DEVELOPMENT OF OXIDATIVE METABOLISM IN THE INTESTINE
Joseph B. Warshaw and R.E. Kimura, Yale Med. Sch., Depts. of Ped., Obs. and Gyn., New Haven, Connecticut

Fatty acids (FA) are the preferred substrate of adult small intestine. We have measured enzymes controlling FA oxidation (palmityl-CoA synthetase and palmitylcarnitine transferase) and FA oxidation itself during development of the rat and bovine intestine. Cytochrome oxidase activity of calf intestinal homogenates was twice that of the fetus, indicating a postnatal increase in mitochondrial number. Oxidation of palmitic and capric acid by bovine intestine increased from 2 to 10nm/mg/hr by three weeks of age and decreased to less than 1nm/mg/hr in the adult. Acetate oxidation by bovine intestine showed a similar developmental profile. Acetyl-CoA synthetase, palmityl-CoA synthetase and palmitylcarnitine transferase showed postnatal increases in activity. Developmental increases in FA oxidation were also observed with isolated rat intestinal mitochondria. Activity increased until 10 days of age, then declined until day 18, and again increased. Palmityl-CoA synthetase activity of rat intestinal mitochondria remained high throughout development whereas microsomal activity was low until day 18 when it increased markedly. Palmitylcarnitine transferase activity of rat intestinal mitochondria increased from 0.5 to 2.8 nm/mg/min by 10 days of age and then showed a decline to adult levels. These changes in FA oxidation may be important for functional maturation of the intestine and also reflect the importance of FA as substrates in the intestine.

SUBSTRATE OXIDATIONS BY EMBRYONIC RAT HEART CELL CULTURES.
Joseph B. Warshaw, Miriam D. Rosenthal, Yale Med. Sch., Depts. of Ped., Obst. and Gyn., New Haven, Connecticut

Beating monolayer cultures of fetal rat heart have been utilized to measure oxidation of labelled substrates to $^{14}CO_2$ and to investigate metabolic interactions of cells in culture. Increased rates of glucose oxidation by proliferating as compared to density inhibited heart cells is due to enhanced pentose phosphate pathway activity during proliferative growth. Oxidation of palmitate by rat heart cultures is independent of stage of growth and is linear with increasing protein concentration and time. Oxidation of palmitate is stimulated by addition of carnitine to the assay medium. Unlabelled palmitate spared the oxidation of glucose-^{14}C to $^{14}CO_2$ by 50%. Unlabelled glucose had no effect on the oxidation of palmitate. Palmitate did not inhibit lactate production by rat heart cell cultures incubated with buffer alone or with glucose. Embryonic rat heart cultures actively oxidized pyruvate-^{14}C to $^{14}CO_2$. As observed with glucose, addition of palmitate to the assay medium inhibited oxidation of pyruvate-^{14}C to $^{14}CO_2$ by 50%. In contrast, palmitate had no effect on $^{14}CO_2$ production from α-ketoglutarate. Similar to the adult heart, palmitate is the preferred substrate of fetal rat heart cultures. The data suggests that inhibition of glucose oxidation by palmitate is related to direct inhibition of pyruvate dehydrogenase. Cultured myocardial cells provide a relatively homogeneous source of tissue for investigation of metabolic interactions and substrate preferences of the heart.

GLUCOCORTICOID EFFECT UPON THYMIDINE KINASE IN DEVELOPING CEREBELLUM. Morton E. Weichsel, Jr.(Intr. by William B. Weil, Jr.) Dept. of Human Development, Col. of Human Medicine, Michigan State Univ., E. Lansing, Mi.

Glucocorticoids have been used in animal models and more recently in human studies as a means to accelerate neonatal lung maturation in order to prevent the respiratory distress syndrome in premature infants. Little attention has been paid to the possible effects of glucocorticoids upon developing organs other than lung. Recent studies of cortisol administration on DNA content in developing rat brain showed an early reduction as well as a permanent defecit in cerebellar DNA. In addition to confirming these findings, we have shown cortisol administration in the early neonatal period to have a profound effect on thymidine kinase, a salvage pathway enzyme for pyrimidine biosynthesis, in rat cerebellum during early development. Thymidine kinase normally peaks in activity in rat cerebellum at approximately 6 days of postnatal age and falls rapidly thereafter. With cortisol administration, enzyme activity is maximally suppressed at 6 days of age using both normal and undernourished controls. These findings suggest that neonatally administered glucocorticoids may have effects which are potentially detrimental to areas of the central nervous system which may be undergoing cell replication during the time the drug is administered. Thus the possible use of glucocorticoids for their salutary effects in the respiratory distress syndrome may necessitate consideration of any hazardous effects, however subtle, upon central nervous system development.

DNA SYNTHESIS AND THYMIDINE KINASE ACTIVITY DURING CEREBELLAR DEVELOPMENT: EFFECT OF THYROXINE. Morton E. Weichsel, Jr.(Intr. by William B. Weil, Jr.) Dept. of Human Development, Col. of Human Med., Michigan State Univ., E. Lansing, Mi.

Thyroid hormone administered from birth has been found to increase cerebellar DNA production in the rat by 6 days of age and to decrease DNA synthesis thereafter. This acceleration of development under hormonal influence has led to studies involving the relationship between thyroxine, DNA and activities of enzymes involving pyrimidine biosynthesis during cerebellar development. Using daily doses of thyroxine, we found rat cerebellar DNA to be increased significantly above control values by age 2 days until age 6 days. Following that, cerebellar DNA synthesis decreased to 78% of control values by age 12 days. The activity of thymidine kinase, a salvage pathway enzyme for pyrimidine biosynthesis, was found to be significantly elevated over control values by age 1 day. This elevation of enzyme activity in cerebella of treated animals continued until age 5 days, at a maximum of 137% of control values, following which activity fell significantly below control values by 9 days of age. Thyroxine thus appears to induce thymidine kinase activity in early cerebellar development and is apparently related to premature decrease of activity. These findings provide evidence that thymidine kinase may be subject to hormonal control, and may be a critical regulatory enzyme for cerebellar DNA biosynthesis.

ACID MUCOPOLYSACCHARIDES AS DISPERSING AGENTS IN LYSOSOMAL DIGESTION. R. Wolfe, M. Philippart, E. Lassila and S. Nakatani. Mental Retardation Unit, Neuropsychiatric Inst., Los Angeles.

Mucopolysaccharides are known to coat cell surfaces. They can thereby reach secondary lysosomes when the cell membrane invaginates to form pinocytic vacuoles. In the mucopolysaccharidoses, lysosomes accumulate enormous quantities of mucopolysaccharides. We propose that this is an exaggeration of a normal process, during which hydrolase activities are generally increased while a few are decreased. One of the intriguing problems of lysosomal physiology is the way in which water-insoluble substances such as lipids are digested. To accomplish this in vitro, detergents are needed. Some micellar system is most likely involved, as suggested by the occasional occurrence of myelin bodies even in normal lysosomes. We have compared sphingomyelinase activity using either Triton-X 100 and chondroitin sulfate or hyaluronic acid. Using a large concentration of mucopolysaccharide (up to 4 mg per ml of incubation mixture), activities close to those obtained with the detergent were observed. Since glycolipids accumulate in mucopolysaccharidoses, it would seem that either dermatan sulfate is not a suitable dispersing agent, or that it combines with the enzyme as suggested by the alterations in electrophoretic mobility which were reported by Kint et al, (Science:181, 352, 1973). (Supported in part by PHS Grant HD-04612.)

INHIBITION OF CELLULAR PROLIFERATION USING AN INHIBITOR OF ASPARTATE TRANSCARBAMYLASE. Takashi Yoshida, Norman Kretchmer, George Stark, and Nicholas Hoogenraad, Dept. Ped. & Biochem., Stanford Univ. Med. Ctr., Stanford, Calif. 94305

There is a direct correlation between the activities of enzymes for pyrimidine nucleotide biosynthesis and the proliferative activities of a wide range of cell types. Experiments with isoproterenol-stimulated salivary glands of mice further show that cellular proliferation is dependent on de novo pyrimidine synthesis. Effective inhibition of the de novo pathway, therefore, should lead to an impairment of cellular proliferation. We have used a transition-state analogue of aspartate transcarbamylase (ATCase), phosphonacetyl-L-aspartate (PALA) to test the dependence of proliferating cells on an elevated de novo pyrimidine synthesis. PALA is a potent ATCase inhibitor, having a K_i of $10^{-8}M$ for the enzyme from mouse spleen, and $10^{-10}M$ for certain tumor cell lines. PALA effectively killed transformed hamster kidney cells when added to a concentration of $10^{-4}M$ to culture medium. The specificity of action of PALA was indicated by the finding that uridine could completely remove the harmful effect of PALA by providing an alternative source of pyrimidine nucleotides. PALA is not metabolized to any significant extent, and is still effective 72 hours after a single injection of PALA (40 μg/g body wt). Preliminary experiments also show that PALA blocks isoproterenol-stimulated incorporation of thymidine into DNA of mouse salivary glands.