kinase C *in vitro* is directly related to the state of kernicterus remains unclear from our present results. We failed to demonstrate an *in vivo* effect of bilirubin on the specific activity of protein kinase C in adult Sprague-Dawley rats (data not shown). However, kernicterus is confined to the very early neonatal period or low birth weight infants; acidosis or hypoalbuminemia also tend to occur in such patients. Acidosis or hypoalbuminemia increases unbound bilirubin levels allowing the accumulation of bilirubin in central nervous tissues (17). Therefore, experiments using premature or very early neonatal animals with hyperbilirubinemia are necessary in order to clarify precisely the role of protein kinases in kernicterus.

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Unsuccessful Attempts to Induce Peroxisomes in Two Cases of Zellweger Disease by Treatment with Clofibrate

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ABSTRACT. The cerebro-hepato-renal syndrome of Zellweger is a fatal hereditary disease and most of the affected infants die before the age of 6 months. Most probably the fatal outcome of the disease is due to an apparent complete lack of peroxisomes in the liver, kidneys, and brain. Treatment with clofibrate is known to increase drastically the number of peroxisomes in mammalian liver. We therefore treated two infants with the Zellweger syndrome with clofibrate (30 and 45 mg/kg body weight, respectively) for 3-4 wk. No clinical effect of the treatment was observed in any of the two cases, and the pattern of abnormal bile acids in serum did not change. No peroxisomes could be

detected by electron microscopy of liver biopsies taken immediately after the treatment. Our failure to induce peroxisomes in the two Zellweger patients is in accord with the hypothesis that the protein missing in this autosomal recessive disease is absolutely essential for the formation of peroxisomes. (*Pediatr Res* 19: 590-593, 1985)

The Zellweger syndrome is a fatal hereditary disease characterized by craniofacial malformations, hypotonus, liver enlargement, renal cysts, central nervous disturbances, and mental retardation (1). In general affected infants die before 6 months of age. The most characteristic histological finding in this disease is an apparently complete absence of peroxisomes in the liver and kidneys (2). Goldfischer *et al.* (2) have also demonstrated

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mitochondrial abnormalities and a defect in the mitochondrial electron transport chain. In some cases, the mitochondria seem to be normal, however, and it has been suggested that the mitochondrial changes are secondary to the lack of peroxisomes (3). The autosomal recessive inheritance of the Zellweger syndrome suggests that it is an inborn error of metabolism affecting one specific protein. On the other hand, it is difficult to understand the apparently complete absence of a cell organelle as a consequence of only one missing protein. One can only speculate that the missing protein may have a regulatory function, maybe necessary for the biogenesis of peroxisomes, or maybe an essential component for membrane synthesis.

Treatment with clofibrate is known to increase drastically the number of peroxisomes in mammalian liver (4). Although the mechanism of this induction is not known, it has been shown that clofibrate induces mRNA for four peroxisomal enzymes (5). It was also recently reported that three clofibrate-sensitive peroxisomal enzymes are synthesized on free polysomes, released into the cytosol, and then transported into peroxisomes (6). If the specific protein missing in the Zellweger disease is absolutely essential for the biogenesis of peroxisomes, treatment with clofibrate cannot be expected to have any effect on the disease. If, however, there is a small number of hitherto undetected peroxisomes in the hepatocytes of affected infants, it should be possible to increase the number of the organelle by treatment with clofibrate. Such an effect may also improve the condition of the patient, although the damage to the central nervous system most probably is irreversible.

Herein, we report unsuccessful attempts to induce peroxisomes in the liver of two infants with the Zellweger syndrome.

MATERIALS AND METHODS

Case 1. This case was the first infant born to healthy young parents without consanguinity. Due to prolonged delivery this was completed with vacuum extraction. The infant, a girl, was born after 40 wk of gestation, birth weight was 2860 g, and length was 49 cm. At birth the dysmorphic features were noticed, and the infant had profound hypotonia. In addition to the typical abnormal skull shape and appearance of Zellweger disease, ultrasonic investigation showed enlargement of the liver. X-ray showed the typical calcification of patellae and acetabulum, and the patient had congenital glaucoma and pathological EEG with pathological visual evoked response. Laboratory investigations showed normal values except for increased serum concentrations of transaminases (ASAT 3.4 ukat/L, ALAT 2.6 ukat/L) and serum lactate dehydrogenase (26 ukat/L). There was a slight transient hyperbilirubinemia during the first weeks, and the serum transaminases increased further to 14 and 10 ukat/L, respectively. There was no increased level of pipecolic acid in the urine. The diagnosis was verified by high serum concentrations of trihydroxycoprostanic acid (7, 8) and the typical C₂₉-dicarboxylic acid (8). A tissue culture of fibroblasts from the patient showed increased levels of very long-chain fatty acids (9, 10). The patient had frequent short-lasting convulsions which were not cured by phenobarbital treatment but reduced the frequency.

The infant gradually deteriorated and died at the age of 4½ months. No autopsy was performed according to the wishes of the parents.

Case 2. This case was a boy, the third child born to healthy parents with no consanguinity. The firstborn sibling was healthy, but the other sibling died at 1 wk of age with multiple malformations: hydrocephalus, pes equinovarus, micrognathia, retentio testis, and cystic kidneys. The present child was born after 39 wk of gestation at normal delivery. The birth weight was 2850 g and the length was 49 cm. At birth the child had tachycardia, areflexia, profound hypotonia, and there was no spontaneous respiration. He was intubated and immediately transferred to a respirator in which he was treated with oxygen supply. There

were extensive pulmonary infiltrations considered to be due to adaptive disturbances. The child had the typical facial appearance of Zellweger disease, abnormal skull shape, epicanthus, and was also hypotonic. Ultrasonic investigation showed dilatation of the lateral and third ventricles of the brain and also suspect renal cortical cysts. Intravenous urography was normal. The patient improved, and the oxygen supply could be gradually decreased. After 2½ months of age the infant was kept in bed. EEG was pathological with a pathological result of visual evoked response. Despite the continuous profound hypotonus and hyporeflexia, the diagnosis was not proved until 3 months later. Increased levels of pipecolic acid was not found in the urine, but the serum contained high levels of trihydroxycoprostanic acid and the typical C₂₉-dicarboxylic acid (7, 8).

The frequency of general convulsions increased despite anti-convulsive therapy, and the patient died at 5 months of age due to interstitial pneumonia. A liver biopsy was taken within 30–60 min after death. Renal cortical cysts, liver steatosis, and a slight atrophy of the adrenals were found at autopsy.

Ethical aspects. Treatment with clofibrate was approved by the local ethical committee of the Karolinska Institute at Huddinge University Hospital.

Histological methods. Needle biopsy was performed according to Menghini (11). For light microscopy specimens were fixed in Histofix and embedded in paraffin and Eponaraldite. Sections were stained with hematoxylin and eosin, periodic acid-Schiff, Perl's iron, and reticulin. For electron microscopy randomly chosen 1-mm³ cubes of liver were fixed in 2% glutaraldehyde containing 0.1 M cacodylate buffer (pH 7.4) and 0.1 M sucrose. The liver samples were postfixed in 1% osmium tetroxide, dehydrated in graded ethanol solutions, and embedded in LX 112 resin. *En bloc* staining was performed with uranyl acetate added to the final ethanol solution. Thin sections were stained with lead citrate.

For ultrastructural demonstration of catalase, slices were sectioned from frozen glutaraldehyde fixed tissue and incubated with DAB at pH 10.0 according to the procedure of Novikoff *et al.* (12). Incubation was performed for 30 and 60 min at 37°C. The slices were curled, embedded, and processed as above. As control specimens, liver biopsy material from a healthy 2-yr-old child was used.

RESULTS AND DISCUSSION

Case 1 was treated with clofibrate in a dose of 30 mg/kg body weight daily for 4 wk prior to death, and case 2 with 45 mg/kg body weight daily for 3 wk prior to death. No clinical effect of the treatment was observed in any of the two cases. The pattern of abnormal bile acids in serum (7, 8) did not change during the treatment. Needle liver biopsies were taken immediately before the treatment and immediately after death.

According to light microscopical findings, the histological appearance of all liver biopsies was essentially normal except for focal areas of hepatocyte rosette formation, discrete fibrosis, and inflammation in the portal areas as well as some Kupffer cell siderosis. Following clofibrate treatment, some small intracytoplasmatic lipid droplets (2 µm) were noticed in periportal hepatocytes as well as mild portal roundcell infiltration. Both phenomena are probably due to the treatment and have been seen also in adults given clofibrate or similar drugs (Björkhem I, Blomstrand S, Glaumann H, Strandvik B, unpublished observation).

According to examinations by transmission electron microscopy, the general appearance of the hepatocytes was quite normal (Fig. 1a). However, mitochondrial irregularities were present, eg, disturbed orientation of the cristae, focal electron-dense deposits, and rounded swollen shapes. Similar alterations have been described in other pathological conditions including α -1-antitrypsin deficiency (11). No peroxisomes could be found in the hepatocytes. This was confirmed by enzyme histochemistry which also

Fig. 1. Electron microscopy of liver biopsies from case 2 before (*a*) and after (*b*) clofibrate treatment. Note absence of peroxisomes. For explanation see the text.

Fig. 2. Electron micrographs from case 2 following clofibrate treatment (*a*) and control liver from a kidney donor (*b*) incubated for histochemical demonstration of catalase. The liver tissue was in both cases obtained about 0.5–1 h postmortem fixed, frozen, and sectioned. Incubation was performed in alkaline DAB. Note absence of peroxisomes in *a* (clofibrate treatment of case 2) in contrast to the prominent staining of peroxisomes of control liver in *b*.

disclosed absence of microperoxisomes. In the control samples numerous peroxisomes (0.6 μ m) were seen together with a few small dense-staining microperoxisomes scattered in the cytoplasm. The latter particles were characterized by a dense matrix surrounded by a lucent or "empty" halo. In the samples from the patients there were autophagic vacuoles in various stages of development and abundant residual bodies. Compared to the control samples, the Golgi complex was difficult to recognize, although present.

Clofibrate treatment did not result in occurrence of detectable peroxisomes or microperoxisomes (Fig. 1*b*). Enzyme histochemistry substantiated this conclusion (Fig. 2*a*). This absence of peroxisomes was not due to postmortem artefacts, since in liver tissues from kidney transplant donors taken 1 h after circulatory arrest, peroxisomes were easily identified (Fig. 2*b*). However, the treatment seemed to cause some enlargement of occasional mitochondria, vesiculation of their cristae, and scattered lipid inclusion bodies in the cytoplasm.

Our failure to induce peroxisomes in the two Zellweger patients is in accordance with the hypothesis that the protein missing in this disease is absolutely essential for the formation of peroxisomes. It cannot be completely excluded, however, that an earlier postnatal treatment would have had an effect.

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Growth and Development of Pediatric Research¹

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ABSTRACT. Pediatric research may be entering a period of failure to thrive. This is despite impressive scientific achievements, significant financial benefits, intriguing unanswered questions and a rewarding life for the pediatric researcher. Many of the difficulties are financial and situational, including small departments and divisions, few pediatric research mentors, impecunious pediatric hospitals and services, ethical constraints on pediatric research and competing responsibilities. Grants to pediatric departments represent about 3% of the total NIH research project support. The two largest federal programs supporting Pediatric Research are the Clinical Research Center (CRC) program (20% of an \$28,000,000/yr budget) and the National Institutes of Child Health and Human Development (17% of a \$123,000,000/yr budget). There are 75 CRCs of which 60 admit children; 10 CRCs are in children's hospital and 6 other CRCs have a clear pediatric focus. Pediatric Departments receive 3% of NIH funds; this represents

10% of funds to clinical departments. By contrast medicine departments receive 17% of NIH funds (47% of funds to clinical departments). NIH research support to pediatrics (\$82,000,000/yr) is centered in a few large departments; 14 pediatric departments receive 343 of the 572 NIH grants (60%). By contrast, 66 of 120 pediatric departments have no (45) or 1 (21) NIH grants. Obstacles are identified and suggestions are offered for future (students and residents), beginning (fellows and assistant professors), established (associate and full professors) and women pediatric researchers. (*Pediatr Res* 19:593-598)

Pediatric research can use all the help it can get. Despite monumental scientific advances and proven cost benefits (eg, elimination of poliomyelitis), pediatric research (and other medical research), has undergone a growth arrest (1). If we are not vigilant, pediatric research can begin a period of failure to thrive. This essay addresses some ways to maintain its growth and development. I shall point out some advantages and disadvantages of pediatric research, discuss some financial aspects of

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