† 265 FACILITATED MATURATION OF THE INTESTINAL MICROVILLUS MEMBRANE (MVM) BY THYROXINE: A CLUE TO THE DEVELOPMENT OF THE INTESTINAL BARRIER.

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It has been noted in the past that the MVM of newborn animals differs from adult MVM in IgG binding, enzyme activity, and membrane fluidity (MF). In this investigation we studied the influence of thyroid hormone (T4) on MVM maturation, particularly in relation to MF and IgG binding, two factors that control antigen uptake and transport in the intestine. Pregnant (17 day gestation) and 11 day old rats were given intraperitoneal T4 (50mcg/100g body weight) for three successive days. Control and T4-treated rats delivered normally and the MVM of the newborns (37 treated, 82 controls) and the 14 day olds (15 treated, 21 controls) were examined for enzyme activities. Results confirmed that sucrase activity can be induced and lactase activity is depressed by T4 induction. MF was performed using electron spin resonance spectroscopy. IgG binding to 0.2 mg/ml MVM protein was done using ^{125}I-rat IgG. Results indicated that: 1) MF is decreased (MVM is more organized) in T4-treated newborns; 2) IgG binding is decreased in T4 treated animals as compared to controls (13% vs 20% in newborns and 19% vs 43% in 14 day olds at 2mcg/ml IgG). IgG binding to MVM is concentration dependent and saturable (range .17-100 mcg/ml IgG) in all groups, demonstrating specificity of binding. In conclusion, T4 induces MVM maturational changes, particularly decreased MF and decreased IgG binding, factors important in macromolecular uptake. This may play a role in mucosal host defense in the newborn period.

† 266 ISOLATION AND CULTURE OF FETAL LUNG ENDOTHELIAL CELLS.

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Endothelial cells are one of the three major cell types of the lung parenchyma and serve as the lung's interface with the circulation. These cells have been previously isolated from adult lung. We have now succeeded in isolation and culture of endothelial cells from fetal lung. Lungs are removed from 19 day (term = 22 d) fetal rats, minced, and dissociated into individual cells with trypsin. Fibroblast numbers are reduced by an initial attachment to plastic culture flasks for 1 hr, after which the remaining cells are centrifuged and incubated as a pellet for 1 hr at 37°C. The cells are then resuspended and injected into collagen sponges (Gelfoam^{R}). Endothelial cells and some fibroblasts float free of the sponges and attach to the underlying flask over the next 24 hr. The fibroblasts are then eliminated by trypsinizing the attached cells and allowing the fibroblasts to attach to a fresh tissue culture flask for 3 hr, following which a pure fraction of endothelial cells remain and are replated. These cells are identified by their characteristic "cobblestone" morphology and positive stain for factor VIII by immunofluorescence. There is strong contact inhibition which diminishes after one week in culture. This method will provide a means to study the role of the endothelial cell in fetal lung development.

(Supported by NIH grants HL-27372 and HL-27352)

267 POSTNATAL ACCUMULATION OF ADENINE NUCLEOTIDES IN RABBIT LIVER MITOCHONDRIA AND THE DEVELOPMENT OF CITRULLINE SYNTHESIS.

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Liver mitochondria (mito) accumulate adenine nucleotides right after birth thereby greatly increasing the matrix ATP+ADP+AMP content (BBA: 681:300, 1982). Bioenergetic function is also enhanced. Overall, there is a large increase in matrix ATP that might be expected to stimulate ATP-dependent pathways within the mito. Carbamyl phosphate synthetase and ornithine carbamyltransferase are enzymes of the urea cycle that are localized in the matrix; they catalyze citrulline (CIT) synthesis from ornithine, NH_3, and CO_2 in an ATP-dependent reaction sequence. We investigated whether the postnatal increase in the matrix ATP pool size affects CIT synthesis. Mito were isolated from rabbit pups at birth and at 2 hrs of age. ATP, ADP and AMP were determined enzymatically in PCA extracts. CIT synthesis (assayed by $^{14}CO_2$ fixation) increased between birth and 2 hrs, from 1.38±.35 to 2.40±.26 umol/hr/mg mito protein. The matrix ATP+ADP+AMP pool size (nmol/mg mito protein) increased from 5.7±.8 at birth to 15.1±1.6 at 2 hrs and the matrix ATP/ADP ratio increased from 1.47 to 3.52. The larger adenine nucleotide pool size together with the higher ATP/ADP ratio resulted in a 3.4-fold increase in matrix ATP concentration. Two moles ATP are required per mole CIT, so the increase in ATP appears to account for the 1.8-fold increase in CIT synthesis. Conclusion: The accumulation of adenine nucleotides and the maturation of energetic functions within the first few hours after birth allow a rapid postnatal increase in the capacity for CIT synthesis. (NIH HD 16936)

† 268 GALACTOSE ALIMENTATION IN THE PUP OF A DIABETIC MOTHER.

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Galactose(GAL) may regulate neonatal carbohydrate assimilation and may not stimulate insulin(INS) secretion. GAL may therefore stabilize glucose(GLU) metabolism in the IDM. Pups born to INS dependent diabetic dogs or controls were fed 0.6g/kg of GAL or GLU (N=5). During fasting IDM pups had equivalent blood GLU levels and systemic GLU appearance rates(Ra), but had elevated INS (7.4±0.3 vs 2.5±0.3 uU/ml, p=0.01) compared to control pups. After GAL feeding to IDM pups, blood GAL increased and peaked to 1 mM at 30 min. The glycemic response in IDM pups was similar after GLU or GAL feeding. Plasma INS increased minimally in the IDM pup after GAL feedings. In contrast, INS increased 2.5 fold after GLU. Ra of GLU in IDM pups after GAL feeding peaked at 30 min. and was lower than after enteric GLU at 30 min. (81.9±6.2 vs 106.2±8.6 µmol/kg/min, p=0.01). Ra after enteric GLU peaked at 60 min. and again was higher (118.2±10.5 vs 67.7±6.7, p=0.01). Hepatic glycogen content increased in the IDM fed GAL compared to fasted controls (634±27 vs 485±27 µmol/g, p=0.01) while GLU feeding had little effect. GAL and GLU feeding had no effect on glycogen synthase activity but the active component of phosphorylase decreased in both IDM groups compared to fasted controls. In summary, enteric GAL alimentation in IDM dogs results in lower INS levels with comparable glycemic responses compared to GLU feeding, attenuates Ra and enhances net glycogen content. In the presence of fasting hyperinsulinemia, GAL alimentation may stabilize GLU metabolism without excessive INS secretion.

269 EFFECT OF SEVERE HYPOXEMIA (H) AND HYPOTENSION (HT) ON NEWBORN CEREBRAL BLOOD FLOW (CBF), AND CEREBRAL OXYGEN CONSUMPTION ($CMRO_2$)

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Cerebral autoregulation (AR) is absent under certain conditions. We studied the effect of H on AR of CBF in anesthetized paralyzed, ventilated newborn dogs using microspheres. Following baseline measurements H was produced by ↓FIO_2 (PaO_2<20 torr) and maintained for the duration of the experiment. In 5 animals HT was produced by acute ↓ in blood volume resulting in a fall of 40% in mean BP. 7H animals served as controls and had no changes in BP or CBF. 2-way ANOVA, Neuman-Keuls, Mean±SD.

	Experimental (n=5)		
	Baseline	H	H+HT
CO ml/k/min	243±57	256±52	89±20*†
BP mmHg	48±11	63±12	39± 8*†
CBF ml/g/min	.22±.09	.50±.1†	.50±.05†
$CMRO_2$ mg/min/100g	1.25±.6	.58±.3†	1.63±.6
PO_2 (torr)	239±34	14±1.2†	20±2†
PCO_2 (torr)	38±2	39±2	31±5*†

†P<0.05 between periods, *P<0.05 exp vs control.

Thus, 1) severe H produces a marked increase in CBF primarily due to cerebral vascular resistance (CVR), 2) in spite of↑ CBF and maximal tissue oxygen extraction, $CMRO_2$ falls by 50%, 3) unlike the adult, AR of CBF in preserved after 10' of severe H, 4) the further fall in CVR due to HT, after maximal vasodilatation due to H, suggests that the mechanisms for H and HT vasodilatation on the cerebral vasculature may be different.

● 270 IMPULSE ACTIVITY DIFFERENTIALLY REGULATES LEU-ENKEPHALIN AND CATECHOLAMINE (CA) CHARACTERS IN THE ADRENAL MEDULLA.

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Trans-synaptic impulse activity has long been known to increase CA biosynthesis and CA synthetic enzymes in the adrenal medulla. Since opiate peptides are co-stored and co-released with CA's in medullary cells, and since medullary opiate peptides may be important modulators of the sympatho-adrenal stress response, we examined trans-synaptic regulation of the putative peptide neurohumour, leucine-enkephalin (ENK) and the CA enzymes, tyrosine hydroxylase (TH) and phenylethanolamine-N-methyl transferase (PNMT) in the rat adrenal medulla in vivo and in vitro. Surgical denervation of the adrenal or pharmacologic blockade of synaptic transmission, treatments known to decrease CA traits, increased ENK-like immunoreactivity to 150% of control. Medullae explanted to culture exhibited a 50 fold rise in ENK in 4 days, whereas TH remained constant, and PNMT decreased to a new baseline. Depolarizing concentrations of K^+ prevented the accumulation of ENK while not affecting TH or PNMT. Our studies suggest that enkephalinergic and catecholamine characters are differentially regulated by impulse activity and depolarization in the adrenal medulla. Independent regulation of adrenal neuropeptides and CA enzymes may allow new therapeutic approaches to shock, hypoxemia, or stress-induced analgesia through alteration of specific neurohumoral mechanisms. (Supported by NIH Grants HL00756, NS10259, and HD12108).