● **277** CHARACTERIZATION OF SURFACTANT PHOSPHOLIPID RELEASE FROM SUPERFUSED FETAL LUNG SLICES AND ISOLATED NEONATAL TYPE II CELLS. Thomas S. Jamison and Joseph B. Warshaw. The University of Texas Health Science Center at Dallas, Department of Pediatrics, Dallas, TX.

While factors controlling the synthesis of surfactant have been studied extensively, less is known about the cellular mechanisms controlling surfactant release. We have adopted an in vitro superfusion method for the study of surfactant release from fetal lung slices and isolated neonatal alveolar type II cells. In these tissues the surfactant phosphatidylcholine (PC) pool is radiolabeled with ^{3}H-choline. Release of PC is then quantitated by determining the level of PC in the perfusate following specific challenges with endocrine and pharmacologic agents. Both the β-agonist isoproterenol and prostaglandin E_2 were found to stimulate surfactant release in a dose-dependent manner. Cyclic AMP (cAMP) and the calcium ionophore A23187 also stimulated surfactant phospholipid release. In addition, the phorbol ester, TPA, was found to be a potent stimulus for surfactant release. The stimulated release of surfactant phospholipid by prostaglandin E_2 could be antagonized by the calcium channel blocker Nifedipine. From these studies it can be concluded that surfactant phospholipid release can be stimulated by a variety of agents acting on the adenylate cyclase-cAMP cascade, either via specific receptors or by activation of a phospholipid sensitive protein kinase C. The process of release is calcium sensitive and appears to be largely dependent on calcium fluxes.

278 RED BLOOD CELL MALONDIALDEHYDE (RBC-MDA) AND VITAMIN E TO TOTAL LIPID SERUM RATIO (E/TL) IN LOW BIRTHWEIGHT INFANTS. L Johnson, S Abbasi, C Dalin, R Dworanczyk. Univ. of PA Med Sch, Dept. Peds. Penn Hosp. Phila PA.

The RBC-MDA assay reflects adequacy of the overlapping antioxidant systems of the RBC, including glutathione, glutathione peroxidase, glutathione reductase, catalase, selenium, vitamin E and methemoglobin reductase. It gives no indication of the antioxidant protection afforded by serum proteins and so underestimates in vivo defenses. Membrane oxidant stress changes with ambient oxygen concentration, level of pro-oxidant metals, such as iron, and level and degree of unsaturation of membrane lipids. Equilibration of RBC membrane lipids with plasma lipids, which change at birth and with progression from parenteral to enteral feedings, requires about 2 weeks time. Membrane lipid equilibration in other tissues occurs more slowly. All of these factors affect tissue peroxidation rates. As seen in the table below, RBC-MDA assays on < 2000 g BW infants, performed at birth and during the first week on hyperalimentation and intralipid feedings, were usually abnormally high (> 150 nM/g Hgb or > 3 SD above normal adult mean), inspite of serum E/TL ratios well above normal for adults. Some degree of oxidant damage to cellular membranes following birth would appear to be inevitable.

	Birth (N = 47)			Week One (N = 41)		
RBC-MDA	E/TL Ratio (mg/g)			E/TL Ratio (mg/g)		
nM/g Hgb	< 1	1 to 2	> 2	< 1	1 to 2	> 2
< 150	0	8	3	0	6	0
150-250	3	9	6	2	4	3
> 250	2	8	8	3	11	12

● **279** SYNERGISTIC EFFECTS OF EPIDERMAL GROWTH FACTOR AND SOMATOMEDIN IN CHICK CHONDROCYTES. Stephen F. Kemp, J. Paul Frindik, Fu-Ju Ma, M. Joycelyn Elders. University of Arkansas for Medical Sciences, Department of Pediatrics, Little Rock, Arkansas.

Multiple growth factors (GF) have been isolated, characterized and shown to stimulate growth in at least one tissue. Some have been termed competence factors, while others are termed progression factors dependent upon their site of action in the cell cycle of 3T3 cells. Somatomedin (SM) is the major regulator of cartilage growth and glycosaminoglycan (GAGS) biosynthesis. Epidermal, fibroblast, platelet derived growth factors, and multiple stimulating activity (EGF, FGF, PDGF, and MSA) are known to be important for growth of other tissues. The combined effects of these GF factors with SM in cartilage cells have not been defined. We have studied the interaction of these GF with SM-C in embryonic chick cartilage cells using radioactive precursors. The cells are grown in Ham's F-12 nutrient mixture with 6% serum for 4-6 days following which the media is removed and replaced with media containing 0.5% albumin and the factor or factors to be studied. Radioactive precursors were added after one hour and incubation continued for 2-3 hours. In 2-12 hour incubations SM-C is stimulatory for $^{35}SO_4$ uptake, [^{3}H]-thymidine into DNA, [^{3}H]-uridine into RNA, and [^{14}C]-serine into protein with no potentiation by EGF, FGF, PDGF, or MSA. However, when the incubations are extended 24-30 hours, there is a synergistic effect of EGF and FGF on DNA, RNA, and protein synthesis with less potentiation of the SM effect on GAGS biosynthesis. The data suggests a synergistic interaction between SM and other GF for optimal cartilage growth.

● **280** ONTOGENETIC CHANGES IN LEUCINE DISPOSAL RATE, OXIDATION RATE AND PROTEIN SYNTHETIC RATE DURING FETAL LIFE. Jan M. Kennaugh*, Alan W. Bell*, Giacomo Meschia*, and Frederick C. Battaglia, Departments of Pediatrics and Physiology, University of Colorado School of Medicine, Denver, Colorado.

The successful chronic catheterization of sheep fetuses at mid-gestation (approx. 75 days) has permitted us to investigate the fractionation of leucine metabolism during early fetal life, with comparisons of similar measurements made by us at later stages of gestation.

Twelve pregnant ewes with gestations of 73-88 days were studied after 3-5 days of recovery from surgery for placement of catheters in the umbilical vein and artery, uterine vein, maternal artery and fetal peripheral vein. Uterine and umbilical blood flows were determined with the steady-state diffusion technique following a tritiated water infusion. ^{14}C-leucine was infused into the fetus at a constant rate and after a 2½ hour equilibration period the leucine disposal rate and the leucine flux to CO_2 and protein synthesis were calculated, employing a three-compartment model to quantify ^{14}C-leucine flux out of the fetal compartment. The leucine disposal rate within the fetal compartment was 9.16 ± 0.66 (SEM) umole kg^{-1} min^{-1}. Leucine oxidation measured by $^{14}CO_2$ production was 3.1 ± 0.57 (SEM) umole kg^{-1} min^{-1}, while leucine flux into protein was 5.96 ± 0.28 (SEM) umole kg^{-1} min^{-1}. The calculated fractional protein synthetic rate (K_s) was 0.214 ± 0.009 (SEM), which is significantly higher than that in late gestation fetuses. The importance of amino acids as fetal fuel is underscored by the quantification of a 30% oxidation rate for leucine at this gestational age.

† **281** THE SUPRA ADDITIVE EFFECTS: TRIIODOTHYRONINE (T_3) AND CORTISONE ON JEJUNAL Na-K ATPASE IN DEVELOPING RAT. R.E. Kimura and T. Campfield (Spon.by M. Simmons) Dept. of Peds., Univ. of Utah Med. Center, Salt Lake City, UT.

We determined the effect of T_3 and cortisone (cort) on jejunal Na-K ATPase in the developing rat. Na-K ATPase in jejunal homogenates increases two-fold at weaning (21 days). Since serum T_3 increases at weaning (20-24 days), we investigated the effect of T_3 on Na-K ATPase . One dose of T_3 (0.1 ug/gm body wt) administered at 17 days of age increases Na-K ATPase in suckling 18 day old pups to postwean levels. This activation does not occur in jejunum of 14 and 16 day old or postwean pups. Since endogenous glucocorticoids increase on days 16-18 of age, these data suggest that jejunum only respond to T_3 after exposure to glucocorticoids. To test this we treated pups at ages 10-13 days with (cort) (15 ug/gm body wt) and on day 15 with one dose of T3. Na-K ATPase was measured on day 16. Treatment with (cort) increased Na-K ATPase in 16 day old pup jejunum. The addition of T_3 and (cort) further stimulated Na-K ATPase to postwean levels in suckling 16 day old pups. These data suggest that the increase in jejunal Na-K ATPase of developing pups is mediated by T_3 and that the T_3 is only effective in enterocytes exposed to glucocorticoids.

Jejunal Na-K ATPase (μmol/mg prot/hr) (values=means±SE (no.))

AGE	TREATMENT			
Days	CONTROL	T_3	Cortisone	Cortisone+T_3
14	1.74±0.25 (4)	1.25±0.16 (4)		
16	1.61±0.16 (5)	1.85±0.24 (4)	2.35±0.11 (4)	2.85±0.30* (4)
18	1.87±0.59 (3)	3.24±0.03 (2)		
>27	3.54±0.42 (4)	3.62±0.56 (4)		

* statistically different when compared to cont and T_3 p<0.05

282 DEVELOPMENT OF FETAL CANINE HEPATIC METABOLISM. RM Kliegman, J Russell, S Morton. Case Western Reserve Univ, Rainbow Babies & Childrens Hosp, Dept Peds, Clev, OH

To determine the maturation of hepatic metabolism during the last week of gestation, 7 premature fetal pups(53d) were compared to 6 term fetal pups(60d); weight was 143±16 vs 253±12 g p<0.001. Hepatic tissue from term pups had greater glycogen (756 ±39 vs 308±11 umol/g p<0.001) and greater total glycogen synthase activity (31.7±4.9 vs 10.9±1.3 umol/min·mg protein p<0.01). Both the active and total glycogen phosphorylase activity was also greater at term. Hexokinase activity did not change, however, glucokinase increased at term (7.80±1.4 vs 4.52±0.62 p<0.05). Hepatic glucose and lactate levels did not change, however, glucose 6-phosphate was lower at term (0.073±0.006 vs 0.167±0.035 p<0.01). Pyruvate (0.188±0.013 vs 0.106±0.021 p<0.01) and phosphoenolpyruvate (0.108±0.013 vs 0.061±0.012 p<0.05) were higher at term while citrate was unchanged. Pyruvate kinase activities (1.3 and 6.6 mM-Km) increased during the last week of gestation while the active component of pyruvate dehydrogenase was also higher in more mature pups (2.67±0.71 vs 0.82±0.35 p<0.02). The gluconeogenic enzyme, phosphoenolpyruvate carboxykinase, did not change during this time. Hepatic energy status was augmented at term as ATP increased (2.28±0.12 vs 1.78±0.16 p<0.02). The increase of hepatic glycogen and ATP together with the developmental pattern of glycogen synthase, glucokinase, pyruvate kinase and pyruvate dehydrogenase suggests increased utilization of circulating glucose for both glycogen synthesis and glycolytic energy production at term. (values m±SE)