229 PHASIC HEART RATE CHANGES IN PREMATURE NEONATES IN RESPONSE TO SENSORY STIMULATION.

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Heart rate may vary as a function of sensory and central processes. Previous studies in term infants have indicated a phasic response to stimulation, with an acceleratory response at 6 wks of age followed by a deceleratory phase later in infancy. Although a deceleratory response to sensory stimulation may be obtained in the term infant in the first few days of life, it is difficult to elicit. In newborn animal studies, the shift of heart rate changes occurs during a time of rapid neurochemical and neurostructural development. The present work evaluates changes in heart rate in healthy premature infants elicited by a startle reflex. Five infants with mean gestational age at birth of 30 wks (range, 28-32 wks) were studied at 34 weeks' conceptual age. Infants were connected to a pneumocardiogram for continuous monitoring of heart rate and respiratory rate. The sensory stimulation consisted of an eye-blink eliciting device consisting of a miniature solenoid with a teflon striker and a miniature photoreflexive densiometer attached to a TDH 39 earphone to assess eyeblinks. During the study period, infants were in active or undifferentiated sleep. A biphasic response of deceleration-acceleration was seen with a mean decrease in heart rate from baseline of 12% (range, 6-21%) followed by a mean increase from baseline of 7% (range, 4-10%). This data suggests that the prematurely born infant is capable of an orientation-attentional process. Although the 34 wk gestation infant is neurodevelopmentally immature, the response to stimulation suggests a maturational change consistent with cortical control over subcortical structures.

● 230 CELL PROLIFERATION IN HUMAN FETAL SMALL INTESTINE P. Arsenault, D. Ménard. Département d'anatomie et de biologie cellulaire, Faculté de médecine, Université de Sherbrooke, Québec, Canada (sponsored by M. Pleszczynski).

Cellular kinetic data are not available for human fetal intestinal tissue. Therefore an investigation was undertaken to establish the tritiated thymidine (^3H -TdR) incorporation into DNA and the labelling indices (LI) in the epithelium (E), mesenchymal (ME) and the muscular (M) layers between 8 and 15 weeks gestation. Explants of small intestine were cultured in serum-free Leibovitz L-15 medium at 37°C in 95% air-5% CO₂. The LI were established on radioautographs of explants cultured for 2 h in presence of 2 μCi of ^3H -TdR per ml of culture medium. The incorporation of ^3H -TdR into DNA was evaluated after a 6 h period and results expressed as DPM/ μg DNA. Between 8 and 10 weeks' gestation, the LI were 28, 14 and 9% for respectively the E, ME and M layers. Between 10 and 12 weeks there was a decrease of the LI which remained more or less constant between 12 and 15 weeks (15, 9 and 6%). Labelled epithelial cells were noted at all levels of the epithelium prior to or at the beginning of villus formation and later on were observed only in the intervillous area. The addition of hydrocortisone (50 ng/ml) to the culture medium during 5 days induced a significant increase (98%) of ^3H -TdR incorporation into DNA. This overall increase of DNA synthesis was reflected at the epithelial cell level where the LI increased by 57%. These experiments provide some basic data on cell proliferation in fetal intestine and indicate that explant culture technique should be useful for the study of the regulators of cell proliferation during the development of human intestine.

231 EXPLANT CULTURE OF HUMAN FETAL ESOPHAGUS, STOMACH AND COLON P. Arsenault, D. Ménard. Département d'anatomie et de biologie cellulaire, Faculté de médecine, Université de Sherbrooke, Québec, Canada (sponsored by M. Pleszczynski).

We recently reported the successful maintenance of human fetal intestinal tissue for 9 days in serum-free organ culture (Ménard and Arsenault, Gastroenterology, in press). This investigation was undertaken in order to evaluate the possibility to extend to other fetal digestive tract tissues this serum-free organ culture technique. Explants of fetal esophagus, stomach and colon (10-15 weeks gestation) were maintained in serum-free Leibovitz L-15 medium at 37°C in 95% air - 5% CO₂. As determined by light and electron microscopy the overall architecture of esophagus, stomach and colon explants was maintained for 9, 21 and 15 days, respectively. Furthermore, the epithelium of the stomach continued to differentiate. The incorporation of ^3H -thymidine and ^3H -leucine continued during the culture period, reflecting a sustained synthesis of DNA and proteins. On the other hand, the incorporation of ^3H -glucosamine increased during the culture, pointing out to the possibility of an accelerated glycoprotein synthesis. As determined by radioautography, the proliferative cells were detected in the basal layer of the ciliated columnar epithelium of esophagus, at all levels of the stomach epithelium and in the intervillous area of the colon. These observations clearly established that the entire human fetal digestive tract can be maintained in the same complete controlled environment. Therefore, this explant culture technique should be a useful tool for the study of the development of human fetal digestive tract.

232 SEX SPECIFIC DIFFERENCES IN RESPIRATORY TOLERANCE IN RAT SUCKLINGS (RS) UNDERGOING ASPHYCTIC EPISODES.

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Postnatally administered asphyctic insults to newborn rats has had an effect on growth & development compared to controls (Growth, 1984, 48, 120-133). Older literature suggests that adult female rats are more resistant to asphyctic episodes. To test this in RS, data were collected from 22 male asphyctic (MA) (day 1: $\bar{X}$ wt. 7.4 $\pm$ 6gm) & 24 female asphyctic (FA) (day 1: $\bar{X}$ wt. 6.8 $\pm$.5gm) RS. The asphyctic process was carried out in single fixed volume 22 ml. airtight capped jars & the outcome measure was the time interval to reach 30" apnea. ANCOVA was done (regressing time to reach 30" apnea on wt. for same day of life) to detect sex-specific time difference in day & wt.-adjusted pups.

TABLE: MEAN WTS. & ASPHYCTIC TIME $\pm$ 1 SD BY SEX & DAY OF LIFE	DAY 1		DAY 2		DAY 3		DAY 4	
	MA	FA	MA	FA	MA	FA	MA	FA
WT. (GM)	7.3 $\pm$ 6.8	6.8 $\pm$ 8	7.5 $\pm$ 8	7.5 $\pm$ 9	8.6 $\pm$ 9.9	8.6 $\pm$ 9.9	9.9 $\pm$ 9.5	9.5 $\pm$ 9.5
UNADJUSTED*	61	69	45	45	30	32	23	24
WT. ADJUST.*	67	64	48	42	32	30	25	23

Results: (See Table) 1) the mean unadjusted time interval to reach 30" apnea was higher for females than males (all days NS)** 2) by ANCOVA, the mean wt. adjusted time interval to reach 30" apnea were greater** in males for all days. The resistance to asphyxia seen in adult female rats could not be shown in asphyctic RS.

*mean time in minutes

**P > .05 (NS)

● 233 INTERACTION OF GLUCOCORTICOID, THYROID HORMONE AND PROLACTIN IN STIMULATION OF PHOSPHATIDYLCHOLINE (PC) SYNTHESIS IN CULTURED FETAL LUNG. Philip L. Ballard,

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To investigate hormonal interactions in regulation of surfactant synthesis, we assayed PC synthesis in organ cultures of fetal rat (18 d), rabbit (23 d) and human (17-25 wk) lung maintained in serum-free Waymouth's medium for 2-6 d. Explants were exposed to no hormones, dexamethasone (Dex, 10-100 nM), T₃ (2 nM), prolactin (PRL, 0.2-2 $\mu\text{g}/\text{ml}$) or combinations thereof. PC synthesis was assayed by incorporation of ^3H -choline into PC for 4 h in 3-11 experiments. Results (mean $\pm$ SE) are shown below.

	PRL	Dex	Dex+PRL	T ₃ +Dex	T ₃ +Dex+PRL
	(% stimulation vs control)				
Rat	0 $\pm$ 0	35 $\pm$ 3	44 $\pm$ 9	102 $\pm$ 23	114 $\pm$ 33
Rabbit	3 $\pm$ 1	113 $\pm$ 19	123 $\pm$ 25	213 $\pm$ 25	197 $\pm$ 31
Human	8 $\pm$ 9	195 $\pm$ 18	196 $\pm$ 20	242 $\pm$ 36	201 $\pm$ 46

In contrast to published reports for rat and human lung, we found no significant effect by PRL either alone or in the presence of other hormones. Results were equivalent for 2 sources of PRL (NIH & Sigma), a range of exposure times (1-6 d), a range of doses (0.001-8 $\mu\text{g}/\text{ml}$), incorporation rate of ^3H -acetate into PC and distribution of cpm among phospholipids (rabbit), incorporation of choline into saturated PC (rat), and rocker vs lens paper/grid culture systems (human). We conclude that Dex and T₃ have optimal effects on surfactant synthesis in cultured fetal lung in the absence of exogenous PRL.

234 ABERRANT ZINC BINDING IN TESTES OF TESTICULAR FEMINIZATION RATS. James M. Bates, Jr., Wai-Yee Chan, Kyung W. Chung and Owen M. Rennett. Departments of Pediatrics and Anatomical Sciences, Oklahoma City, OK.

Testicular feminization (Tfm) is an inherited form of male pseudohermaphroditism. In the rat, this syndrome is manifested by an absence of androgen dependent differentiation, small cryptorchid testes and infertility. Previous studies in our laboratory had established that cryptorchid testes of these rats showed drastically reduced zinc content and failure of zinc retention. The present studies attempt to further characterize this aberrant metabolism of zinc in the Tfm rats. Testes from normal littermates were used as control in all experiments. Distribution of endogenous zinc in the testicular cytosol was examined by chromatography through a Sephadex G-75 column (1.5X90 cm) equilibrated with 10 mM Tris-HCl, pH 8.0. Both zinc content and 280 nm absorbance of fractions collected were determined. Normal control testes showed typically four zinc peaks: peak 1 at void volume, peak 2 corresponded to M_r 30k, peak 3 corresponded to M_r 10k and peak 4 at the wash volume. Peak 2, however, was missing when cytosol of cryptorchid testes of the Tfm rats were analyzed. Column fractions corresponding to zinc peak 2 of the cryptorchid and normal control testes were pooled and concentrated separately and examined by electrophoresis through 12% polyacrylamide gel containing 0.1% SDS. A protein band with M_r 23k was missing in the cryptorchid testes. Whether the missing protein of the cryptorchid testis was related to the absence of zinc peak 2 and the decreased testicular zinc content in the Tfm rat is under further investigation. (Supported in part by NIH grant HD 16730).