248 FETAL CEREBRAL RESPONSES TO VENTILATION AND OXYGENATION. Christine A. Gleason, Robert H. Notter, M. Douglas Jones, Jr. The Johns Hopkins Hospital, Baltimore, MD and University of Rochester, Rochester, NY.

Previous studies have shown that cerebral oxygen consumption ($CMRO_2$) increases by nearly 50% at birth. The perinatal factors responsible for this increase are unknown, but onset of ventilation and increased arterial PO_2 (PaO_2) are one possibility. In 7 fetal sheep (131-138d gestation) we inserted vascular catheters and an endotracheal tube in utero. After 1-3d recovery, we measured cerebral blood flow (CBF) with radioactive microspheres, and arterial and sagittal sinus oxygen contents (CaO_2, CvO_2). We calculated $CMRO_2$ (CaO_2-CvO_2 x CBF), oxygen transport (OT = CBF x CaO_2) and fractional oxygen extraction (E = $CMRO_2$/OT). Measurements were made during nonventilated control (C), ventilation with 3% O_2 (V) and ventilation with an FiO_2 sufficient to raise PaO_2 to 73 Torr (O). A calf lung surfactant extract was instilled intratracheally prior to ventilation to maintain stable pH and $PaCO_2$. Results, mean (S.D.):

	PaO_2	$CMRO_2$ ml/100g/m	OT ml/100g/m	CBF ml/100g/m	E
C	19.4 (1.3)	4.11 (0.7)	11.8 (4.4)	109 (42)	.37 (.1)
V	19.4 (1.7)	4.17 (1.8)	9.1 (3.7)	87 (36)	.46 (.07)
O	73.0 (13.9)*	4.64 (1.4)	10.9 (5.7)	56 (25)*	.45 (.09)

* $p < 0.05$, compared to control

Although CBF fell, OT and $CMRO_2$ were maintained during 1-2 hrs of fetal oxygenation. Therefore, these results show that fetal $CMRO_2$ is apparently not limited by fetal PaO_2.

▲249 ONTOGENY OF RECEPTORS FOR EPIDERMAL GROWTH FACTOR IN MAMMALIAN KIDNEY. Paul R. Goodyer, J. Fata, Z. Kachra, and C. Goodyer. McGill University, The Montreal Children's Hospital, Divisions of Pediatric Nephrology and Endocrinolgy, Montreal, Quebec.

Sponsored by Kaplan, B.S.

Epidermal growth factor (EGF) is synthesized in abundance in mammalian kidney and appears in urine (Reg. Pep. 10:37, 1984). It is apparently trophic for the intestine in the postnatal period (Ped Res 19:213, 1985) but little is known about its function in kidney. We have characterized the high affinity binding sites for EGF in membrane fractions from rat and human kidney at various stages of development. Crude membrane fractions sedimenting in buffered 0.25 M sucrose between 8000-15000 x g were prepared from pooled fetal and postnatal whole kidneys. Specific binding of I^{125}-labelled murine EGF rose from 2.32% (% of total CPM bound/300 ug prot) in 16-18 day fetal rats to 3.3% just prior to birth at 21-22 days. Thereafter, EGF binding fell rapidly to lower levels (0.5%) by one month of postnatal age. Specific binding of I^{125}-labelled human EGF was low (0.41%) in pooled kidney membranes obtained obtained at the time of therapeutic abortion by D and C (16-18 weeks gestation). In 2 infants (36 and 40 weeks gestation, respectively) who died in the first 2 weeks of life, EGF binding was 0.67% and 1.95%, respectively. In one 60 year old adult male, specific EGF binding was 0.37%.

These findings suggest that renal EGF receptors are most abundant in late fetal life and may play a role in kidney development at that stage.

†250 15-HYDROXYEICOSATETRAENOIC ACID (15-HETE) PROMOTES MIGRATION OF HUMAN RETINAL MICROVESSEL ENDOTHELIAL CELLS (HME) IN VITRO. Janet E. Graeber, Marie J. Stuart, Bert M. Glaser. Depts of Ped and Ophth, The Johns Hopkins Hospital, Baltimore, MD and Upstate Medical Center, Syracuse, NY.

Angiogenesis is an important process in vasoproliferative disorders, but its control remains unclear. Endothelial cell migration is a crucial step in this process. Previous work in our laboratory has shown that 15-HETE, a major arachidonic acid (AA) product of vascular endothelium and lymphocytes, enhances the migration of fetal bovine aortic endothelial cells (FBAE) at high concentration (200 μM) in the presence of serum-containing media. The current study was undertaken to determine if 15-HETE at lower concentrations would enhance migration of human retinal microvessel endothelium (HME). Retinal cells were obtained by a modification of the method of DelVecchio et al (Invest Ophth Vis Sci 25:247). HME expressed Factor VIII related antigen and synthesized plasminogen activator. Migration was measured by the modified Boyden chamber method of Glaser et al (Nature 288:247). Lower wells received HME in minimal essential media (MEM). Chambers were sealed, inverted, and cells attached to a collagen coated membrane. Chambers were placed upright and upper wells received 15-HETE in MEM (10^{-5} to 10^{-10}M), or MEM alone. 15-HETE (10^{-7} and 10^{-6}M) significantly stimulated HME migration to 142% ± 23 (S.D. $p < 0.01$) and 134% ± 20 ($p < 0.05$) of baseline respectively (n=6). Further studies with FBAE in this same serum-free system demonstrated similar results. The AA metabolite 15-HETE stimulates HME migration and may play a role in vasoproliferative disorders of the eye.

251 AMINOGUANIDINE IMPROVES FETAL GROWTH IN STREPTOZOTOCIN-INDUCED MATERNAL DIABETES. Robert E. Greenberg, Tracy Dunham and James Wogenrich, University of New Mexico School of Medicine, Department of Pediatrics, Albuquerque.

When streptozotocin-induced diabetes is produced early in gestation in rats, fetal growth is markedly compromised. Cross-linking of proteins is increased in the diabetic, due to formation of advanced non-enzymatic glycosylation products (AGP). Aminoguanidine (A), a nucleophilic hydrazine compound, prevents formation of AGP and cross-linking of arterial proteins in vivo in diabetic rats. We administered aminoguanidine (25 mg/Kg IP) daily to control and diabetic rats from day 5 of gestation to sacrifice on day 21.

	Fetal wt. (grams)	Placental wt. (grams)	Maternal Blood Glucose	%Incr. Maternal Weight
Control	5.22±.26	.568±.04	153±42	60.6
Control + A	4.94±.18*	.589±.07	181±28	48.4
Strept. only	3.75±.53**	.863±.09	572±44	34.1
Strept. + A	4.33±.17*	.790±.04	571±17	38.8

(*p=<.05 **p=<.001)

Aminoguanidine produces increased fetal growth in diabetic pregnancies, while slightly retarding fetal growth in controls. Increased placental size in diabetic pregnancies is unaffected by aminoguanidine. These data suggest that reduced fetal growth in diabetic pregnancies may result from non-enzymatic glycosylation.

252 CALCIUM DOES NOT REGULATE PROTEIN DEGRADATION IN FETAL MUSCLE. Robert E. Greenberg and James Wogenrich, University of New Mexico School of Medicine, Department of Pediatrics, Albuquerque.

The rate of protein degradation in adult skeletal muscle in vitro is markedly enhanced by treatments that increase intracellular Ca^{2+}. Fetal diaphragm degrades protein at much lower rates than adult muscle, and is unaffected by ambient Ca^{2+}. In order to further define regulation of proteolysis during development, tyrosine release from skeletal muscle, incubated in vitro, was used as an index of protein degradation.

Tyrosine release in fetal muscle is unaffected by Ca^{2+} concentration, the calcium ionophore A23187 or muscle depolarization produced by elevated extracellular K^+. Proteolysis in fetal muscle is reduced 35-40% by inhibitors of thiol proteinases (leupeptin or Ep-475) in the absence of Ca^{2+}. Inhibition of ATP-dependent proteolysis by NaF or DNP occurs in fetal muscle independent of Ca^{2+}. Methylamine, an inhibitor of lysosomal proteolytic activity, impedes ionophore-induced proteolysis in adult muscle but has no effect on fetal muscle. Ca^{2+}-facilitated proteolysis is enhanced by nutrient deprivation in adult but not fetal muscle. The activities of lysosomal enzymes (cathepsin B, galactosidase, glucosaminidase and acid phospatase) in muscle homogenates do not vary during development.

These results indicate that proteolysis in fetal muscle is independent of calcium. Thus, the overall rate of protein breakdown in fetal muscle remains unaffected by physiologic or pathologic states that alter intracellular Ca^{2+} concentration.

●253 EVIDENCE OF THE ROLE OF PROTEIN KINASE C IN PERINATAL DEVELOPMENT OF THE SURFACTANT SYNTHESIS. Mikko Hallman and Matti Romu. Univ. of Helsinki, Department of Pediatrics, Helsinki, Finland

Protein kinase C is a regulatory element in intracellular signal transduction. Diacylglycerol, produced in response to extracellular signals by turnover of phosphoinositides, Ca++, and phosphatidylserine synergistically activate protein kinase C. Phorbol esters, such as 12-O-tetradecanoylphorbol-13-acetate (TPA) directly activate protein kinase C. In this study we investigated whether 1) the degree of maturity influences the effect of inositol (INO) in potentiating the hormone-induced synthesis of surfactant phosphatidylcholine (SPC); 2) the INO effect is due to activation of protein kinase C. Lung explants from 21 or 28 day-old rabbit fetuses were cultured in serum-free minimal essential medium for 4 days in the presence of 0.1 μM dexamethasone (DX), 0.1 μM thyroxine (T4), 100 ng/ml TPA, or 1.5 mM INO. Choline incorporation into surfactant phosphatidylcholine (SPC) (CPM/μg DNA) was analyzed (mean+SD,n=4):

	None	INO	DX+T4	DX+T4+INO	TPA	TPA+DX+T4+INO
21 days:	12± 4	16± 7	21± 9	247± 13	285±11	310± 29
28 days:	310±14	329± 34	604±24	810± 39	756	869

TPA, diacylglycerol, or INO together with hormones, induced SPC synthesis in 21-day-old fetuses. In explants from 28-day-old fetuses, dibutyryl cyclic AMP was as effective as TPA in stimulating SPC incorporation. We propose that protein kinase C is involved in the differentiation of the surfactant system, and that high INO concentration facilitates intracellular transmission of hormonal signals in very immature alveolar cells.