The pathologic findings were remarkably uniform in both cases and will be described together, any differences being noted.

The adrenals weighed 1.6 g and 1.0 g in Cases 1

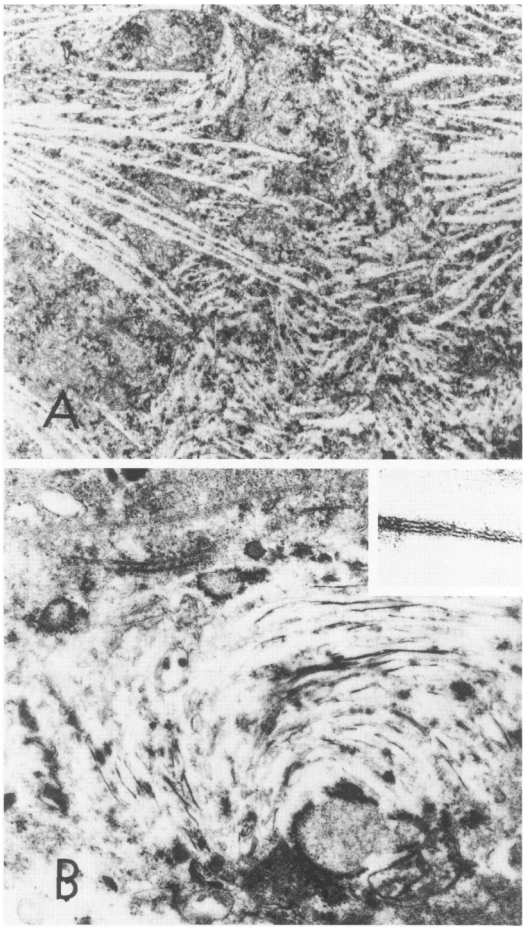

Figure 3A—Electron micrograph of adrenal cortical cell shows the needlelike clefts from which the crystalline lipid has been leached out. (×5900) **B**—Membranous whorls are separated from each other by clefts. (×17,000) **Inset**—Detail of the membranes seen in **B** to show the trilaminar structure. (×114,000)

and 2, respectively, where the expected weight for both adrenals at this age in our institution would be 4.5 g. The glands were tiny, and the cortex was replaced by vague nodules consisting of very large cells with abundant waxy-appearing cytoplasm (Figure 1). Extracapsular cortical nodules were composed of the same cells. The medulla was present and unremarkable. Normal lamination of the adrenal cortex could not be seen, although the zona glomerulosa was represented, at least in places, by nests of smaller, foamy, more normal-looking cells. Some of the large cortical cells, irregularly dispersed in the nodules, stained for lipid with oil red O; and in hematoxylin–eosin-stained sections these cells had a fibrillar, striated appearance in the cytoplasm where the lipids had been leached out. Polarization of fresh frozen sections showed the lipid to be largely in crystalline form (Figure 2). Small amounts of fibrous tissue, mononuclear inflammatory cells, and macrophages with ingested lipids were found between nodules of adrenocortical cells. The solubility of the lipid inclusions was similar to that previously described,[17] that is, the crystals resisted extraction with acetone, ethanol, and methanol but were no longer present when viewed by polarized light after immersion in chloroform, xylene, or propylene oxide. Electron-microscopic examination of the striated adrenocortical cells showed parallel and whorled lamellas separated by spaces that presumably contained the lipids before extraction (in propylene oxide) (Figure 3). In some instances, large numbers of radiating clefts were unassociated with the membranous lamellas. The lamellar-lipid profiles in both cases were not as striking nor as well formed as those seen in the more usual childhood ALD.[10] The complex bundles of lamellar inclusions seen in macrophages in other organs, as described by Manz et al[14], were not found in the adrenals.

The thymus in both cases contained a most striking enlargement of macrophages at the corticomedullary junction as well as along the fibrous trabeculas (Figure 4). These cells were often multinucleate, reached a diameter of 450 μ in the largest cells seen, and had coarsely clumped cytoplasm. Few crystalline lipid inclusions could be seen on polarized frozen sections. The cells had the staining properties of lipofuscin (ceroid). The cytoplasm was intensely PAS-positive after diastase pretreatment, was acid-fast on Ziehl-Neelsen stain, stained positively with Sudan black B, and displayed intense yellow-green autofluorescence under ultraviolet light. The crystalline inclusions could be readily extracted in xylene or chloroform, but the complex lipochrome was resistant to these solvents. Electron-microscopic examination of these cells showed bundles of lamellar inclusions, the shape of which corresponded to the coarse granulation evident on light and fluorescent microscopy (Figure 5). The packages were themselves membrane-bound, and amorphous electron-dense masses suggestive of lipofuscin were seen both within and between these

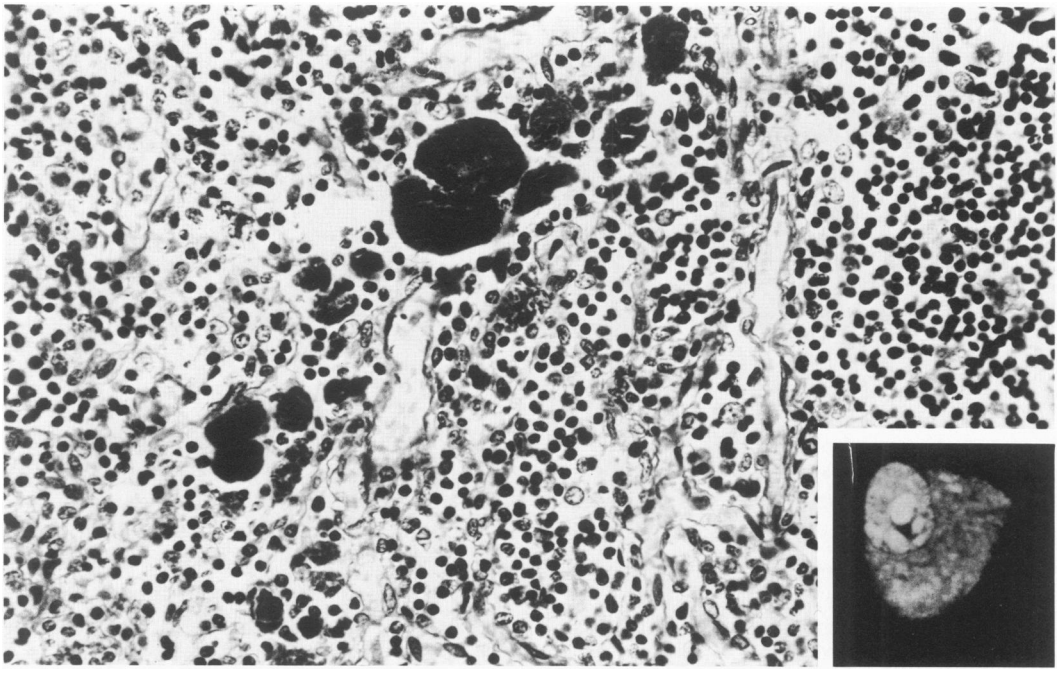

Figure 4 – The thymus contains aggregates of very large cells having the cytoplasmic staining characteristics of ceroid. (PAS, ×615) **Inset** – Autofluorescence of one of these large thymic macrophages shows the packaging of the complex lipid material in the cytoplasm. (×970)

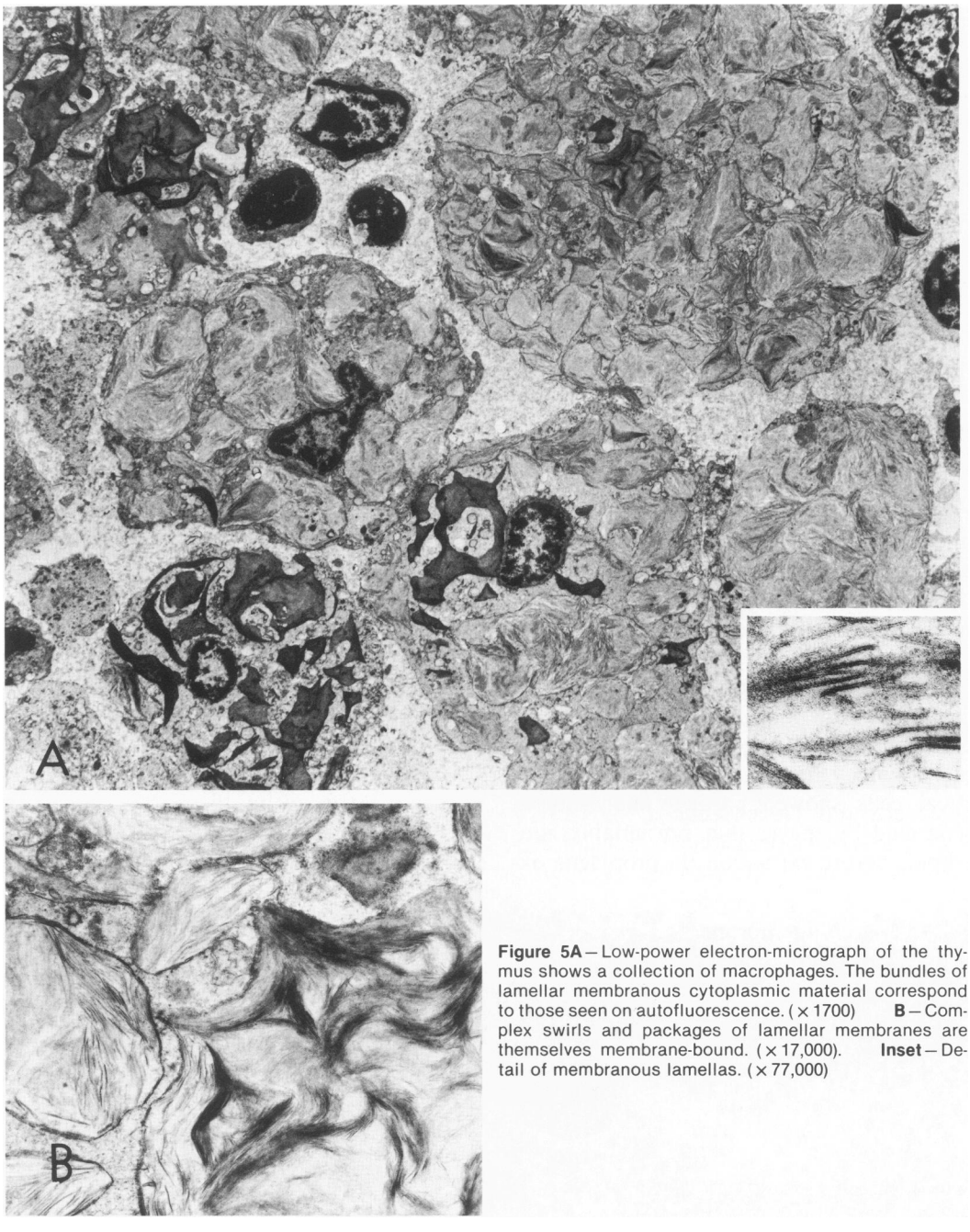

Figure 5A — Low-power electron-micrograph of the thymus shows a collection of macrophages. The bundles of lamellar membranous cytoplasmic material correspond to those seen on autofluorescence. (× 1700) **B** — Complex swirls and packages of lamellar membranes are themselves membrane-bound. (× 17,000). **Inset** — Detail of membranous lamellas. (× 77,000)

structures. These striking macrophages in the thymus are similar to those previously shown in lymph nodes[14] in a case of neonatal ALD. The thymus was otherwise normal; in fact, the cortex showed hardly any of the depletion of small lymphocytes so common in children with debilitating illness.

The liver in both children showed extensive fibrosis, with linking of adjacent portal areas and a consequent irregular nodularity (Figure 6). In contrast to previous reports,[14,15] PAS-positive macrophages (of the type seen in the thymus) were not seen in the liver in either of the 2 cases, nor were polarizable inclusions found on frozen section.

Accumulation of lipochrome within macrophages was conspicuous in both cases in the germinal centers of lymphoid tissue in lymph nodes, spleen, and even gastrointestinal tract. Skin, lung, splenic red pulp, testes, ovaries, and kidney showed no morphologic changes. The pituitary was normal by weight and as seen by light microscopy in both instances. Immuno-

staining for growth hormone, luteinizing hormone, and ACTH showed a striking prominence of ACTH-containing cells when compared with those indexes in age-matched controls.

Neuropathology

Case 1

The brain was heavy (1300 g) and showed diffuse polymicrogyria of frontal and parietal lobes. The white matter appeared sparse but without any recognizable focal lesions. The inferior olives and dentate nuclei were poorly defined. Microscopy confirmed polymicrogyria and showed cortical heterotopias in white matter, diffuse gliosis, and neuronal loss in thalamus, inferior olives, and dentate nuclei. A sharply demarcated area of rarefaction surrounded by fibrillary gliosis was present in the occipital white matter. Microcalcifications and very focal perivascular lymphocytic cuffing were minor features.

Demyelination was patchy throughout the cerebral white matter, especially posteriorly and in the cerebellum. U-fibers were spared. A diffuse gliosis with large hypertrophic astrocytes was present in these areas, but axons in the same areas were relatively spared. Occasional perivascular areas contained macrophages that stained positively for fat with the oil red O stain performed on frozen sections. Polarization of these same cells showed occasional needlelike crystalline inclusions (Figure 7). Electron-microscopic examination showed these macrophages to contain the same complex lipid and membrane aggregates seen in the thymus. Corticospinal tract demyelination and gliosis was the only finding on numerous sections of spinal cord. Peripheral nerves were unremarkable, and no inclusions were found in Schwann cells.

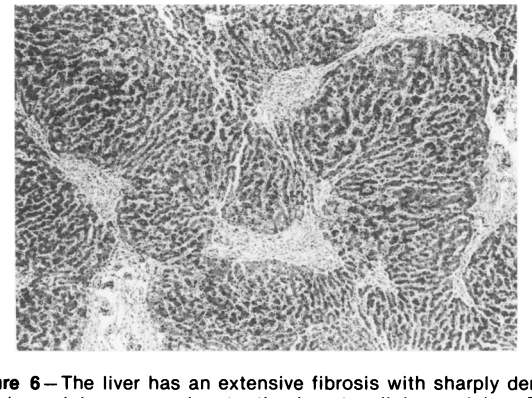

Figure 6—The liver has an extensive fibrosis with sharply demarcated serpiginous margins to the hepatocellular nodules. Both Cases 1 and 2 were identical. (Masson trichrome, ×32)

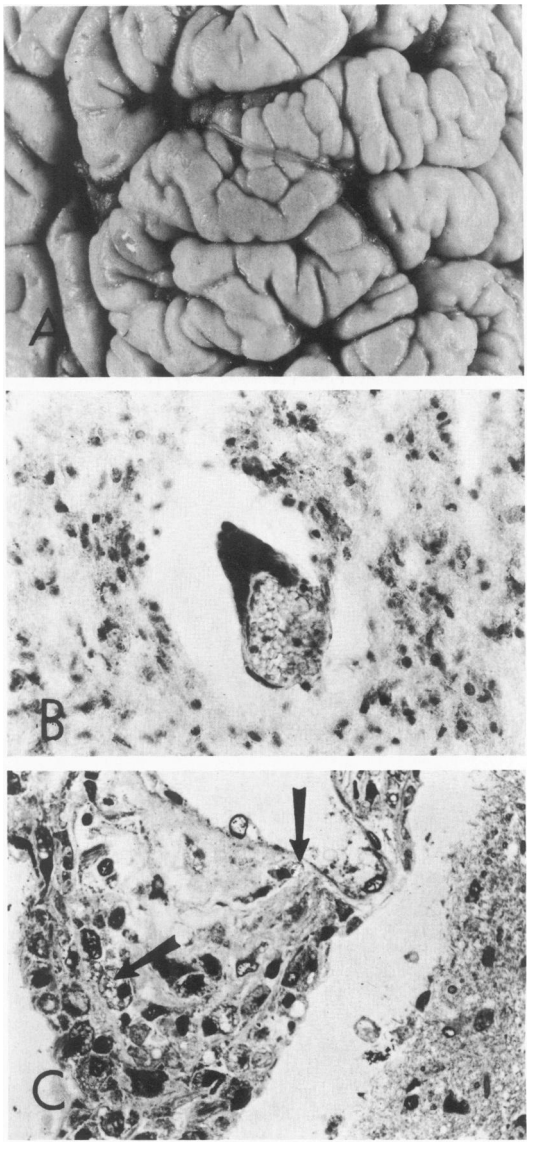

Figure 7A—Parietal cortex from Case 1 shows the polymicrogyria. Similar findings were seen in Case 2. **B**—Frozen section of occipital lobe stained for the presence of lipid shows a collection of lipid-laden macrophages around a blood vessel. (Oil red O, ×213) **C**—One-micron epon-embedded section of the collection of perivascular cells seen in **B**. Many of the cells have lipid vacuoles. Part-polarization demonstrates the presence of occasional needlelike crystals even after plastic processing (*arrow*). (Toludine blue, ×440)

Case 2

The weight of the fresh brain was 1120 g. Polymicrogyria of the superior temporal gyrus and occipital lobes was present. The subcortical white matter and corpus callosum were meager. Gray discoloration was found around the lateral ventricles in the occipital horn and the hilum of the dentate nucleus was cavitated bilaterally (Figure 8). Microscopy confirmed the demyelination, most prominent in the occipital lobes with sparing of U-fibers. Large numbers of macro-

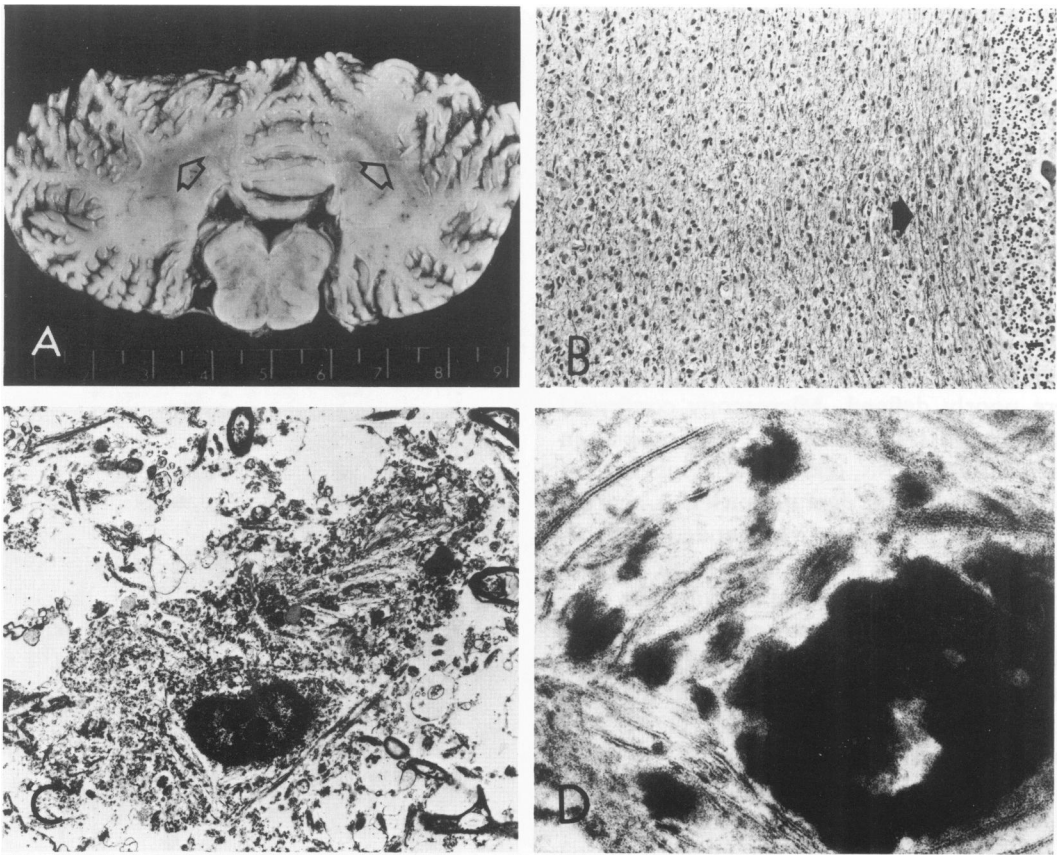

Figure 8A – Section through cerebellum and brainstem of Case 2 shows bilateral symmetrical demyelination and cavitation in the area of the dentate nuclei (*arrows*). **B** – Photomicrograph of the cerebellar white matter with myelin stain shows some residual myelin just internal to the granular area (*arrow*), while the entire section shows severe gliosis. (Woelke, × 183) **C** – Low-power electron micrograph from this area shows a macrophage in a zone of demyelination to have the same cytoplasmic clefts seen in the adrenal and thymus. (× 3900) **D** – Laminar membrane stacks are present around central lipochrome. (× 89,000)

phages with some lymphocytic perivascular cuffing were also seen. Diffuse gliosis was a feature. Calcospherites were found in periventricular white matter. Areas of demyelination with slight perivascular cuffing were also found in the cerebral peduncles, cerebellar white matter, and cortico-spinal tracts. Neuronal loss was demonstrated in the deep sulcal cortex, cuneate and gracile nuclei, and dentate nuclei. Loss of Purkinje cells was also present. Many neurons in the hypothalamus, tegmental nuclei, and throughout the spinal cord contained large amounts of golden brown pigment with staining characteristics of lipofuscin: ie, PAS-positivity, Sudan black B positivity, Ziehl-Neelsen acid fastness, and strong autofluorescence. Frozen sections showed some lipid in perivascular macrophages, and in the occipital area crystalline needlelike inclusions were found. Peripheral nerves showed endoneurial fibrosis, but axons were well-preserved, and no inclusions were noted. Examination of the eyes showed bilateral optic atrophy, demyelination and gliosis of the optic nerves, and gliosis of the retinal nerve fiber layer, with degeneration of the ganglion cells. No inclusions were observed. A pyramidal cataract was present in the right eye.

Biochemical Findings

Table 1 shows that the plasma and cultured skin fibroblasts of both affected children contained an excess of C26:0 fatty acid, in the range observed in ALD.[7] In contrast, both parents showed normal levels of very long chain fatty acids in both the plasma and in skin fibroblasts. The very long chain fatty acid levels in the postmortem tissues of Case 2 were elevated (Table 2). This table also compares the levels with those found in the childhood form of ALD and in a fetus with ALD. The C26:0 levels appear to be higher in liver and adrenal than those usually found in childhood ALD, while levels in the thymus are equivocal. More detailed studies of the spectrum of

Table 1— Very Long Chain Fatty Acids in Cultured Skin Fibroblasts and Plasma

	Fibroblasts			Plasma			
	C22:0 (μg/mg protein)	C26:0 (μg/mg protein)	C26:0 / C22:0	C26:0 (% Total fatty acid)	C26:0 (μg/ml)	C24:0 / C22:0	C26:0 / C22:0
Case 1	0.546	0.300	0.545	0.200	2.79	2.03	0.169
Case 2	0.635	0.291	0.458	0.106	1.83	1.109	1.90
Father	0.415	0.052	0.125	0.011	0.312	0.763	0.011
Mother	1.33	0.060	0.045	0.014	0.381	0.864	0.017
Normal	0.90	0.074	0.081	0.011	0.328	0.836	0.013
	± 0.4	± 0.038	± 0.034	± 0.008	± 0.184	± 0.082	± 0.0094
ALD	0.642	0.411	0.686	0.074	1.62	1.63	0.072
Hemizygotes	± 0.32	± 0.15	± 0.193	± 0.037	± 0.87	± 0.20	± 0.022
ALD	0.722	0.271	0.400	0.051	0.70	1.20	0.038
Heterozygotes	± 0.26	± 0.17	± 0.23	± 0.027	± 0.39	± 0.24	± 0.020

fatty acid composition in lipid subclasses are in progress.

Table 3 shows that the oxidation of C24:0 in cultured fibroblasts from Cases 1 and 2 was impaired to the same extent as in the childhood form of ALD.

Discussion

There are a number of unique features in the findings in these two cases. Previous females with neonatal onset adrenoleukodystrophy have been described.[15,16] Unlike the few female heterozygote carriers who were slightly or partly symptomatic with adrenomyeloneuropathy,[5,20,21] our patient showed the same devastating clinical and extensive pathologic features as her brother. The lipid studies, particularly the demonstration of raised C26–C22 ratio in skin fibroblasts, constitute the first demonstration of the biochemical homology between the neonatal form on the one hand and both childhood adrenoleukodystrophy and adult adrenomyeloneuropathy on the other. We had originally suspected that the widespread systemic involvement might signify a different, but closely related, biochemical defect.[22]

Table 2—C26:0 Levels in Postmortem Tissues Expressed as Percentage of Total Fatty Acids

	Case 2	ALD	ALD fetus	Control
Total lipids				
Brain white	0.73			
Cerebral cortex	0.30			
Adrenal	19.4	0.63 ± 0.48		0.020
Liver	0.079	0.057 ± 0.013		0.026 ± 0.045
Thymus	0.045*	0.023 ± 0.002*		0.0005 ± 0.0001*
Cholesterol esters				
Brain white	3.9 ± 1.5	6.3 ± 3.1†		1.25
Cerebral cortex	6.7 ± 2.8			0.86
Adrenal	39.7	5.2 ± 5.1†	32.5 ± 6	0.022
Liver	4.21 ± 2.1	0.617 ± 0.27	0.77	0.049
Thymus	1.16		2.15	0.59

Where multiple assays were done, mean and SD are listed. In most instances the determinations were performed in quadruplicate. The control samples were from children 1–5 years old with known metabolic disorders. The ALD samples were from 4 children 6–8 years old with the usual clinical form of ALD. The ALD fetus was aborted at 23 weeks gestation.[23]

* These liver samples were subjected to mild alkaline hydrolysis rather than the acid hydrolysis procedure described in the methods section. Alkaline hydrolysis releases fatty acids from glycophosphatides and some cholesterol esters but leaves sphingolipids intact.

† Values from Igarashi et al.[2]

Our patient showed the same devastating clinical and extensive pathologic features as her brother. The lipid studies, particularly the demonstration of raised C26–C22 ratio in skin fibroblasts, constitute the first demonstration of the biochemical homology between the neonatal form on the one hand and both childhood adrenoleukodystrophy and adult adrenomyeloneuropathy on the other. We had originally suspected that the widespread systemic involvement might signify a different, but closely related, biochemical defect.[22]

The elevated level of C26:0 in tissues and cultured fibroblasts and the impaired ability to oxidize C24:0 clearly indicate that these patients had an abnormality of very long chain fatty acid metabolism. While this abnormality is similar to that seen in the childhood form of ALD, the data are not yet sufficient to indicate that the defects are identical. The evidence presented here indicates only that fatty acid oxidation is impaired; isolation and characterization of the specific steps involved are yet to be achieved. We will need more detailed analyses to determine whether the apparent differences between the patterns of tissue lipid accumulation of Case 2 and those of classical childhood ALD are consistent.

Some of the hexacosanoate found in the brains of children with adrenoleukodystrophy is of dietary origin.[3] Yet both of our cases were symptomatic from birth. Two possible explanations are suggested. First, the biochemical defect in these severely affected children may vary somewhat from that of classical adrenoleukodystrophy, and there could be greater local production of long chain fatty acids. Alternatively, the lipids may be derived from the maternal diet and cross the placenta. In that case the dietary therapy of affected children must be extended to the pregnant mother of affected siblings. Both possibilities are currently under investigation.

Table 3—Fatty Acid Oxidation to CO_2 by Cultured Skin Fibroblasts

Case	Stearic acid	Lignoceric acid
Control	100%	100%
Case 1	51%	5%
Case 2	39%	5%
ALD 1	13%	13%
ALD 5	13%	4%

The neonatal systemic form differs from the childhood and adult forms in other ways as well. The systemic nature of the disorder is impressive. Intracytoplasmic inclusions have been found in macrophages in a number of organs, particularly in macrophages in the liver, lymphoid germinal centers, and thymus. The bulk of the accumulation of lipids in these cells is in the form of lipofuscin, with ultrastructural evidence of origin from intracellular membranes. The exact relationship between the lipid crystals, the membranous lamellas, and the lipofuscin is not clear, though it would appear that progressive polymerization and oxidation of the membrane-bound long-chain fatty acids leads to lipofuscin deposition. The lamellar arrays would then be derived from intracytoplasmic membranes damaged by the crystalline inclusions.

The neuropathologic findings in these two children suggest a concomitance of a number of processes by the time of death. The findings of demyelination, particularly in the posterior cerebrum and cerebellar white matter with perivascular lymphoid infiltrates, lipid-laden perivascular macrophages, and polarizable crystalline lipid inclusions were attributable to the adrenoleukodystrophy. Patient 1 had relatively sparse demyelination, although he was symptomatic from birth. There seemed to be a discrepancy between the generalized but only moderate demyelination and gliosis and the devastating neurologic handicap present since birth. The periventricular rarefactions and microcalcifications suggest perinatal periventricular leukoencephalopathy. The role of protracted and refractory seizures in producing much of the neuronal loss and gliosis can only be surmised. The significance of macrocephaly in both children and that of polymicrogyria in both our cases and in previous cases[13,16] is obscure.

The adrenals in both children were tiny, with striated ballooned cells as described by Schaumburg,[1] and contained the characteristic inclusions.[16] The electron-microscopic findings were less well developed than those classically described.[10] There was no evidence of adrenocortical failure in these two children, though the ACTH stimulation test in Case 2 was done at the age of 8 months, a year prior to death. This is in accordance with the other five published cases of neonatal onset adrenoleukodystrophy,[13-16] in only one of which was there biochemically evident adrenocortical hypofunction. It is possible that longer survival and careful testing would eventually have shown adrenocortical deterioration. In this regard, the finding of large numbers of corticotrophs in the pituitary could be regarded as tentative evidence of presymptomatic corticosteroid depletion with compensatory hyperplasia in corticotrophs.

The hepatic fibrosis could not, in these cases, be attributed to the deposition of lipid-laden macrophages and consequent hepatic damage.[14] No polarizable lipid was present in the liver at all, although extractable lipids did show the excess hexacosanoate. The pattern of fibrosis was not only unusual but so closely similar in both children that it is easier to accept an intrinsic explanation than an effect of the many anticonvulsive drugs.

The mode of inheritance of neonatal ALD remains unresolved. There is no doubt that the childhood form of ALD is X-linked. This is based on pedigree analysis and upon the demonstration of two types of clones in women heterozygous for ALD.[10] In fact, the ALD mutation has been mapped on the X-chromosome.[10] The observation that both male and female infants appear to be affected with equal frequency and severity in neonatal ALD suggests at first glance autosomal recessive rather than X-linked inheritance. In support of the autosomal recessive mode of inheritance, it is pointed out that 35 out of 37 female obligate heterozygotes for childhood ALD demonstrated increased levels of very long chain fatty acids in plasma and/or fibroblasts.[23] Neither parent of our 2 patients, on the other hand, demonstrated an increased fatty acid level.

The autosomal recessive inheritance is not the only plausible explanation. Severely symptomatic childhood ALD heterozygotes have been reported by Heffungs et al,[24] and symptomatic females shown to be heterozygous by clonal analysis have also been observed.[11] The unusual observation has been made that in ALD tissue cultures there appears to be selective favoring of the mutant allele,[11] a possible explanation for X-linked symptomatic heterozygotes. The mode of inheritance in our 2 cases has thus not been elucidated, although there should be great interest in determining whether it is possible to cross-correct between cell lines in neonatal ALD and childhood ALD.

References

1. Schaumburg HH, Powers JM, Raine CS, Suzuki K, Richardson EP Jr: Adrenoleukodystrophy: A clinical

and pathological study of 17 cases. Arch Neurol 1975, 32:577–591
2. Igarashi M, Schaumburg HH, Powers J, Kishimoto Y, Kolodny E, Suzuki K: Fatty acid abnormality in adrenoleukodystrophy. J Neurochem 1976, 25:851–860
3. Kishimoto Y, Moser HW, Kawamura N, Platt M, Pallante SL, Fenselau C: Adrenoleukodystrophy: Evidence that abnormal very long chain fatty acids of brain cholesterol esters are of exogenous origin. Biochem Biophys Res Commun 1980, 96:69–76
4. Singh I, Moser HW, Moser AB, Kishimoto Y: Adrenoleukodystrophy: Impaired oxidation of long chain fatty acids in cultured skin fibroblasts and adrenal cortex. Biochem Biophys Res Commun 1981, 102:1223–1229
5. Moser HW, Moser AB, Kawamura N, Migeon B, O'Neill BP, Fenselau C, Kishimoto Y: Adrenoleukodystrophy: Studies of the phenotype, genetics and biochemistry. Johns Hopkins Med J 1980, 147:217–224
6. Kawamura N, Moser AB, Moser HW, Ogino T, Suzuki K, Schaumburg H, Milunsky A, Murphy J, Kishimoto Y: High concentration of hexacosanoate in cultured skin fibroblast lipids from adrenoleukodystrophy patients. Biochem Biophys Res Commun 1978, 82:114–120.
7. Moser HW, Moser AB, Kawamura N, Murphy J, Suzuki K, Schaumburg H, Kishimoto Y: Adrenoleukodystrophy: Elevated C_{26} fatty acid in cultured skin fibroblasts. Ann Neurol 1980, 7:542–549
8. Moser HW, Moser A, Frayer KK, Chen W, Schulman JD, O'Neill BP, Kishimoto Y: Adrenoleukodystrophy: Increased plasma content of saturated very long chain fatty acids. Neurology (Minneap) 1981, 31:1241–1249
9. Ropers H-H, Burmeister P, von Petrykowski W, Schindera F: Leukodystrophy, skin hyperpigmentation, and adrenal atrophy: Siemerling-Creutzfeldt disease: Transmission through several generations in two families. Am J Hum Genet 1975, 27:547–553
10. Powers JM, Schaumburg HH: Adreno-leukodystrophy (Sex-linked Schilder's disease): A pathogenetic hypothesis based on ultrastructural lesions in adrenal cortex, peripheral nerve and testis. Am J Pathol 1974, 76: 481–500
11. Migeon BR, Moser HW, Moser AB, Axelman J, Sillence D, Norum RA: Adrenoleukodystrophy: Evidence for X-linkage, inactivation and selection favoring mutant allele in heterozygous cells. Proc Natl Acad Sci 1981, 78:5066–5070
12. Griffin JW, Goren E, Schaumburg H, Engel WK, Loriaux L: Adrenomyeloneuropathy: A probable variant of adrenoleukodystrophy: I. Clinical and endocrinological aspects. Neurology (Minneap) 1977, 27:1107–1113
13. Ulrich J, Herschkowitz N, Heitz P, Sigrist T, Baerlocher P: Adrenoleukodystrophy: Preliminary report of a connatal case: Light- and electron microscopical, immunohistochemical and biochemical findings. Acta Neuropathol (Berl) 1978, 43:77–83
14. Manz HJ, Schuelein M, McCullough DC, Kishimoto Y, Eiben RM: New phenotypic variant of adrenoleukodystrophy: Pathologic, ultrastructural and biochemical study in two brothers. J Neurol Sci 1980, 45:245–260
15. Haas JE, Farrell DF, Johnson ES: Adrenoleukodystrophy variant in a female (Abstr) Lab Invest 1981, 44:1
16. Benke PJ, Reyes PF, Parker JC: New form of adrenoleukodystrophy. Hum Genet 1981, 58:204–208
17. Johnson AB, Schaumburg HH, Powers JM: Histochemical characteristics of the striated inclusions of adrenoleukodystrophy. J Histochem Cytochem 1976, 24:725–730
18. Sternberger LA: Immunocytochemistry. 2nd edition. New York, John Wiley and Sons, 1979
19. Folch-Pi J, Lees M, Sloane-Stanley GH: A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1967, 266:497–509
20. Marmion LH, O'Neill BP, Schafer RA, Schafer NJ, Grekin RJ, Feringa ER: The adrenoleukomyeloneuropathy complex: A clinical evaluation of disease expression in four generations of a kindred (Abstr). Neurology (Minneap) 1979, 29:560
21. O'Neill BP, Moser HW, Marmion LH: The adrenoleukomyeloneuropathy (ALMN) complex: Elevated C_{26} fatty acid in cultured skin fibroblasts and correlation with disease expression in three generations of a kindred (Abstr). Neurology (Minneap) 1980, 30:352
22. Jaffe R, Zaias B, Hashida Y: Adrenoleukodystrophy: Possible nonspecificity of morphologic findings (Abstr). Pediatric Pathology Club Annual Meeting, March 1978, International Academy of Pathology, Atlanta, Ga
23. Moser HW, Moser AB, Brown FR III, Chen WW, Powers JM: Adrenoleukodystrophy: Prenatal diagnosis and heterozygote detection (Abstr). Ann Neurol 1981, 10:284
24. Heffungs W, Hameister H, Ropers HH: Addison disease and cerebral sclerosis in an apparently heterozygous girl: Evidence for inactivation of the adrenoleukodystrophy locus. Clin Genet 1980, 18:184–188

Acknowledgments

The assistance of Rocco Agostini in the ultrastructural studies is greatly appreciated, as always.