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ORGANOTYPIC CULTURES OF RAT PULMONARY EPITHELIAL CELLS: MORPHOLOGIC DIFFERENTIATION. Cynthia Daugherty, Carol Dion, Jeffrey Whitsett. Children's Hospital Medical Center, Cincinnati, Ohio

Surfactant is synthesized by Type II pneumocytes which arise by differentiation of pre-Type II cells. In the present study, differentiation of pre-Type II cells was characterized in organotypic cultures of lung epithelial cells isolated on day 18 and 19 of gestation. Cells were incubated in 10% carbon-stripped fetal calf serum on collagen pads for 3-4 days in presence and absence of (1 μ M) T₃ and/or hydrocortisone (HC); epithelial cells were then plated in monolayers. Morphologic differentiation was assessed after isolation from the collagen matrix. Synthesis and secretion of surfactant apolipoproteins A (apo A) was used as a biochemical marker of differentiation. Epithelial cells from cultures in carbon stripped media were poorly differentiated consisting of uniform populations of cells in clumps with abundant cytoplasmic polyribosomes and glycogen. Cells (co-cultured with fibroblasts) in the presence of HC with and without T₃, demonstrated marked differentiation of purified epithelial cells. Morphologic markers of differentiation included: 1) acinar structures, microvillous lined lumens, defined junctional complexes surrounded by spindle cells, 2) loss of cytoplasmic glycogen, 3) appearance of lamellar bodies and tubular myelin. Synthesis and secretion of apo A was demonstrated by 2D-IEF-SDS-PAGE. Differentiation increased with time of culture, was enhanced by T₃ and hydrocortisone, maximal effects on differentiation observed with both hormones. We demonstrate *in vivo* differentiation of Type II cells in organotypic culture and stimulatory effects of HC and T₃ on Type II cell differentiation.

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EFFECT OF BETAMETHASONE (B), TRIIODOTHYRONINE (T₃) OR B+T₃ ON MATERNAL (M) AND FETAL (F) GLUCOSE (GLU)-GLYCOGEN (GLY) METABOLISM. U. Devaskar, J. Church, V. Chechani, F. Sadiq and S. Devaskar. (Spon. by W. J. Keenan). St. Louis University, School of Medicine, Cardinal Glennon Children's Hospital, St. Louis, MO.

A potential for B+T₃ therapy in prevention of IRDS of the newborn has been recently proposed (J.C.I. 74,898,1984). Some of the major growth and metabolic effects of B and/or T₃ therapy on the M and F were determined. B (85 μ g/kg), T₃ (175 μ g/kg), B+T₃ (85 and 175 μ g/kg) or the vehicle were administered I.M. on d 25 and 26 of pregnancy to rabbit doe. F wt, F and M plasma GLU, insulin (I) and T₃ concentration, F and M cardiac (Ca) and hepatic (H) GLY content were quantitated on d 27. All data $\bar{X}$ ±SEM (*P < 0.05 vs control (C)). n = No. of litters.

(n)	Fwt (gm)	GLU (mg/dl)		I (μ U/ml)		T3 (ng/ml)		μ mol-GLY-U/gm					
		M	F	M	F	M	F	Ca			H		
C-16	29 $\pm$ 1	123 $\pm$ 3	68 $\pm$ 5	7 $\pm$ 4	19 $\pm$ 4	2 $\pm$ 1	.9 $\pm$.1	3 $\pm$ 1	16 $\pm$ 2	45 $\pm$ 8	28 $\pm$ 3		
B-10	*22 $\pm$ 1	146 $\pm$ 6	*93 $\pm$ 6	15 $\pm$ 3	23 $\pm$ 4	2.6 $\pm$.5	*2 $\pm$.6	5 $\pm$ 1	*2 $\pm$ 1	*74 $\pm$ 37	*8 $\pm$ 3		
T3-6	29 $\pm$ 1	130 $\pm$ 9	*48 $\pm$ 15	8 $\pm$ 2	13 $\pm$ 4	30 $\pm$ 4*	.3 $\pm$.1	3 $\pm$ 1	*4 $\pm$ 1	*13 $\pm$ 4	*7 $\pm$ 1		
T3+B-5	30 $\pm$ 2	143 $\pm$ 6	*59 $\pm$ 6	5 $\pm$ 1	15 $\pm$ 6	*18 $\pm$ 5	*4 $\pm$.1	5 $\pm$ 1	*6 $\pm$ 1	*18 $\pm$ 5	*7 $\pm$ 1		

Conclusions: 1) Runting effect of B is prevented when administered with T3 2) Major differences in M and F are noted:
(a) T3 and/or B cause F but not M hyperglycemia (b) T3 and/or B deplete F but not M Ca GLY (c) B deplete F but increase M H GLY and (d) T3 deplete M and F H GLY.

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AN ADVENTAGEOUS ASYMMETRY IN THE BURSTING PRESSURE OF FETAL MEMBRANES. Rolf R. Engel, James Levine, and Debra Abrams. Hennepin County Medical Center, Department of Pediatrics, Minneapolis, MN.

The association between prematurity and early rupture of amniotic membranes prompted studies of the tensile strength and elastic limit of human fetal membranes. Chorio-amniotic membranes were obtained from 14 uncomplicated, term pregnancies at the time of elective, repeat C-section. None of the mothers had been in labor, all of them had intact membranes prior to the section, and all of the infants were normal. Multiple sites were sampled from each amniotic sac by mounting samples between two circular surfaces (O.D. 3 cm) with a central tapered round hole (diameter 0.45 cm). Progressive increases in the pressure provided by a tank of compressed nitrogen were plotted by a X-Y plotter against the displacement of a rod by the stretched membrane up to the point of bursting.

Human membranes had marked (>3 fold) variability in bursting pressure and elastic limit as compared to synthetic membranes of silicone or polyethylene polymer. There was a positive correlation between the pressure and the elastic limit at the point of bursting for samples of amnion (r=.53, p<10⁻⁶), chorion (r=.53, p<10⁻⁶), and the intact bilayer of the two membranes together (r=.27, p<10⁻⁶) but not for the synthetic membranes. The combination of amnion and chorion was about 7% stronger (p<.003) when pressure was applied from the fetal side as opposed to the maternal surface, although the bursting pressure of amnion or chorion alone was independent of the direction from which it was applied.

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CARCASS ANALYSIS OF THE FETAL AND MATERNAL GUINEA PIG, William A. Engle, James A. Lemons, Indiana University School of Medicine, Indiana University Hospitals, Department of Pediatrics, Indianapolis.

The guinea pig is a small animal species in which the conceptus constitutes a large proportion of maternal weight at term, thereby imposing a major metabolic demand on the mother during pregnancy. In addition the neonatal fat content is similar to the human, making the guinea pig an interesting model for comparative physiologic study. The purpose of our study was to describe the fetal and maternal physical/chemical growth characteristics of the Hartley albino guinea pig throughout the latter half of gestation. Sixty-seven adult and 122 fetal guinea pigs were sacrificed at intervals through gestation and the carcasses analyzed for a variety of growth parameters. Fetal growth is exponential with the fetal mass comprising 25% of the maternal mass at term. Fetal fat and protein content increased exponentially during gestation comprising 9.8% and 16.3% respectively, of the fetal mass at term. Water content diminished exponentially with the 30 day fetus containing 91.6% water versus 67.8% at term. Accretion of energy in the fetal tissue was also exponential, with the energy content of the term fetus being 6025±138 cal/g dry weight. In contrast to the changes in fetal composition, maternal fat, protein, energy and water content remained relatively constant throughout pregnancy. This compositional analysis of the fetal and maternal guinea pig has provided important baseline data for further *in vivo* metabolic investigation into the nature of fetal growth aberrations.

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THE INDUCTION OF OXIDATION BY THYROXINE AND CORTISONE IN RAT PUP JEJUNUM. Steven H. Erdman, Gerald Reinersman and Robert E. Kimura, (Spon. by Michael A. Simmons), Dept. of Pediatrics, Univ. of Utah, SLC, UT.

We determined if increased jejunal glucose oxidation is related to physiologic surges of glucocorticoids (16-20 days) and thyroxine (T₄) (20-24 days) during weaning. We administered cortisone (15 μ g/gm body wt IP) on days 10, 11, 12 and 13. Thyroxine (1.0 μ g/gm body wt SubQ) was given on days 14 and 15, or day 15. Littermate controls received saline injections on the same days. At sacrifice on day 16, jejunal slices were incubated with [1-¹⁴C] glucose, and CO₂ production was measured.

GLUCOSE OXIDATION (nmoles/gm wet wt/hr±SEM)					
Control	Cortisone T ₄ (15d)	T ₄ (14,15d)	Cortisone-T ₄ (14,15d)	T ₄ (14,15d)	Cortisone-T ₄ (14,15d)
# of Pups	8	5	4	6	3
	0.61±.07	0.83±.05	0.85±.04	1.21±.17	2.12±.58

Cortisone-T₄ (14,15d) group is significantly higher when compared to all other groups (p<.05). Thyroxine given on day 15 produces no change in glucose oxidation. Treatment with cortisone increases oxidation of CO₂ (35%). Thyroxine given at 14 and 15 days increases oxidation (200%). Administering T₄ on days 14 and 15 to cortisone treated pups increases oxidation in 16 day old suckling pups to postwean levels (23 day old; 2.24±0.10 n=6). We conclude cortisone and T₄ have a synergistic effect on the early induction of glucose oxidation which was not seen when either hormone is given separately. We speculate metabolic changes during the weaning period in rat jejunum are mediated by the combined effect of glucocorticoids and thyroxine.

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THE VALIDITY OF ACTIVE AVOIDANCE OPERANT LEARNING & RETENTION TESTING IN DETECTING ASPHYCTIC (ASPHX) EFFECTS IN RAT SUCKLINGS (RS). Angelo Ferrara, Yucel Atakent, David Quartermain, Julie Topsis, Pratibha Ankola, Mathilda Klupsteen. New York University School of Medicine, Departments of Pediatrics and Neurology, New York, N.Y.

Difficulty has been noted in learning (maze performance, etc.) in adult rats who have had neonatal asphyxia. Growth & development changes have also been cited. This study planned to detect learning problems in asphx RS by an active avoidance operant test. 25 sex-specific asphx RS (subjected to 4 days of asphyxia at 24 hrs. apart) & 30 controls were studied. All were then trained to avoid a .6m Amp foot pad shock in a V-trough chamber with light stimulus. The # of trials needed to avoid being shocked 9 out of 10 times was then calculated & compared. Retention testing was done at 24 hr. & 1 wk. post training.

Table 1: # of Training Trials by Rx Group & Day of Life		
Day	Asphx $\bar{X}$ ± 1 SD	Control
18	15.3 ± 4 (N=3)	16.8 ± 6.2 (N=5)
19	15.2 ± 4.8 (N=5)	14.7 ± 4.5 (N=6)
21	15.2 ± 3.2 (N=9)	14.5 ± 3.9 (N=10)
22-28	16.4 ± 4.8 (N=8)	16.1 ± 5.1 (N=9)

Results: 1) no signif. diff. was noted between the mean # of trials of all asphx & controls (N=55). 2) females needed fewer trials up to 21 days of life (13.9±4.7) compared to males (16.1±4.0) P<.1, a NS diff. 3) on A retention test, there was NS diff. between asphx, controls & naive controls. Passive avoidance or other models need to be explored as valid instruments for asphx NB rats.