**● 259** STIMULATION OF FETAL LUNG PHOSPHATIDYLCHOLINE SYNTHESIS BY CONDITIONED MEDIUM FROM FETAL LUNG EXPLANTS. Ian Gross, Christine M. Wilson. Yale School of Medicine, Dept. of Pediatrics, New Haven, CT.

Explants of fetal rat lung develop in vitro in the absence of exogenous hormones in the culture medium. This suggests that the fetal lung itself produces a factor or factors which initiate and regulate lung maturation. We have examined the effects of medium obtained from fetal rat lung organ cultures on lung development in vitro. Serum-free conditioned medium (CM) was collected from cultures of 20d lung (term is 22 days) and tested in explants of 16 or 18 day lung. Exposure to CM for 48 or 72h enhanced the rate of choline incorporation into phosphatidylcholine in a dose dependent fashion. About 70% stimulation was observed with undiluted CM. The activity was not destroyed by freezing, heating to 60°C, or charcoal treatment (suggesting that it was not due to low M.W. hormones), but it was obliterated by exposure to trypsin, consistent with the stimulatory factor being a protein or polypeptide. Stimulatory activity was also not decreased by incubation of CM with monoclonal antibody to fibroblast pneumonocyte factor (kindly provided by Drs. Post & Smith), but there was synergism with both dexamethasone and $T_3$. When CM was collected for successive 24h periods from explants of 16 day lung, there was a progressive increase in stimulatory capacity with increasing time in culture. Fetal rat lung appears to produce a factor or factors which regulate its own development. Isolation and characterization of this factor is in progress.

**† 260** THE ADENINE NUCLEOTIDE (AdN) TRANSLOCASE (T) : A ROLE IN DEVELOPMENTAL CHANGES. Daniel E. Hale and John R. Williamson, Depts. of Peds and Biochem., Univ. of Pennsylvania School of Medicine, Philadelphia, PA.

Although developmental changes occurring during the first days of extrauterine life have been well-characterized in terms of enzymatic activities, little attention has been given to overall cellular energy metabolism. Investigations of energy metabolism in guinea pig liver mitochondria (m) have shown that m from the newborn (N) have membrane pH gradients and membrane electrical potentials similar to those found in adults. During the first 24 hours after birth, State 3 rates of oxygen consumption (OC) increase 3 fold whereas the uncoupled rates of (OC) increase only 30%. The m AdN increases 3 fold with no change in the ATP/ADP ratio. Titrations with carboxyatractyloside (CAT), a stoichiometric inhibitor of the m AdNT show that the N has only 50% of the AdNT activity of the adult. Furthermore, it is possible to evaluate the role of the AdNT on the regulation of respiration using flux control theory (Eur. J. Biochem. 42:97-105). Briefly, the degree of control (control strength) exerted by a particular step in a pathway is defined as the fractional change in pathway flux produced by a fractional change in the activity of the enzyme comprising that step; the sum of all control strengths in a pathway being equal to 1.0. By applying this theory to inhibitor titrations of the AdNT, it has been shown that at any rate of OC the AdNT has greater control strength over respiration in the N than in the adult. At maximal rates of OC the AdNT has a control strength of 0.98 in the N and 0.56 in the adult. Since the AdNT plays a central role in cellular metabolism and the N period is a time of high energy demand, it seems probable that the AdNT plays a critical role in development.

**261** NEWBORN RAT PUP ADRENAL CATECHOLAMINE (CA) DEPLETION BY COLD STRESS. J.Hannigan,L.Witek-Janusek,W.P.Zeller C.L.Anderson,K.Ozog and R.M.Hurley (Supp R&E#050-15-211) Loyola University,Stritch School of Medicine,Foster G.McGaw Hospital, Departments of Pediatrics and Physiology, Maternal-Child Nursing, Maywood, IL 60153 (Spon. by Lewis E. Gibson)

Stressors, such as hypoxia and asphyxia induce CA secretion by the newborn's adrenal medulla. The effect of cold-stress upon adrenal CA content in the neonatal rat was assessed by exposing day old fed rat pups to either control temperature (25°C) or cold-stress (4°C) for 5 mins. The pups were dispatched and adrenal glands were immediately processed for measurement of norepinephrine (NE),epinephrine (EPI) and dopamine (DA) using high performance liquid chromatography and electrochemical detection.

Mean ± SEM nmoles/gland

| | NE | EPI | DA |
|---|---|---|---|
| Control (n = 13) | 0.51 ± 0.05 | 0.74 ± 0.07 | 0.16 ± 0.02 |
| Cold-Stress (n = 10) | 0.29 ± 0.03* | 0.57 ± 0.10* | 0.22 ± 0.01* |

(* denotes p < 0.05 vs Control)

There was a significant depletion of both NE and EPI with a significant increase in DA($p<0.05$). The cold-stress used produces neither hypoxia nor respiratory acidosis (Barlow & Santulli, Surgery 77: 687-690,1975). The decreased adrenal CA content is then a direct response of the animal to the cold-stress. In conclusion, cold-stress,like hypoxia and/or asphyxia, depletes adrenal EPI and NE in the day old fed pup. The elevated DA suggests a compensatory CA synthesis in response to cold-stress. Full characterization of the CA response needs to be established.

**● 262** EFFECT OF TRIIODOTHYRONINE ON EPIDERMAL GROWTH FACTOR CONCENTRATION IN NEONATAL MOUSE EPIDERMIS AND BINDING KINETICS IN WHOLE SKIN ORGAN CULTURE AND MEMBRANE PREPARATIONS. S.B. Hoath, J. Lakshmanan, D.A. Fisher, Dept. of Pediatrics, Harbor-UCLA Medical Center, UCLA School of Medicine, Torrance, CA.

Littermate Swiss-Webster mouse pups were divided into control and T3 treated (500 ng sc/d x 5d) groups. Following sacrifice on the 5th day, dorsal skins were dissected and floated for 90 min in phosphate buffered saline (PBS), pH 7.3, containing .01M dithiothreitol. Epidermal sheets were separated, homogenized and assayed for EGF by homologous RIA. T3 treatment increased EGF levels (307±25 vs 159±10 pg EGF/mg Lowry protein; mean±SEM, $p<.01$). Circular sections (area 0.7 $cm^2$) of dorsal skin were suspended in 400 µl PBS+2.5% glucose containing ∿200,000 cpm $I^{125}$-EGF. Analysis of binding curves showed increased uptake of label in skin of T3-treated pups after 30 and 60 min incubation (1.26 and 1.27 x controls ($p<.01$). Binding data were normalized per mg wet weight of skin. Membranes were prepared from homogenate supernatants by centrifugation (2 spins for 30 min at 40,000 g) with resuspension of pellets in PBS+0.5% BSA. 75-125 µg membrane protein were incubated for 30 min with $I^{125}$-EGF. There was increased binding in the T3 group after passage of membranes over millipore filters (1.40 x control, $p<.03$). Summary and conclusions: 1) In vivo T3 treatment elevates EGF levels in neonatal mouse epidermis. 2) T3 increases $I^{125}$-EGF binding to whole skin in organ culture and to crude skin cell membrane preparations. 3) The increase in skin EGF induced by T3 may be due to T3 induction of EGF receptor binding.

**263** ARE SLEEP STATES INFLUENCED BY APNEA AND PREMATURITY Toke Hoppenbrouwers, Joan E. Hodgman, Kazuko Arakawa Nancy Alba, Manuel Durand and Luis A. Cabal. Univ. of So. Calif. Sch. of Med., Los Angeles County-USC Med. Ctr., Dept. of Pediatrics, Los Angeles.

Sleep and waking were studied in normal term and normal and apneic premature infants at 44 wks post-conceptual age. Percentages of active sleep (AS), quiet sleep (QS) and wakefulness (AW) were determined from polygraphic tracings obtained between 6-10 p.m. Term infants were selected by absence of disease and gestational age between 38-42 wks. Normal prematures were selected by absence of disease between 32 and 36 wks post-conceptual age. Apneic prematures were similar, except they had 2 or more apneic episodes ≥ 20 sec. within an 8 hour period after the first week of life for which no etiology could be found.

| | Number | % Total Sleep | % QS | % AS | % AW |
|---|---|---|---|---|---|
| Normal Term | 14 | 72.4* | 25.0 | 47.3* | 21.4* |
| Normal Premature | 9 | 56.6* | 24.9 | 31.7* | 32.8* |
| Apneic Premature | 8 | 70.3 | 26.0 | 44.3* | 22.0* |

*$p < 0.05$

In normal prematures total sleep time was decreased at the expense of AS, and wakefulness was increased. Apneic prematures were not different from normal term infants. Since QS was similar in all groups, maturation of the forebrain does not appear to be delayed. The data suggest a lower threshold for arousal in the normal premature. Stress produced by apnea in the premature infant appeared to accelerate development toward normal term patterns rather than to cause a delay.

**264** ENDOGENOUS CORTICOSTEROIDS AND LUNG MATURATION IN THE FETAL RABBIT. Raimund Huemmelink and Philip L. Ballard. University of California, Department of Pediatrics and Cardiovascular Research Institute, San Francisco.

Endogenous glucocorticoids are known to influence pulmonary development. We studied factors affecting the availability of cortisol (F) and corticosterone (B) to lung tissue of fetal rabbits during the last week of gestation. The adrenal content of F+B was 3.33 ± 0.7, 6.34 ± 1.2 and 8.74 ±0.5 ng/adrenal at 23, 27 and 30 d, respectively, with an F:B ratio of 0.5:1. Plasma F+B was maximal (2.32 ± 0.11 µg/dl) at 23 d, and decreased progressively to 0.72 ± 0.05 at 28-30 d; the F:B ratio was 3.7:1 between 21 and 28 d and 1.5:1 at 29-31 d. Corticosteroid binding globulin (CBG) capacity decreased from 36.0 µg F bound/dl at 23 d to 2.1 ∿g/dl in the newborn, whereas maternal levels increased from 6.2 ±0.6 to 40.8 ± 0.8 µg/dl. Fetal levels of albumin, a low affinity binder, increased from 1.13 to 2.29 g/dl. Plasma free F (by charcoal absorption) was 0.04 µg/dl at 23 d and 0.02 at 31 d. The dissociation constant ($K_D$) of F and B for nuclear binding in cultured explants of lung was 7.3 ± 0.1 and 70.6 ± 9.2 nM, respectively, and binding was decreased in the presence of either albumin or serum. Receptor concentration assayed both in vitro and in culture increased 48% from 23-30 d.

We conclude that F is the physiologically important corticosteroid in the fetal rabbit, but unlike other species, there is no developmental increase in the free circulating concentration. We suggest that factors other than adrenal production and serum binding determine whether there is an increase in F within the lung during development.