272 ONTOGENY OF ERYTHROPOIETIN (EP) KINETICS IN FETAL, NEWBORN, AND JUVENILE SHEEP. Thomas A. Malone, John A. Widness, Alan Mufson, William Oh. Brown University, Women and Infants Hospital, Department of Pediatrics, Providence, Rhode Island, and Genetics Institute, Cambridge, Massachusetts

EP regulates erythropoiesis in the fetus (F), newborn (N), and juvenile (J). Delineating the developmental changes that occur in EP kinetics would be important in explaining the possible role that EP plays in the pathophysiology of anemia and polycythemia. We investigated the differences in EP kinetics in the F, N, and J by infusing ^{35}S labelled human Ep into 5 F (126 day), 6 N (7-8 day), and 4 J (270 day) sheep. After bolus infusion, timed blood samples were obtained and the plasma was precipitated with 20% trichloroacetic acid. Plasma half life ($T_{\frac{1}{2}}$), volume of distribution (V_d) and EP clearance (C_{ep}) were determined from the beta decay curves of ln EP vs time:

Group	$T_{\frac{1}{2}}$ (hours)	V_d (ml·kg^{-1})	C_{ep} (ml·kg^{-1}·min^{-1})
Fetus (F)	*16.3 ± 4.7	516 ± 182	0.36 ± 0.05
Newborn (N)	11.2 ± 3.6	------	-------
Juvenile (J)	9.2 ± 1.7	320 ± 66	0.42 ± 0.17

* $p<0.05$ F vs N and F vs J --- = not determined

F Ep $T_{\frac{1}{2}}$ is prolonged when compared to N and J ($p<0.05$). $T_{\frac{1}{2}}$ decreases as F and N mature ($r= -0.53$, $p<0.05$). We conclude that the F, N, and J have differences in Ep kinetics. These differences, which reflect maturational processes, may play a role in the pathophysiology of anemia and polycythemia.

†273 MULTIPLE β-ADRENOCEPTORS ARE PRESENT IN ADULT BUT NOT FETAL SHEEP KIDNEYS. Tony McKelvey, Christian Felder, Kenneth Nakamura, Jean Robillard, and Pedro Jose. Georgetown Univ and Univ Iowa Med Ctr, Depts of Peds, Iowa City, IA and Washington, DC

β_2-adrenergic induced renal vasodilatation is greater in fetal than adult sheep. To determine a mechanism involved in this age related response, we characterized the β adrenoceptor in fetal and adult renal cortical membranes (30,000 xg fraction containing both vascular and tubular membranes) using radioligand binding studies. Specific binding of the non-selective β adrenergic antagonist ^{125}I-pindolol (defined by 1 uM (-)-propranolol) was 80%. Receptor density from Rosenthal plots (B_{max}, fmol/mg protein), inhibition constant (K_i,M), and % distribution of receptor subtypes using the β_2 adrenergic antagonist, ICI 118,551, from competition experiments (M±SE) are:

	K_i	B_{max}	% subtype β_1	% subtype β_2
Fetus (n=5)	$2\pm1E^{-8}$M	22.8±3.8	0	100
Adult (n=5)	$7\pm2E^{-9}$M	19.6±3.5	52±4	48±4

Ki for (-)-isoproterenol and the β_2 agonist zinterol were similar in both fetus and adult. However, stereoselectivity was greater in adults than fetuses: similar K_i for (-)-propranolol but K_i for (+)-propranolol less in fetus ($6E^{-8}$M) (n=3) than adult ($1E^{-7}$M)(n=3),($p<.05$ ANOVA).

We conclude that β adreceptors in fetal kidneys are less differentiated than those in adults. The greater density of β_2 receptors in fetal kidneys may explain in part the greater β_2 vasodilatory response in fetal than adult kidneys.

274 EVIDENCE FOR THE PRODUCTION OF TROPHIC FACTORS FOR NEURAL CELLS BY ANEURONAL SEGMENTS OF EMBRYONAL CHICK GUT. J.H. Carel Meijers, Dick Tibboel, Arthur W.M. van der Kamp, Jan C. Molenaar (Spon. by Thom E. Lobe). Erasmus University School of Medicine, Dpt. of Pediatric Surgery; Dpt of Cell Biology and Genetics, Rotterdam, The Netherlands.

A striking property of neural crest cells is their migratory behaviour. Eventually they aggregate in the gut. We investigated whether myenteric and submucous clusters of neural cells in the gut still can invade aneuronal gut.

Aneuronal chick gut (E4) in combination with innervated quail gut of different developmental stages (E6, E10, E12) was cultured on the chorioallantoic membrane of the chicken embryo: an in vivo culture system. We were able to demonstrate the presence of quail neural cells in chick gut using nucleolar differences of chick and quail cells (visualised with Feulgen DNA staining).

We observed that neuronal cells of quail gut are able to invade aneuronal chick gut.

Our results indicate that neural crest cell aggregation is not an irreversible process. We postulate that aneuronal segments of the gut produce a diffusible factor, which has a trophic effect on neural cells in neighbouring innervated gut.

†275 PUTATIVE MESENCHYMAL RECEPTOR CELLS FOR MIGRATING NEURAL CREST CELLS IN ANEURAL SEGMENTS OF EMBRYONAL CHICK GUT. J.H. Carel Meijers, Dick Tibboel, Arthur W.M. van der Kamp, Jan C. Molenaar (Spon. by Thom E. Lobe). Erasmus University School of Medicine, Dpt of Pediatric Surgery. Dpt of Cell Biology and Genetics, Rotterdam, The Netherlands.

To clarify the regulation of the neural crest cell migration in the gut, we investigated whether cell adhesion molecules, that are expressed by neural crest cells (NCAM, NgCAM, HNK-1), are also expressed by other cells in aneuronal segments of the gut. We therefore cultured isolated segments of aneuronal gut of four and five day old embryos on the chorioallantoic membrane. Immunostaining, with antibodies directed against cell adhesion molecules, revealed a diffuse band of HNK-1 stained mesenchymal cells, located inside the developing smooth muscle layer. We call this HNK-1 mode I expression. Immunostaining of cultured proximal, innervated segments of the gut of E5 embryos revealed a concentration of the HNK-1 epitope at the sites of the myenteric and submucous plexus. We call this HNK-1 mode II expression. The E/C8 antibody, directed against a neurofilament associated protein, was used to demonstrate presence or absence of neural crest cells in the gut. To determine whether the difference in HNK-1 expression was related to the presence of neural crest cells, we cultured aneuronal gut together with the neural anlage (neural tube and neural crest). Immunostaining revealed that in these co-cultures HNK-1 mode II replaced HNK-1 mode I expression. These results suggest the presence of a subpopulation of mesenchymal cells in the gut, that is involved in neural crest cell migration and aggregation.

276 GLYCOPROTEIN SYNTHESIS IN HUMAN FETAL ESOPHAGUS, STOMACH, SMALL INTESTINE AND COLON. Daniel Ménard, Pierre Arsenault, Département d'anatomie et de biologie cellulaire, Faculté de médecine, Université de Sherbrooke, Québec, Canada. (Spon. by Marek R. Pleszczynski).

Glycoproteins represent important components of the cell. No data are available concerning glycoprotein synthesis in esophagus (E), stomach (S), small intestine (SM) and colon (C) between 12 and 17 weeks' gestation. Explants of E, S, SM and C were cultured in serum-free Leibovitz L-15 medium at 37°C (J. Pediatr. Gastroenterol. Nutr. 1985; 4:893). Glycoprotein synthesis was evaluated by the incorporation of ^3H-glucosamine (^3H-Glu) into total protein during a 6 hour period and results expressed as DPM/µg protein. Explants were also processed for radioautography in order to visualize glycoprotein synthesis in the epithelial as well as in the non-epithelial compartments. The incorporation of ^3H-Glu into total protein remained more or less constant between 12 and 17 weeks' gestation. The levels were similar in E, S and C and the SM exhibited the highest ^3H-Glu incorporation. Radioautographs illustrated newly formed glycoproteins in the epithelial and non-epithelial compartments of the different tissues of the gastrointestinal tract. Explants from E, S, SM and C were cultured during 5 days and glycoprotein synthesis evaluated at the beginning and the end of the culture (6 hours in presence of 10 µCi ^3H-Glu per ml). In all cases, glycoprotein synthesis drastically increased during the culture, the increases representing 196, 183, 66 and 337% respectively. In the SM, the increase of the total glycoprotein synthesis was reflected in part by an increment of important membrane glycoproteins, that is the brush border membrane hydrolytic enzymes. Such correlation was not found in the colon. Whether the increased synthesis observed in culture represents an accelerated maturation of fetal tissues remains to be elucidated. However, the present data strongly suggest the presence of glycoprotein synthesis inhibitor(s) in utero during human gastrointestinal tract development.

●277 **CEREBRAL ANTI-OXIDANT MECHANISMS OF THE FETAL GUINEA PIG BRAIN DURING GESTATION AND THE EFFECT OF MATERNAL HYPOXIA.** **Om P. Mishra and Maria Delivoria-Papadopoulos. Univiversity of Pennsylvania School of Medicine, Dept. of Physiology, Phila., PA 19104**

The anti-oxidant enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP), glutathione reductase (GR), and glucose-6-phosphate dehydrogenase (G6PD) were measured in the developing fetal guinea pig brains. Following anesthesia, one group (n=36) of fetal guinea pigs were removed from normoxic mothers and another group (n=36) from mothers subjected to hypoxia (FiO_2 7% for 40 min). Fetuses of 30, 35, 40, 45, 50 and 60 days gestation were studied. Fetal brains were removed, homogenized, and analyzed for enzyme activities. The activity of the anti-oxidant enzymes remained constant during 30, 35, 40, and 45 days but increased sharply after 45 days of gestation: CAT from 3.04±0.53 to 5.17±0.75 umoles H_2O_2/min/mg protein (pr); GP from 25.40±3.50 to 49.66±3.29 nmoles NADPH oxidized/min/mg pr; GR from 18.9±2.30 to 32.54±4.83 nmoles NADPH oxidized/min/mg pr; G6PD from 28.90±4.60 to 38.69±1.39 nmoles NADPH formed/min/mg pr; and SOD from 25.46±4.57 to 32.11±2.85 units/mg pr between 45 and 60 days of gestation. In hypoxic fetal brains at 45 and 60 days of gestation, the enzyme activities were (units as above): CAT 2.67±0.54 and 5.13±1.27; GP 18.80±3.30 and 53.70±2.56; GR 20.60±2.90 and 32.84±3.81; G6PD 24.60±3.90 and 37.97±6.05; and SOD 28.77±4.93 and 27.74±2.21. The data shows that maternal hypoxia leading to fetal hypoxia did not affect the activity of anti-oxidant enzymes of the fetal brain. Although the guinea pig brain is enzymatically mature at term, it is proposed that the brain during early gestation has underdeveloped anti-oxidant enzyme defense mechanisms and is at potential risk from oxidative free radical reactions during normoxia as well as hypoxia. (NIH Grant # 5-RO1-HD 20337).