247 DETERMINATION OF FETAL LUNG MATURITY BY FLUORESCENT POLARIZATION (FP) OF AMNIOTIC FLUID. John F. Vogt, Steven A. Golde, Luis A. Cabal, Steven Gabbe, Joan E. Hodgman. Univ. of So. Calif. School of Medicine/Los Angeles County Medical Center, Dept. of Pediatrics and Obstetrics/Gynecology, Los Angeles.

The intrinsic fluidity of lipid aggregates in amniotic fluid (AF) can be determined by FP, a spectral technique which monitors molecular motion. FP of lipids in AF has been found to correlate with other measures of lung maturity. In order to extend these observations and provide clinical correlation, FP was determined on amniotic fluid obtained during therapeutic abortion, by diagnostic amniocentesis, or at delivery. Samples contaminated with meconium or blood were excluded. Eighty-seven clear samples of AF were analyzed by FP with Lecithin/Sphingomyelin (L/S) ratios determined on 41. FP values correlated with gestational age (GA); 24 of 26 samples before 34 weeks were above 0.345, while after 36 weeks all except 1 were below 0.345. FP values also correlated significantly with L/S ratios above and below 2 ($p=.01$). All delivered infants have been greater than 34 weeks GA with FP values below .345 prior to delivery and none have developed Hyaline Membrane Disease. Determination of FP values on amniotic fluid appears to be a promising technique for evaluation of lung maturity. It has the advantages of ease of performance and reproducibility. The correlation of mature values with clinical outcome was good. Further study of amniotic fluid from premature deliveries will clarify the reliability of FP values in predicting pulmonary immaturity in Hyaline Membrane Disease.

248 REGULATION OF CHOLESTEROL BIOSYNTHESIS IN CULTURED CELLS OF NEURAL ORIGIN. Joseph J. Volpe and Sally W. Hennessy, Wash. Univ. Sch. Med., Depts. Peds. & Neurol., St. Louis.

Biosynthesis of cholesterol, a major component of cellular membranes, is of particular importance in developing neural tissue. Because of the difficulties inherent in in vivo studies of whole brain, we have turned to cultured C-6 glial and neuroblastoma cells, which exhibit many properties of differentiating cells. Regulation of cholesterol biosynthesis and the major rate-controlling enzyme, HMG-CoA reductase, by the low density fraction (LDL) of serum lipoproteins (LP) was demonstrated. When glial cells were grown in LP-poor serum, a 22-fold increase in enzymatic activity was observed within 24 h. A qualitatively similar but quantitatively less impressive effect was noted in the neuronal cells. That the important component in LDL is cholesterol was shown by defining a 60% reduction in reductase activity in glial cells grown for 6 h in the presence of 50 µg/ml of pure cholesterol. Desmosterol was even more effective than cholesterol, producing an 80% reduction in activity at a concentration of only 10 µg/ml. Associated with the suppression of HMG-CoA reductase by LDL and by desmosterol was a stimulation of cholesterol ester synthesis. These observations suggest important roles in regulation of cholesterol synthesis for desmosterol and cholesterol esters, which are present in relatively high concentrations in brain only early in development. ------ The data have provided important information about the regulation of cholesterol biosynthesis in developing glial and neuronal cells and revealed differences in this regulation between the two cell types.

249 GLUCOSE AND FATTY ACID OXIDATION BY DEVELOPING LUNG. Joseph B. Warshaw and Mary L. Terry, Yale University School of Medicine, Dept. of Pediatrics, New Haven, Conn.

Although the lung is active metabolically during late fetal development and in the immediate postnatal period because of large energy requirements for growth and the synthesis of surface-active phospholipids, there is little information available concerning substrate oxidations by the developing lung. We have measured the capacity of lung slices obtained from 17 day fetuses to adults to oxidize glucose and fatty acids. Glucose oxidation to CO_2 decreased between 17 and 20 days of fetal development. Coincident with birth CO_2 production from glucose increased and remained constant until weaning when activity again increased possibly in association with the shift to higher carbohydrate intake. The ratio of glucose -1-^{14}C: Glucose -6-^{14}C oxidation to $^{14}CO_2$ was highest during late fetal development suggesting increased glucose oxidation via the pentose phosphate pathway at that time Oxidation of palmitate in fetal, newborn and adult lung was negligible even in the presence of carnitine. However, oxidation of capric acid (10 carbon) was significant and increased from 55 n moles/g/hr at 17 days gestation to 130 n moles/g/hr by the 5th postnatal day. Activity then declined to adult levels which were approximately 25% that of glucose oxidation. The data suggests that while the developing lung can oxidize glucose and medium chain fatty acids, palmitate oxidation is limited possibly because of requirements for its utilization as a substrate for pulmonary lecithin.

250 EXPERIMENTAL HYPOTHYROIDISM: RELATIONSHIP BETWEEN CEREBELLAR CELL DIVISION AND ENZYMES INVOLVING NUCLEIC ACID METABOLISM DURING DEVELOPMENT. Morton E. Weichsel, Jr., Brian R. Clark, and Russell E. Poland, UCLA Sch. of Med., Harbor Gen. Hosp. Depts of Pediatrics and Psychiatry, Torrance, Calif.

Perinatal hypothyroidism in the rat results in a delay in the developmental spectrum of cerebellar cell replication with a shift to a later age in the developmental spectrum of activity of thymidine kinase (TK), a salvage pathway enzyme active in replicative DNA synthesis. In the present experiments hypothyroidism was induced by administration of propylthiouracil (PTU) to the mother from the 18th day of gestation. We measured cerebellar activities of uridine kinase (UK), aspartate transcarbamylase (ATC), and thymidylate synthetase (TS). These enzymes are associated with the salvage of uridine, de novo synthesis of uridylate, and conversion of uridylate to thymidylate respectively, and their peak activities normally occur near the time of most rapid cerebellar DNA synthesis. Activities of ATC at age 3 days and TS at age 5 days were significantly decreased to 91.3% and 94% of control respectively, while activities were significantly elevated to 116% and 142% of control by day 15. UK activity was unaffected by hypothyroidism after 5 days of age. The shift to a later age in the developmental spectrum of ATC and TS supports the possibility that the de novo and interconversion pathways relate closely to replicative DNA synthesis, whereas the salvage pathway for uridine may relate more closely in brain to the synthesis of RNA and the sustaining of non-dividing cells.

251 HUMAN PLACENTAL Ca-Mg STIMULATED ATP'ase ACTIVITY. Jeffrey Whitsett, Reginald C. Tsang, Leonard I. Kleinman. U. of Cincinnati Col. of Med., Cincinnati.

Calcium and magnesium are transported against a concentration gradient in the human placenta from mother to fetus. Ca-Mg ATP'ases have been described in numerous tissues and organelles and have been associated with the active transport of calcium. The properties of Ca-Mg stimulated ATP'ase activity were investigated in mitochondrial and microsomal fractions prepared from human placental villus tissue in 12 placentas. Ca-Mg ATP'ase was always 2-3 fold higher in the microsomal fraction. Ca stimulated activity was less in each preparation than for Mg; Ca ATP'ase was $\bar{X}$=13.1 µmole PO_4 mg^{-1} 30 min^{-1} (range 10.2-23.9) and Mg ATP'ase $\bar{X}$=20.2 (13.3-28.3). Microsomal fractions had lower glutamate dehydrogenase and higher alkaline phosphatase activity than mitochondrial fractions. Sucrose gradient fractions of microsomal protein showed that Ca-Mg ATP'ase were enriched together and associated with the plasma membrane marker 5' nucleotidase rather than an endoplasmic reticulum marker NADH oxidase. pH max found to be 7.8-8.3 for both Ca and Mg ATP'ase. ATP was hydrolyzed >ADP and 5'AMP was not hydrolyzed. Ethacrynic acid 1-5mM caused inhibition of both Ca-Mg ATP'ases. Sodium azide inhibited mitochondrial Ca-ATP'ase more than microsomal. Inhibition of Ca-ATP'ase was minimal in the microsomal fraction in 10mM azide. Both mitochondrial and microsomal Mg-ATP'ase were equally and markedly inhibited by azide (5-20mM). The human placenta is rich in Ca-Mg ATP'ase activity at term and this activity is associated with plasma membrane markers; its role in human placental Ca and Mg transport remains to be determined.

252 DEVELOPMENT OF CYCLIC AMP DEPENDENT PROTEIN KINASE IN HUMAN NEWBORN ADIPOSE TISSUE. Paul B. Wieser, Milan Novak, Maria Buch, Univ. of Miami School of Medicine, Dept. of Ped., Miami, Josef Skala, Univ. of British Columbia Centre for Developmental Medicine, Vancouver.

Human newborn adipose tissue possesses a high degree of metabolic activity. The rates of lipolysis and glycogenolysis are elevated to provide fatty acids and glucose for the increased energy demands of extrauterine life. Both of these processes are mediated in part by cyclic AMP dependent protein kinase.

The activity of cyclic AMP dependent protein kinase was determined in a group of normal infants ranging in age from 2 to 72 hrs. There was a significant increase in protein kinase activity after the first day of life, 0.23 ± 0.02 nmoles ^{32}P incorporated/mg/min in the absence and 0.41 ± 0.02 in the presence of cyclic AMP for less than 24 hrs old and 0.29 ± 0.01 in the absence and 0.54 ± 0.02 in the presence of cyclic AMP for greater than 24 hrs. However the protein kinase activity ratio, i.e. activity —cyclic AMP/activity + cyclic AMP was highest during the first 12 hrs of life indicating that the enzyme was in a highly activated state immediately after birth. The activity ratio for 6 newborns less than 12 hrs old was 0.61 ± 0.02 compared to 0.51 ± 0.01 for 4 newborns in the 13 to 24 hr age range.

The effect of high concentrations of sodium chloride on the equilibrium between active and inactive protein kinase was also determined on the enzyme isolated from adult adipose tissue.

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