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ABSTRACTS

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Special Sessions

NEUROLOGY

Effect of Xylocaine on infant rat brain mitochondria. Arnold L. Smith. *Children's Hosp. Med. Ctr., Boston, Mass.* (Intr. by David H. Smith).

Local anesthetics are widely used in obstetric practice to facilitate painless delivery. Their usage has been associated with neonatal respiratory depression and, in the case of fetal injection, with neonatal seizures. Because of these observations the effect of Xylocaine on infant rat brain mitochondrial enzymes was investigated.

The neurochemical development of 5- to 10-day-old infant rats approximates that of the human newborn. Xylocaine was administered intraperitoneally to infant rats at this age in doses calculated to produce blood levels comparable to those in human newborns with toxic signs and symptoms. Two hours after injection there was a 65% decrease in brain D(—)-beta-hydroxybutyrate dehydrogenase (HBDH) activity and a 40% decrease in succinic dehydrogenase (SDH) activity. Brain cytochrome oxidase (CyO) activity was not significantly decreased. Temporal examination of these enzymes after injection suggested that HBDH was the first enzyme inhibited. These observations are in contrast to the lack of inhibition of liver HBDH, SDH, and CyO. The inhibition of oxidative enzymes in infant rat brain mitochondria persists up to 5 hours after injection. This enzyme inhibition is also demonstrable in measurements of respiration of intact brain mitochondria. Oxygen consumption of normal infant rat brain mitochondria is best supported by D(—)-beta-hydroxybutyrate (BOH), but succinate was also effective. BOH dependent respiration of infant rat brain mitochondria was inhibited by Xylocain in vivo and in vitro. The data suggest that further studies of Xylocaine usage and effects in the perinatal period are indicated.

Multiple sulfatase deficiencies, the enzymatic basis of a new disorder. Jerome V. Murphy, Marcia Williams, and Hugo W. Moser (Intr. by R. Michaels). *The Mass. General Hosp., Boston, Mass.*

Since 1961 seven patients have been reported with both generalized neuronal storage and metachromatic degeneration of white matter. These patients have the onset of their degenerative disorder during their second year of life with loss of motor skills. Deafness and seizures ensue. Associated findings include icthyosis, minor skeletal anomalies, and hepatosplenomegaly. Alder-Reilly bodies are seen in the white blood cells and the urine contains excessive amounts of cerebroside sulfate and glycosaminoglycans.

Frozen post mortem tissues from two unrelated patients with this disorder were available for enzymatic studies. Aryl sulfatases A and C were absent in all tissues studied. Aryl sulfatase B was deficient in liver, but relatively absent in brain and kidney. Steroid, cholesterol and estrone sulfatase activities were absent in liver. Mixing experiments failed to reveal a factor inhibiting these sulfatases. Enzymatic study of parental white blood cells failed to identify the carrier state. Several accumulated substrates could be specifically associated with an enzymatic deficiency.

Explanations for the absence of these lysosomal and microsomal enzymes are theoretical, but other disorders of multiple enzyme deficiencies have been reported.

Brain glucose metabolism in undernourished rats. H. Peter Chase, Vijay Kumar, Denis O. Rodgerson, and Geoffrey P. Cheung. *Univ. of Colo. Med. Ctr., Denver, Colo.*

Glucose is the primary substrate used by the infant brain either as a source of energy or for lipid and amino acid synthesis. The in vivo conversion of glucose U-^{14}C, 2.5 μCi/60 g of body weight injected intraperitoneally, to lipids and amino acids was measured in the brains and livers of poorly nourished and control infant rat pups at ages 6, 10, 17, and 24 days, and in adult animals fed a control or 8% protein diet for 3 months. Brain lipid formation was decreased 50 to 75% from ages 6 to 17 days, and brain amino acid formation was reduced 60% in 10-day-old rat pups. Reduced in vivo hepatic conversion of glucose U-^{14}C to lipids and amino acids was found only at ages 6 and 10 days. In vitro production of $^{14}CO_2$ from glucose U-^{14}C incubated with brain slices was not altered in 10- or 20-day-old malnourished animals (1.58 ± .26 *vs.* 1.14 ± .07). Brain ATP, phosphocreatine, and glycogen levels were not reduced at either age 10 or 20 days. Mitochondrial glutamate dehydrogenase activity catalyzes the formation of amino acids from α ketoglutarate in Krebs cycle, and was reduced 21 to 30% in the brains of malnourished animals, and 49 to 81% in the livers. Supernatant NADP-isocitric dehydrogenase activity, believed to produce TPNH for lipid synthesis, was not reduced in the brains, but was reduced in the livers of malnourished infant rats. Pyruvate kinase, one of the rate limiting enzymes of glycolysis, showed reduced activity in liver and muscle of malnourished animals, but not in brain.

Decreased glucose utilization in the brains of malnourished animals may explain the previously described alterations in lipid and protein synthesis resulting in permanent brain structural deficits.

In utero induction and development of brain β-hydroxybutyrate dehydrogenase (BDH). M. MICHAEL THALER and MAX M. CHAIT. *Univ. of Calif., San Francisco, Calif.*

Ketones become the major source of energy for brain metabolism during prolonged fasting. The energy-yielding conversion of β-hydroxybutyrate to acetoacetate is catalyzed by the substrate-inducible enzyme, BDH. The capacity of fetal brain for utilization of β-hydroxybutyrate, and effects of maternal starvation on development of BDH activity in immature brain were studied in rat fetuses, and in newborn rats and rabbits. In rats, BDH activity appeared on the 17th day of gestation, and tripled within 2 days. A second rise in BDH began 12 hours after birth, doubling activity by 48 hours. BDH developed more rapidly in fetuses from pregnant animals fasted for 3 days. At 21 days gestation, their activity was 42.5 ± 6.4 units compared with 24.7 ± 4.3 units in fetuses from fed controls. At birth offspring of fasted rats had approximately twice the BDH activity of normal newborns. When the former were nursed by fed animals, BDH activity decreased to normal values within 24 hours. Normal rabbits fasted for 24 hours after birth had 130% higher BDH activity than fed littermates. These results show that fetal and newborn brain can utilize ketones. The development of this capacity is accelerated by maternal or postnatal starvation. Thus, the immature brain appears extremely responsive to qualitative changes in nutrients before and after birth.

Neuropathological and ocular changes in the cerebrohepatorenal syndrome. JOSEPH VOLPE, DAVID WALTON, MARK ROGERS, and LEWIS HOLMES. *Harvard Med. Sch., Boston, Mass.* (Intr. by John W. Littlefield).

We have found in a four-month-old girl with the cerebrohepatorenal syndrome two abnormalities that may provide insight into the basic nature of the disorder. These are: (1) a profound defect of neuronal migration, and (2) iron storage in ocular structures. This girl had the following neurological findings: no visual responses with normal pupillary reactivity, no auditory responses, no suck, severe hypotonia, no reflexes and multifocal clonic seizures. Pathological findings included diffuse polymicrogyria and pachygyria, severely disordered deeper layers of cerebral cortex, tremendous aggregates of neurons in cerebral white matter, hypoplastic corpus callosum, dysplastic inferior olivary and dentate nuclei, numerous heterotopic Purkinje cells in cerebellar white matter, disordered cerebellar cortex (nodulus) and a dysgenesis of the grey matter of the lumbosacral spinal cord. Myelination was only slightly delayed for age, and spinal roots, peripheral nerves and muscle were normal. These neuropathological findings suggest that the primary genetic defect may involve the programming of neuronal migrational events. Also she had abnormal retinal pigmentation and glaucoma. Electroretinogram showed normal retinal signals but no measurable occipital signals. Her eyes showed reduplication of the pigment epithelium and iron deposits in the corneal epithelium and ciliary body. These unique iron deposits may be due to abnormalities of iron metabolism but not simply to increased serum iron (SI). She never had an elevated SI; 2 weeks before death, her SI was 122 μg%, TIBC 608 μg%.

Timing of intracranial bleeding in newborn infants. N. DYER, R. GUTBERLET, J. RAYE, G. FAXELIUS, S. SWANSTROM, A. BRILL, and M. STAHLMAN. *Vanderbilt Univ. Sch. of Med., Nashville, Tenn.*

Intracranial hemorrhage is a frequent finding at autopsy in very immature infants. Because of the possibility of therapy in whom DIC seems to be present, it was thought important to be able to time the intracranial bleed to see if it had already occurred before therapy could have been initiated. Infants at high risk for intracranial bleeding have been transfused as early in the course of their illness as possible with red cells tagged with ^{50}chromium. ^{50}Cr is a stable tracer which can be activated in vitro to ^{51}Cr and counted. If the infant died and had an intraventricular clot in which >70% of the red cells were tagged, it was assumed that bleeding occurred after tagging. If the clot contained <30% tagged cells as compared to the sample of blood taken at tagging or just prior to death, it was assumed that bleeding had occurred prior to tagging. 28 such tagged infants have died and had intracranial clots analyzed for ^{50}Cr. 16 infants had HMD, 7 extreme immaturity, the remainder died of other disorders. 18 were <1250 g. B.Wt., 23 were <32 wks. gestation. Median age of tagging was 6.3 hrs. while median age at death was 33.2 hrs. ^{50}Cr concentrations in clot samples indicated that 25 infants had clearly bled after tagging and only 2 had already had their major bleed before tagging. 4 infants presumably had some bleeding before tagging, but continued to bleed afterwards. Investigations of the possible exchange of ^{50}Cr tag after clot formation did not indicate significant exchange between tagged circulating red cells and those in the clot. 12 infants had clear-cut DIC, 11 possible, and 5 had no evidence of DIC. All but 1 without DIC had grossly abnormal second stage clotting values. It is concluded that most of these infants' intracranial hemorrhages occurred after birth and after their disease process was clearly established.

In vitro studies of *post mortem* neural tissue in subacute sclerosing panencephalitis (SSPE). JOHN F. GRIFFITH and SAMUEL L. KATZ. *Duke Univ. Med. Ctr., Durham, N. C.*

Brain and spinal cord from 2 cases of SSPE were obtained within one hour of death and cultures were established from multiple sites including frontal, temporal and parietal lobes, pons, cerebellum and cord. These were serially passaged and remained viable for many months. Their growth rates, cytologic detail, and survival varied depending on the region of brain sampled. Only certain of these cultures, derived from specific neuroanatomical sites showed evidence of measles antigen or yielded a viral agent when appropriately studied.

Cell cultures derived from cortical regions showed intranuclear and intracytoplasmic inclusion bodies even before a monolayer had formed. These inclusions were both type A and B, and in one case the cells showed significant hemadsorption in a distribution indicating the presence of viral antigen in terminal processes.

Two cell types could be identified in early cultures from the cortex but with passage, one of these, a small round cell with a hyperchromatic nucleus, disappeared and the other, a larger cell, seemed to elongate and divide rapidly, resulting in the fibroblastic monolayer. The cells derived from the cerebellum and spinal cord were slower to grow, had a shorter survival and different morphology.

Despite widespread pathologic changes in both brains, cytopathology developed only in the cultures of the frontal lobe in