259 HEART RATE AND RESPIRATORY RESPONSES TO HYPOXIA IN UNANESTHETIZED NEWBORN MAMMALS. J. P. Finley, C. Kelly, (Spon. by R. B. Goldbloom), Dalhousie University, Dept. of Pediatrics, Halifax, N.S., Canada.

The cardio-respiratory response to hypoxia is the subject of intense research in human infants, but is mostly limited to non-invasive studies. Animal investigation is an attractive alternative, but most animal studies have involved anesthetized or non-intact preparations, which may not reflect human physiology. The cardio-respiratory responses to hypoxia are not well documented in unanesthetized intact newborn animals. In particular, there is little information on the periodic breathing (PB) response to hypoxia and, hence, on the practicality of an animal model for PB.

We studied heart rate (HR) and respiratory responses during quiet sleep to 17% FIO_2 in unanesthetized full-term newborns of five species; lamb, piglet, puppy, kitten, and rabbit (N=31). ECG, HR and respirogram monitoring was used. There was no significant change in mean HR and respiratory rate (RR) with hypoxia for any species. Brief apneas >5 sec. were frequent(5-8/hr) both in 21% and 17% O_2, but only in lambs and puppies. No sustained periodic breathing was induced by hypoxia; although in three species, there was one brief episode.

We conclude that mild hypoxia has little effect on HR and RR in these unanesthetized newborns. Respiratory patterns showed some apneas in only two species, but these were not significantly affected by hypoxia. Periodic breathing was not frequent enough to make an animal model practicable. This may indicate considerable maturity of respiratory control in full-term newborns of these species.

260 FETAL NUTRITION: CALCIUM REGULATES HUMAN PLACENTAL UPTAKE OF AMINO ACID. Stanley E. Fisher, Peter I. Karl (Spon: M.C. Rattazzi). Cornell Univ Med Coll, North Shore University Hospital, Dept. Pediatrics, Manhasset, NY

Intracellular calcium regulates numerous cell functions, including amino acid uptake by rat diaphragm and hepatocytes (Arch Biochem 23:96; Proc Nat Acad Sci 77:5953). Therefore, we studied the effect of calcium (Ca) on the uptake of ∝-amino isobutyric acid (AIB) by human placental slices. During 45 minutes preincubation, tissue calcium was maintained (CaM), or was depleted (CaD) by 2.0 mM EGTA in Ca-free medium. Tissues were then incubated with 125 uM 14C-AIB for 60 minutes in medium with (+) or without (-) Ca. AIB uptake, expressed as the ratio of intracellular to extracellular AIB concentrations (Ci/Co), was 3 53 ± 0.13 (mean ± SE) in controls (CaM+), but was markedly reduced in CaD slices incubated without Ca repletion (CaD-): Ci/Co = 1.66 ± 0.08 ($p<0.001$). Ca repletion during incubation (CaD+) resulted in partial restoration of 60 min AIB uptake: Ci/Co = 2.64 ± 0.11 ($p<0.01$, compared to either CaM+ or CaD-). In time-course experiments, the slope of AIB uptake for CaD+ was parallel to CaM+ by 15 minutes and linear through 60 minutes. However, CaD- showed no AIB uptake after 30 minutes. Finally, 10 uM verapamil, a calcium channel blocker, reduced 60 minute AIB uptake by 17.5% ($p<0.05$). CONCLUSION: Calcium regulates human placental uptake of the actively transported, neutral amino acid, AIB. SPECULATION: The placenta may serve as a model for studying calcium regulated amino acid transport in human tissue.

● **261** ENDODERM-SECRETED FACTOR WHICH STIMULATES GROWTH OF STEM CELLS OF THE H6 EMBRYONAL CARCINOMA CELL LINE. Emily L. Germain and John W. Littlefield. Johns Hopkins University School of Medicine, Developmental Genetics Laboratory, Department of Pediatrics, Baltimore.

Interactions between undifferentiated and differentiated cells may be important in regulating the growth of the embryo. Undifferentiated stem cells of the embryonal carcinoma cell (ECC) line called H6 can be induced to differentiate to parietal endoderm-like cells by retinoic acid ($3x10^{-6}$ M). We have now found that these endoderm-like cells secrete a factor which stimulates the growth of stem cells.

A dilute inoculum of H6 stem cells grew very poorly in 0.5-1.0% fetal calf serum-supplemented Eagle's MEM (a few small, unhealthy colonies), but in the presence of H6 endoderm-like cells they grew as well as in 5% serum-supplemented medium (39% plating efficiency). There was no reciprocal stimulation of endoderm by stem cells, unlike a recently defined factor of the PC13 ECC line (Heath and Isacke, CSH abst., 9/84). Furthermore, the incorporation of ^{3}H-thymidine into the DNA of stem cells growing on a coverslip in 0.5-1.0% serum-supplemented medium was stimulated up to 40-fold by the presence of endoderm underneath the coverslip. Considerably less stimulation occurred when stem cells or fibroblasts were substituted for endoderm. Finally, 0.5-1.0% serum-supplemented medium conditioned by exposure to endoderm for 20 hours gave a 2-fold stimulation of ^{3}H-thymidine incorporation into stem cells as compared to unconditioned media. Again, less stimulation occurred with medium conditioned by stem cells or fibroblasts.

† **262** GLUCOSE AND INSULIN INHIBIT FETAL LUNG MATURATION IN VITRO. Ira H. Gewolb and William Merdian (Spon. A.R. Fleischman). Albert Einstein Coll. of Med., Bronx, NY

Delayed fetal lung development is a feature of the diabetic pregnancy. Although hyperglycemia and/or hyperinsulinemia have been suggested as causes of this pulmonary maturational delay, they are difficult to evaluate independently in vivo. We therefore studied the effect of glucose (G) and insulin (I), alone and in combination, on lung development in fetal rat lung in culture.

19-day fetal rat lung explants were cultured in F-12 medium (10mM G; ~300 mosm) and in F-12 media with a total of 50 mM G, 100 mM G, I (1 unit/ml), I + 50 mM G, and I + 100 mM G. Explants were cultured for 48 hours, then pulsed with H^3-choline for 4 hours. Incorporation of choline into phosphatidylcholine (pmol/hr/mg protein) was used as an index of fetal lung maturation.

10mM G	50mM G	100mM G	I	I+50mM G	I+100mM G
3410±578	2788±436*	2480±420*	2087±318**	2106±285**	2198±300*

paired t-test; * $p<.025$ vs. 10mM G, ** $p<.01$ vs. 10mM G

Explants cultured in media containing 40 and 90 mM added mannitol (osmolar controls) did not evidence the inhibition seen under the high G conditions. Moreover, uptake of choline from the media into the non-lipid fraction was not affected by changes in G, I, or osmolarity.

CONCLUSIONS: Choline incorporation is decreased 20-30% by increased G, and approximately 40% by I. There was no additive effect of high G and I in the doses used. We conclude that both G and I have a direct inhibitory effect on fetal lung maturation in vitro.

† **263** HISTOLOGIC ABNORMALITIES IN THE DIABETIC RAT PLACENTA Ira H. Gewolb, William Merdian, Joseph B. Warshaw, and Allen C. Enders. Albert Einstein Coll. of Med., Bronx, NY, U. of Texas Health Sci. Cntr., Dallas, TX, and U. of California Sch. of Med., Davis, CA.

In previous work with the diabetic (DM) pregnant rat we found placentas to be larger and fetuses smaller than normal. To study cellular differences that might contribute to the disparity in size and function of DM placentas, we analyzed by light and electron microscopy DM and control placentas on days 14, 18, and 22 (term). DM was induced using IV streptozotocin (45 mg/kg).

DM placentas (days 18 and 22) were marked by the presence of large numbers of glycogen-distended cells in the junctional zone. The trophoblastic layers in the interhemal membrane were also significantly thicker in the DM group, and large accumulations of lipid droplets were prominent. Since there is normally a striking thinning of the labyrinthine placental barrier by day 22, the thickness of age-matched DM placentas is more impressive, and possibly increases the diffusion distance in the DM placentas. Moreover, 22-day DM placentas contained more glycogen and rough endoplasmic reticulum in the inner trophoblastic layer, a feature similar to 18-day control placentas.

Thus, DM placentas have a number of features that are normally seen earlier in gestation. Histologic evidence confirms increased glycogen and lipid in both the junctional zone and in the cellular barrier between maternal and fetal blood. We speculate that the thickening and modifications of the cellular barrier in DM may affect fetal growth and nutrition by increasing the diffusion distance between the maternal and fetal circulations.

= **264** STIMULATION OF PHOSPHATIDYLCHOLINE (PC) SECRETION BY LEUKOTRIENES C_4, D_4 AND E_4 IN TYPE II CELLS ISOLATED FROM ADULT RAT LUNG. Alasdair M. Gilfillan and Seamus A. Rooney. Dept. Ped., Yale Univ., New Haven, CT.

We previously reported that incubation of type II cells with 1-10 μM arachidonic acid (AA) stimulated PC secretion and that this effect was blocked by 0.1 μM nordihydroguaiaretic acid and 10 μM indomethacin (I) but not by 0.1 μM I or 10 μM flufenamic acid, ibuprofen or naproxen. Since these data suggested that lipoxygenase products of its metabolism mediated the effect of AA we examined effects of leukotrienes on PC secretion in type II cells. Cells isolated from adult rats were cultured for 20 h in medium containing serum, antibiotics and ^{3}H-choline, transferred to fresh serum-free medium and incubated with leukotrienes for 90 min. ^{3}H-PC in cells and medium was then measured. LTC_4, LTD_4 and LTE_4 but not LTB_4 stimulated PC secretion and this effect was concentration dependent in the range 10^{-12}-10^{-6} M. In 7 experiments LTC_4, LTD_4, and LTE_4 (1 μM) increased the rate of PC secretion by 63%, 81% and 100% from 0.7±0.04 (^{3}H-PC in the medium as % of that in cells + medium) in the control cultures to 1.14±0.08 ($P<0.001$), 1.27±0.12 ($P<0.002$) and 1.40±0.12 ($P<0.001$), respectively. The concentration of LTE_4 which produced half maximal stimulation (EC_{50}) was $5x10^{-12}$ M. Leukotriene receptors have been reported in intact lung tissue. Whether these effects are mediated by specific leukotriene receptors in type II cells remains to be established.

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