**● 289** ENDOGENOUS EPINEPHRINE (E) SECRETION REGULATES SURFACTANT RELEASE. J.F. Padbury, H.C. Jacobs, R.W. Lam A.H. Jobe, D.A. Fisher, UCLA School of Medicine, Harbor-UCLA Medical Center, Dept. of Pediatrics, Torrance, CA

Adrenergic mechanisms are important in the regulation of pulmonary surfactant metabolism. We gave SKF29661, a specific inhibitor of adrenal epinephrine synthesis to 15 pregnant New Zealand White rabbits to study the role of fetal adrenal epinephrine in the regulation of alveolar surfactant (AS) content in the immediate neonatal period. Does were given 0, 25, 50 and 100 mg/kg/d starting at 20 days gestation. Fetuses were delivered at 31 days by C-section (term:31 days). One-half of each litter was sacrificed immediately and the remainder were allowed to breathe for one hour and then sacrificed. After sacrifice, the adrenals were immediately removed; one was assayed for catecholamine content, and the other for PNMT activity. A thorough alveolar wash was then performed to recover the AS. Total AS phosphatidylcholine (PC) was determined by thin layer chromatography. SKF 29661 caused significant reduction in adrenal E content at each dosage ($p<.001$); maximal effect was 57% reduction at 100 mg/kg/day. There was a progressive decrease in alveolar PC with increasing dosage of SKF29661 ($p<.01$). Furthermore, adrenal E in both treated and control animals correlated positively with alveolar PC ($r=0.450$, $p<.001$). Conclusions: 1) SKF29661 crosses the placenta and inhibits fetal adrenal E production; 2) reduced fetal adrenal E is associated with decreased alveolar PC. These results suggest that in the neonatal period, endogenous E release plays an important role in the regulation of AS possibly by increasing secretion.

**● 290** GLUCOCORTICOID INDUCTION OF MATURATION OF GLUCOSE METABOLISM IN JEJUNAL CELLS. Gerald T. Reinersman and Robert E. Kimura (Spon. by M. Simmons). Dept. of Peds., University of Utah Medical Center, Salt Lake City, Utah.

[1-$^{14}$C] glucose is metabolized to $^{14}CO_2$ by the glycolytic and the pentose phosphate pathways and [6-$^{14}$C] glucose releases $^{14}CO_2$ primarily through glycolysis. We determined that glucose metabolism to $CO_2$ by intestine of suckling rat is low. After weaning at 20 day, [1-$^{14}$C] and [6-$^{14}$C] glucose oxidation to $^{14}CO_2$ rises by 24 day. The ratio of [1-$^{14}$C] glucose oxidation to [6-$^{14}$C] glucose oxidation (C1/C6 ratio) decreases indicating a relative increase in glycolysis at the time of weaning. Since an increase of endogenous glucocorticoid before weaning mediates changes in rat intestinal sucrase activity and mitotic index, we determined if these changes in glucose metabolism are mediated by glucocorticoids. After administering cortisone (1.5 mg/100 g body wt., ip.) on days 10 to 13 of age, [1-$^{14}$C] and [6-$^{14}$C] glucose oxidation did not rise prior to weaning, but increased 3-5 fold compared to control rate by 24 hours after weaning. The C1/C6 ratio decreased to postwean levels by 21 days. These results suggest that cortisone induces glycolytic enzymes, but metabolic rates do not change until diet changes with weaning.

| Age (days) | 16-20 | | 21 | | 23-24 | |
|---|---|---|---|---|---|---|
| substrate | control | Rx | control | Rx | control | Rx |
| | (nmoles $^{14}$CO/mg wet weight/hr) | | | | | |
| [1-$^{14}$C]glu | 0.51±.04 | 0.55±.06 | 0.49±.1 | 1.55±.3* | 1.3±.02 | 1.56±.33 |
| [6-$^{14}$C]glu | 0.21±.03 | 0.24±.02 | 0.16±.05 | 0.94±.1* | 0.86±.11 | 1.09±.16 |
| C1/C6 | 2.71±.31 | 2.51±.24 | 3.45±.75 | 1.66±.2* | 1.58±.22 | 1.46±.14 |

*t test ($p<0.01$) when comparing control and treated.

**● 291** ABLATION OF THE MATERNAL SUPRACHIASMATIC NUCLEI DISRUPTS THE TIMING OF THE FETAL CIRCADIAN CLOCK S.M. Reppert and W.J. Schwartz, Children's and Neurology Services, Massachusetts General Hospital, Boston, MA.

We have proven in the rat that a functional circadian clock oscillates in the fetal suprachiasmatic nuclei (SCN) and that the maternal circadian system coordinates the phase of the fetal clocks to ambient lighting conditions (Science 220:969, 1983). As the first step towards elucidating the mechanism of maternal coordination, we examined the effects of maternal SCN ablation. The $^{14}$C-deoxyglucose (DG) method was used to monitor metabolic activity of the fetal SCN. Timed pregnant rats were exposed to diurnal lighting throughout pregnancy. On gestational day 7, dams either received bilateral electrolytic SCN lesions or sham lesions. On gestational day 18, all were placed in constant darkness; dams were injected i.v. with 145 µCi/kg DG during either mid-subjective day or mid-subjective night on gestational day 21. Autoradiographs of serial sections of fetal brains from sham-operated dams revealed that the fetal SCN were all metabolically active during subjective day (n=18) and all inactive during subjective night (n=18). In contrast, after histologically confirmed complete SCN ablation in the dam, there was no significant day-night variation of SCN metabolic activity in the fetuses; activity was scattered over the inactive to active range within each of the 3 litters studied (8-10 fetuses/litter) at each of the two injection times. The results indicate that the maternal SCN are essential for coordination of the fetal circadian clock. (Supported by PHS Grant HD14427)

**● 292** THE DEVELOPING CIRCADIAN TIMING SYSTEM: FUNCTIONAL APPEARANCE OF LIGHT-DARK ENTRAINMENT. S.M. Reppert Children's Service, Mass. General Hosp., Boston, MA.

In rats, it is not until postnatal day 4 that the retinohypothalamic tracts, which convey entraining information about lighting from the retina to the circadian clock in the suprachiasmatic nuclei (SCN) in the adult, begin to innervate the SCN. We thus examined when during development retina-mediated entrainment occurs. Circadian output was monitored in rat pups by measuring the rhythm in pineal N-acetyltransferase (NAT) activity. Timed pregnant rats were exposed to diurnal lighting throughout pregnancy. In one study, on the day of birth half the pups in each of 10 litters and all dams were blinded (bilateral enucleation); all were then exposed to a reversed lighting cycle until day 10 when they were placed in constant darkness. The population profiles of NAT activity on postnatal day 11 were clearly rhythmic for both groups; the intact pups were entrained to reversed lighting while the blind pups were still in phase with the prenatal (diurnal) lighting cycle. In another study, on the day of birth only the dams were blinded. The blind dams with their intact pups were then exposed to reversed lighting until either postnatal day 6 or 8 (6 litters for each group) when the animals were placed and thereafter kept in constant darkness. Analysis of pup NAT profiles on postnatal day 10 showed that the pups exposed to reversed lighting until day 8 were synchronized to reverse lighting while those exposed to reversed lighting until day 6 were only partially synchronized to reversed lighting. Thus, extraretinal mechanisms do not entrain the developing circadian system and retina-mediated entrainment is evident by postnatal day 6.

**● 293** SURFACTANT RELEASE FROM ISOLATED TYPE II EPITHELIAL CELLS: ROLE OF MICROFILAMENTS (ACTIN). Ward R. Rice, Kevin C. Osterhoudt, Jeffrey A. Whitsett, Children's Hospital, University of Cincinnati

Alteration of cytoskeletal elements of secretory cells is associated with exocytosis, presumably by regulating microfilament (actin) and microtubule structure and function. We used cytochalasins (to promote microfilament depolymerization), rhodamine-conjugated phalloidin and fluorescein-conjugated monoclonal antibodies against actin and tubulin to partially characterize the role of the cytoskeleton in surfactant secretion by Type II epithelial cells in primary culture. Surfactant pools were prelabeled with 3H-choline to follow release of tritiated phosphatidyl choline (3H-PC). Cytochalasins A,B,C, and D enhanced release 2-3-fold, EC50 of 1,1,.5, and .1 uM, respectively, but did not enhance cytosolic cyclic AMP levels. Only A caused significant cytotoxicity as determined by release of intracellular lactate dehydrogenase. Dose response of 3H-PC release by C and D was biphasic (maximum response, 1.0-0.5 uM) decreasing toward control levels >1uM. D caused release by 1 hr and progressively increased release up to 3 hr. D-induced release was additive to that induced by terbutaline and forskolin. By fluorescent labeling, microfilaments were associated with lamellar bodies and D caused a change in cell morphology and microfilament/microtubule structure. Cytochalasin-induced release of 3H-PC is independent of cyclic AMP synthesis and is associated with alterations in cell morphology and organization of the cytoskeleton associated with lamellar bodies, further supporting the role of the cytoskeleton in surfactant release.

**294** GESTATIONAL AGE AND CORD SERUM BILIRUBIN BINDING. Dorothy A. Ritter, John D. Kenny (Spon. by V. H. Auerbach) Temple Univ. Sch. of Med., Temple Univ. Hosp., Dept. of Pediatrics, Philadelphia, PA.

The effect of gestational age on bilirubin binding by albumin was studied using cord serum obtained from 22 preterm infants, 13 term infants and samples obtained from 17 adults. Using the horseradish peroxidase method, the apparent unbound bilirubin concentration (AUBC) was measured with increasing amounts of total bilirubin (TB). Serum albumin was also determined. The resultant bilirubin titration curve was analyzed using the least squares fit of the empiric equation $Y = aX^b$ and calculation of the association constants (Ka) using Scatchard plots.

After correction for albumin concentration by plotting AUBC against the molar ratio of TB/albumin (R), term and preterm infants had identical curves while both were different than adults. At R of 0.6, AUBC for preterm infants was 46.1 nM/L ± 1.7 SEM; for term infants it was 49.8 nM/L ± 3.5 SEM (P=NS) and for adults it was 29.8 nM/L ± 1.6 SEM (both $P<0.001$). Using Scatchard plot analyses, the $Ka_1$ for preterm infants and term infants was 0.39 $X10^9M^{-1}$ ± 0.05 SEM and 0.48 $X10^9M^{-1}$ ± 0.15 SEM (P=NS); for adult albumin $Ka_1$ was 1.45 $X10^9M^{-1}$ ± 0.29 SEM (both $P<0.01$).

We conclude that after correction for albumin concentration, bilirubin binding does not change with gestational age. Molecule for molecule the binding of cord albumin is the same for preterm and term infants. This suggests that infant albumin binds bilirubin less effectively than adult albumin.