●236 PHOSPHATIDYLCHOLINE (PC) SECRETION BY CULTURED FETAL RAT TYPE II PNEUMOCYTES. April R. Dworetz, Mitchell J. Kresch, Seamus A. Rooney, Ian Gross Yale Univ Sch of Med, Dept. of Pediatrics, New Haven, CT.

Purinoceptor and B-adrenergic agonists have been shown to stimulate PC secretion by adult rat type II cells. We have studied the influence of these agents on secretion by fetal type II cells. The cells were isolated by explant culture, cell dissociation, differential adhesion, type II cell aggregation, and monolayer culture. To study secretion the cells were incubated with [^{3}H]-choline for 20h, followed by 90 minutes of exposure to varying concentrations of ATP or terbutaline. Purity of type II cell cultures ranged from 85 to 90%. Post culture viability was 97.3±0.2%. LDH release averaged 1.5%; no difference was observed between control and treated cultures.

Baseline secretion of PC from the fetal cells was 0.63±0.11%. We observed dose dependent enhancement of secretion by both ATP and terbutaline. Maximal stimulation by ATP occurred at 10^{-4}M. At this dose secretion was 1.24±0.28% (97% increase). Maximal stimulation by terbutaline was observed at 10^{-5}M (75% increase). These data differ from those in adult rat type II cells in which baseline PC secretion ranged from 1% to 3%. ATP and terbutaline produced greater stimulation in adult cells (approximately 400% and 100% respectively) than in fetal cells.

We conclude that fetal type II cells secrete PC. Baseline and stimulated secretion is lower in these cells than in adult cells. This suggests that although fetal and adult cells are morphologically similar, they are functionally different. (Supported by HD 07094, HL 19752, HL 31175.)

†237 MATURATION OF ANTIOXIDANT ENZYME ACTIVITY IN THE RAT SMALL INTESTINE. Elizabeth L. Engelhardt, James C. Beggs, Josef Neu. Spons. by Donald V. Eitzman. University of Florida College of Medicine, Department of Pediatrics, Gainesville, Florida.

Free radicals have been implicated in the pathogenesis of ischemic-reperfusion injury in the bowel. The protective role of the antioxidant enzymes has been studied, but no information exists on the development of these enzymes in the small intestine (SI). The ontogeny and glucocorticoid-enhanced maturation of superoxide dismutase (SOD), catalase (C), and glutathione peroxidase has been defined in the lung. We measured the activities of C, SOD, and the superoxide generating enzyme xanthine oxidase (XO), in the rat SI. Pregnant rats at 18, 19, and 20 days gestation received 4 injections of 0.2 mg/kg of dexamethasone or an equal volume of saline (S) over 48 hours. Rats aged 14-104 days were injected with 50 mg/kg of hydrocortisone or S. The rats were killed 12 hours after the last injection. Assays were done using homogenates of the SI. Glucocorticoid administration hastened the attainment of adult values for maltase and sucrase, but had no effect on the activity of C, SOD, or XO. The table presents the mean enzyme activity ± SEM expressed as units/mg of DNA.

AGE = days from term	XO(U/mg DNA)x10^{-2}	SOD (U/mg DNA)	C (U/mg DNA)
-2 (7)	4.1 ± 2.9	67.9 ± 12.5	116.2 ± 30.2
-1 (8)	8.5 ± 2.7	90.2 ± 11.6	133.0 ± 27.0
Term (11)	13.5 ± 2.3	84.8 ± 9.9	175.5 ± 23.9
+ 16 (18)	8.0 ± 1.8	135.3 ± 7.8	463.4 ± 18.7
+ 21 (20)	20.0 ± 1.7	151.3 ± 7.5	375.8 ± 18.1
+ 106 (12)	25.2 ± 2.2	166.0 ± 9.6	477.7 ± 23.2
( ) = n	p = 0.0001	p = 0.0001	p = 0.0001

The increase in activity with maturity suggests a less effective defense system in younger animals placing them at increased risk for free radical injury to the intestine. Exogenous glucocorticoids do not alter the activities of these enzymes.

238 LONG-LASTING BEHAVIORAL IMPACT OF NEONATAL ASPHYCTIC EPISODES IN RAT MALE SUCKLINGS. Angelo Ferrara, Yucel Atakent, Walter Lewis, David Quartermain. NYU School of Medicine, Departments of Pediatrics and Neurology, New York, N.Y.

It is well known that toxic and asphyctic insults in neonatal rat models have produced behavioral changes (in activity, memory and learning). The persistence of these effects longitudinally has not been well documented. 100 male pups from 22 Sprague-Dawley dams were randomly assigned in equal numbers to an asphyctic (7 asphyctic episodes in 4 days in individual vials) and to a control group, at 5 activity testing intervals (7,14,21,30,50 days of life), where independent groups of 20 animals were placed singlely in a drum actometer to record 10 min (5 trials of 2 min each) of learning activity. In addition, conditioned active avoidance response learning was examined, at 45-50 days of life. ANOVA with repeated measures (where needed) was used for analysis. $P < .05$ was considered significant. RESULTS: 1) the asphyctic group demonstrated a significant ($P < .05$) hyperactivity at 21 days of life (compared to control) and then a significant ($P < .05$) hypoactivity at day 50 of life. There was no difference in activity at 7,14 or 30 days of life. Intertrial habituation was intact in both groups at all ages. 2) There was no significant difference in active avoidance (number of trials to criterion & escape latency times) learning between asphyctic and controls and in memory testing. These behavioral findings suggest that postnatal asphyctic events in these model may inhibit the cholinergic system and induce early hyperactivity and late (50 days) hypoactivity (possibly) mediated by norepinephrine), but not alter the acquisition of conditioned avoidance responses.

239 EXOGENOUS INSULIN AND FETAL ABLATION: MECHANISMS FOR 2 MODELS OF FETAL MACROSOMIA IN THE RAT. Sandra L. Finley, Edward S. Ogata. Northwestern Univ. Med. School, Depts. of Peds., Ob/Gyn,, Chicago.

While insulin is a critical growth stimulating hormone during the newborn period, other factors as litter size affect fetal growth. We compared the effect of insulin alone versus reducing litter size upon growth. In one model of macrosomia, we injected fetal rats in situ with insulin (I) or saline (S) on day 18 of gestation (term 21.5). In the other, we reduced fetal number by selectively ligating (L) arteries in the secondary cascade of uterine vessels on day 15. Fetuses of sham operated mothers were controls (C). L reduced litter size to 5±2 fetuses, while C, I, and S averaged 8-10 fetuses. Newborn I pups weighed 5.21±.05g (C 5.01±.09g). I and L pups developed hypoglycemia to the same extent compared to their controls but at different points during the newborn period: I pups at 120 and 240 min (30.0±.3 v 45.9±5.7 mg/dl, p .01). L pups at 0, 20, and 60 min (73.2±2.6 v 91.0±4.9 mg/dl, p .01). At these times, I and L pups were hyperinsulinemic compared to their controls (I 46.8±9.6 v S 22.7±3.7 uU/ml, p .01; L 111.0±20.2 v C 52.5±12.0 uU/ml, p 01). Plasma glucagon did not differ between I and L and their controls during fetal and neonatal life (485.6→860.0 pg/ml). I and L pups had significantly elevated hepatic glycogen at birth and 20 minutes. Providing excess insulin without extra metabolic fuels can enhance fetal growth. Limiting litter size also accelerates fetal growth. The surprising finding of hyperinsulinemia and neonatal hypoglycemia in L suggesting that limiting litter size may enhance fuel provision to the remaining fetuses.

▲240 TRANSFORMING GROWTH FACTOR (TGF) ACTIVITY IN OVINE FETAL KIDNEY: POSSIBLE ROLE FOR TGF IN FETAL DEVELOPMENT. Michael Freemark and Marty Comer, (Spon. by Stuart Handwerger). Duke University Medical Center, Department of Pediatrics, Durham, NC

Recent studies demonstrating that epidermal growth factor (EGF) has biologic effects in the fetal lamb suggest a role for EGF or the EGF-like transforming growth factors (TGFs) in ovine fetal development. However the presence of EGF or TGF in the ovine fetus has not been established. To determine whether ovine fetal tissues contain EGF or TGF-like activity, an acid-ethanol extract of ovine fetal kidney was tested for the ability to induce growth of rat kidney fibroblasts in soft agar. The fetal kidney extract (20-800 ug protein/ml) stimulated a dose dependent increase in the number of soft agar colonies (>8 cells). Approximately 70% of these colonies measured ≥ 3750 uM^2. The fetal kidney extract was dissolved in 1 M acetic acid and chromatographed on Bio-Gel P10. Two peaks of TGF-like activity, with apparent MW 14K (peak 1) and 8K (peak 2), eluted from the column. Half-maximal effects of pooled peaks 1 and 2 were achieved using 100 and 20 ug protein/ml, respectively. Peaks 1 and 2 also competed with ^{125}I-mouse EGF for binding to EGF receptors in ovine fetal liver but had no activity in a homologous mouse EGF RIA. Neither TGF activity nor EGF receptor binding activity was detected in Bio-Gel fractions co-eluting with ^{125}I-EGF. These findings demonstrate that ovine fetal kidney contains TGF activity. The absence of EGF receptor activity in fractions co-eluting with ^{125}I-EGF suggests that TGF may predominate in the ovine fetus and may play an EGF-like role in ovine fetal development.

†241 INCREASED EPIDERMAL GROWTH FACTOR CONCENTRATIONS IN DONAHUE'S SYNDROME (LEPRECHAUNISM). Joseph P. Frindik, Stephen F. Kemp, M. Joycelyn Elders. University of Arkansas for Medical Sciences, Pediatric Dept. Little Rock, Arkansas.

Epidermal growth factor (EGF) is a well characterized polypeptide factor known to be important in growth and proliferation of multiple cell types. We have described a patient (Lep Ark I) with Donahue's syndrome whose phenotypic characteristics, i.e., severe acanthosis nigricans, hirsutism, pachyderma, precocious development of teeth and breast buds, and gut hypertrophy are consistent with hypersecretion or hyperfunction of epidermal growth factor. We previously demonstrated that this patient has increased secretion of epidermal growth factor in her urine (151.9±59.4 ug EGF/gm cr compared to 26.7±4.3 ug/gm in control children) but were unable to quantify plasma concentrations at that time. We have now developed a heterologous, double antibody radioimmunoassay (RIA) based on mEGF to estimate plasma levels of EGF in human plasma. Dilutions of the patient's plasma displace ^{125}I-EGF in a linear fashion producing a line parallel to that of the EGF standards. Mean plasma concentration levels of EGF in normal adults by this method are 4.8±1.7 (N=5) and in children 8.4±0.3 (N=5). In contrast, the mean plasma concentration of EGF in Lep Ark I is 20.2±3.3 ng/ml (N=4). Reported blood levels of EGF in humans have ranged from 273±46 pg/ml to 1.89±0.5 ng/ml. Our mean plasma concentrations for children were higher than those previously reported but were less than half the value found in Lep Ark-I. We conclude that the elevated urinary excretion of EGF in Lep Ark-I reflects hypersecretion in this patient, thus providing a disease state for this important growth factor.