218 CELL PROLIFERATION IN HUMAN FETAL GASTROINTESTINAL TRACT. Pierre Arsenault, Daniel Ménard. Département d'anatomie et de biologie cellulaire, Faculté de médecine, Université de Sherbrooke, Québec, Canada (Spon. by Marek R. Pleszczynski).

Cellular kinetic data are not available for developing human gastrointestinal (GI) tract. Therefore an investigation was undertaken to establish tritiated thymidine (^{3}H-TdR) incorporation into DNA and labeling indices (LI) in the epithelium (E), mesenchyme (ME) and the muscular (M) layers between 10 and 17 weeks' gestation. Explants of esophagus, stomach, small intestine and colon were cultured in serum-free Leibovitz L-15 medium at 37°C in 95% air - 5% CO_2 (J. Pediatr. Gastroenterol. Nutr. 1985; 4:893). The incorporation of ^{3}H-TdR into DNA was evaluated during a 6 hour period and results expressed as DPM/µg DNA. The LI were established on radiòautographs of explants cultured for 2 hours in presence of 2 µCi of ^{3}H-TdR per ml of culture medium. The incorporation of ^{3}H-TdR into the total DNA was highest at 12 weeks gestation in the esophagus and stomach and decreased steadily over the next 4 weeks, while the total DNA synthesis remained relatively constant in the small intestine and colon. However, the establishment of LI showed the highest epithelial LI in the small intestine (28.8%) and colon (18%), and the lowest in the esophagus (11.8%) and stomach (9.4%). This pattern remained during development although the LI decreased (16 weeks: 9.2, 11.4, 5.2 and 5.4%, respectively). On the other hand the ME and M Layers of the stomach always exhibited LI comparable to that of their respective epithelia (12 weeks: E, 9.4; ME 9.5; M, 9.3; 16 weeks E, 5.4; ME, 5.5; M, 5.3%). In the small intestine, the LI was always highest in the E and lowest in the M. This pattern was acquired by the colon once the villi had developped. The localization of the labeled cells was correlated with the morphogenesis of the different tissues of the GI tract. This study provides for the first time basic data on cell proliferation in fetal GI tract, and indicates that explant culture should be useful for the study of the regulators of cell proliferation during human development.

219 DECREASED LYMPHOCYTIC BETA ADRENO-RECEPTOR BINDING CAPACITY IN APNEA OF INFANCY. David Bader, S Buckley, TG Keens and D Warburton, Developmental Lung Biology Research Center, Childrens Hospital of L.A., USC School of Med., L.A., C.A.

High catecholamine levels can inhibit respiratory drive. Infants with idiopathic apnea of infancy (AOI) have elevated circulating catecholamine levels. Therefore we hypothesize that infants with AOI may have decreased adreno-receptor binding capacity, different receptor affinity or different receptor function. We studied 10 infants with AOI (mean age 8±3 months, $\bar{x}$ ±SE) and 10 healthy controls (mean age 10±3 months:NS) in order to measure: (a) circulating catecholamine levels, (b) maximal binding capacities, (Bmax) for β receptor on lymphocyte (BS/L) and for α receptor on platelets (BS/P), (c) adreno-receptor affinity to specific antagonist ^{125}ICYP (K_D), and (d) Agonist-induced formation of CAMP: isoproterenol stimulated to basal CAMP concentration (I/B).

	Nor Epi (pg/ml)	Epi (pg/ml)	β Bmax (BS/L)	β K_D (pm)	αBmax (BS/P)	α K_D (pm)	I/B
AOI	733±253	415±141	507±60	28±6	167±8	2.6±0.3	1.50±0.16
CON	285±38	280±69	904±82	56±10	191±7	2.1±0.3	1.51±0.17
P	< 0.01	NS	< 0.01	< 0.05	NS	NS	NS

We conclude that infants with AOI have decreased lymphocytic beta-adrenergic binding capacity, lower K_D and therefore increased affinity. There was no difference in agonist induced formation of CAMP. We speculate that decreased adreno-receptor binding capacity and increased affinity may play a role in the etiology of AOI.

220 REDUCED NEONATAL C-REACTIVE PROTEIN RESPONSE Robert D. Baker (Spon. by Peter Newberger)

C-reactive protein (CRP) is an acute phase liver protein used clinically to indicate the presence of infection in premature and full-term infants. I found neonatal rabbits respond much less vigorously to a CRP stimulus (IM injection of turpentine) than do adult rabbits. Sera from stimulated and control animals, both neonatal and adult, were depleted of albumin and subjected to 5-15% gradient SDS-PAGE under reducing conditions. Equal amounts of total protein were loaded in each lane. Gels were stained with Coomassie blue. In adult stimulated serum there was a prominent band at about 23,000 daltons, the published molecular weight of reduced CRP. This band was absent in control sera and faintly visible in the lane corresponding to neonatal stimulated serum. To further demonstrate a diminished CRP response in neonatal rabbits, sera were analyzed by Ouchterlony against anti-CRP and anti-alpha$_2$macroglobulin (anti-A2M). A vigorous adult stimulated serum CRP response was found. However only trace amounts of CRP were detected in neonatal stimulated serum and no CRP was detected by this method in either neonatal or adult control sera. In contrast, all four types of sera demonstrated strong precipitin lines with anti-A2M. The observed reduced CRP levels were not due to a shift in response time or dose-response phenomenon. Maximal CRP response for neonates and adults was found to be at 72 hours. Dose response experiments demonstrated a maximum response at 0.58 ml/kg for neonates and 0.45 ml/kg for adult rabbits. Development of the CRP response in human neonates is not known, however these studies suggest that developmental stage may be a factor in interpreting CRP levels in neonates.

●221 SURFACTANT SYNTHESIS IN ISOLATED LUNG CELLS. Philip L Ballard, Helen G Liley, Linda W Gonzales, Robert Ertsey, Samuel Hawgood, Dept Peds and Cardiovasc Res Inst, Univ Calif San Francisco, Dept Peds, Mt Zion Hosp, San Francisco, CA.

Explant culture and hormone treatment increase synthesis of saturated phosphatidylcholine (PC) and surfactant apoprotein SP 28-36 in fetal type II cells. We asked whether these effects were maintained during cell monolayer culture. Human fetal lung (18-22 wk gestation) was cultured as explants for 5 d with 10 nM dexamethasone plus 2 nM T_3; then type II cells (85% pure) or mixed cells (54% type II) were prepared and maintained as monolayers in MEM+ 10% fetal calf serum for 1-5 d.

	Day 1	Day 5	p
Choline → PC (nmol/mg DNA/4 h)	36.8 ± 6.3	32.2 ± 12.9	NS
Saturation (%)	34.9 ± 3.2	25.6 ± 2.6	<.05
Acetate → PC (nmol/mg DNA/4 h)	16.1 ± 1.1	7.1 ± 2.4	<.05
Saturation (%)	37.5 ± 2.4	26.4 ± 2.7	<.05
SP 28-36 (µg/mg DNA)	1.8 ± 0.4	< 0.1	<.05

During culture the distribution of acetate changed (decrease into PC and lyso PC and increase into most other phospholipids) and the number of lamellar bodies in type II cells decreased progressively. The changes were similar in type II and mixed cell populations and were not prevented by the presence of T_3 plus dexamethasone. In fibroblasts (87% pure) choline incorporation increased from d 1 to d 5 (17.1 ± 6.1 to 39.3 ± 7.7 nmol/mg DNA/4 h) with no change in saturation (25.5 ± 0.8 to 22.6 ± 2.5%) consistent with membrane PC synthesis. We conclude that synthesis of both lipids and protein of surfactant rapidly ceases (dedifferentiation) when fetal type II cells are cultured as monolayers.

●222 ETHANOL INDUCED DEPRESSION OF DNA SYNTHESIS IN ASTROCYTES. W. Thomas Bass and Joseph J. Volpe. Washington University School of Medicine, St. Louis Children's Hospital, Departments of Pediatrics, Neurology, Biological Chemistry, St. Louis, MO.

The principal neuropathological abnormality in fetal alcohol syndrome is a disorder of neuronal migration. This developmental process is directed by radial glial (astrocytic) cells. One potential deleterious effect of ethanol on these cells is an inhibition of proliferation. To address this possibility we utilized primary cultures of dissociated neonatal rat brain, which we and others have shown to consist of >90% astrocytes. Cell proliferation was assessed by measurement of DNA synthesis (from [^{3}H]thymidine) and protein deposition. The effect of ethanol, added on day 3 (the time of first cell attachment), on DNA synthesis on day 7, the time of peak cell proliferation in control cells, and on protein deposition on day 10 is shown in the Table, as percent of control. The activity of glutamine synthetase (an astrocyte specific enzyme) on day 18 is also shown.

mM Ethanol:	8.6	17.2	86	172
DNA synthesis	100 ± 1%	80 ± 2%	72 ± 1%	54 ± 2%
Total protein	100 ± 1%	94 ± 2%	81 ± 3%	66 ± 6%
Glutamine synthetase activity			77 ± 1%	49 ± 2%

The relative specificity of the effect of ethanol was shown by demonstrating no comparable effect on glycoprotein synthesis (from [^{3}H]mannose) or on sterol synthesis (from [^{14}C]acetate). The data thus show a significant decrease in DNA synthesis in developing astrocytes at concentrations of ethanol observable within the range of human intoxication (10-40mM). (NIH-HD-07464)

†223 EFFECTS OF HYDROCORTISONE ON HUMAN FOETAL KIDNEY IN SERUM-FREE ORGAN CULTURE. Lyne Bertrand, Normand Brière. (Spon. by Marek R. Pleszczynski). Département d'anatomie et de biologie cellulaire, Faculté de médecine, Université de Sherbrooke, Québec, Canada.

The influence of hydrocortisone (HC) on the in vitro maturation of human foetal kidney was studied. Following legal therapeutic abortions, explants (2 x 2 mm) of renal cortex from foetuses aged 12-18 weeks, were placed on a lens paper covering a stainless steel grid lying over the central well of a Falcon culture dish. The explants were cultured for 2 and 5 days in serum-free Leibovitz L-15 medium at 37°C in a mixture of 95% air-5% CO_2, without hormones (controls) or with HC at concentrations of 12.5, 25 or 50 ng/ml. During the studied period, the overall architecture of the renal structures was preserved without evident signs of nephrogenesis. DNA synthesis was measured by incorporation of ^{3}H-thymidine and was increased on day 5 by 80% with the addition of HC at 12.5 ng/ml and by 131% with 50 ng/ml. The sites of ^{3}H-TdR incorporation were the same after HC addition. The activity of some brush border hydrolases was modified by HC. Gamma-glutamyltranspeptidase activity was reduced by 68% on day 2 and by 42% on day 5 with 12.5 ng of HC. Maltase activity was increased by 19% on day 2 with 50 ng of the hormone. Trehalase activity was reduced by 61% with 12.5 ng and by 50% with 50 ng of HC, on day 5. Alkaline phosphatase activity was decreased on day 2, by 50% with 12.5 ng and by 39% with 25 and 50 ng of HC. This study provides for the first time, basic data on the direct effects of hydrocortisone on proliferation and brush border enzyme activities in the human foetal kidney maintained in organ culture. (MRC of Canada)