

- **300** GROWTH OF THE FETAL GUINEA PIG: PHYSICAL AND CHEMICAL CHARACTERISTICS. John W. Sparks, Jean R. Girard, Soomant Callikan and Frederick C. Battaglia. College de France, Paris, and University of Colorado School of Medicine, Department of Pediatrics, Denver

Achievement of the intrauterine growth rate is a goal frequently used in nutrition of preterm infants. Data on the chemical composition of the human are limited and there is curiously little similar data in experimental animals. We studied physical and chemical macronutrient accretion in the fetal guinea pig, a species in which the high fat content and rapid growth rate emphasize the demands of fetal growth upon maternal nutrition. We studied 32 fetuses in 12 litters ranging from 39 days to term (67 d), analyzing the fetuses for wet weight, dry weight, carbon, nitrogen, fat content, caloric content (bomb calorimeter) and amino acid composition. Body weight increased exponentially with gestational age at 7.1%/d. Dry weight concentration tripled from 10% to 30% by term and total dry weight increased at 10.1%/d. Fat concentration rose from 2% at 45 days to 12% at term and fat content increased 13.5%/d. The total caloric accretion rate was 100 kcal/kg/d at 45 days, increasing to 220 kcal/kg/d at term. Over 60% of the caloric accretion was due to fat accretion. Caloric values of fat and non-fat dry weight were 9.5 and 4.5 kcal/g respectively. Amino acids represented 80% of the total body nitrogen and 46% of the total body carbon. These data represent the first systematic study of chemical growth during fetal life in any experimental animal, provide accretion measurements unavailable in human studies and demonstrate the importance of fat accretion in caloric demands of fetal growth.

- **301** RENAL-HEMODYNAMIC CHANGES IN HYPOXEMIC NORMOCARBIC PIGLETS. Jose Strauss, Salha Daniel, Paulo J. Dickstein, Oswaldo Trindade, Eduardo Bancalari, L. Stanley James and Gaston Zilleruelo. Departments of Pediatrics and Anesthesia, University of Miami, Miami, Florida and Columbia University, New York, New York.

Hypoxemia has been reported to induce a decrease or an increase in renal function depending on various factors. This study assessed some hemodynamic and renal functions in hypoxemic neonatal pigs maintained at a normal PaCO<sub>2</sub>. These results were compared with those of a similar study (Rowe, M.I., Strauss, J.; *Pediatr. Res.* 7:411/183, 1973) in which PaCO<sub>2</sub> and PaO<sub>2</sub> were decreased. Eleven piglets anesthetized with pentobarbital and mechanically ventilated were studied for a control period of 60 min. at a mean PaO<sub>2</sub> of 76.1 (range 60-96) mmHg and after 60 min. ventilation with 10-12% O<sub>2</sub> to attain a mean PaO<sub>2</sub> of 33.4 (range 24-51) mmHg. The following increases were documented after 60 min. of hypoxemia; cardiac output, from 0.72 to 0.85 L/min; mean arterial blood pressure from 102.5 to 117 mmHg; urine volume from 0.027 to 0.063 ml/min/kg. Arterial blood pH decreased from 7.450 to 7.392. All changes were significant at p < 0.05. These urinary results are similar to those obtained in piglets under Ketamine anesthesia, breathing spontaneously 10% O<sub>2</sub>, and whose PaCO<sub>2</sub> decreased markedly but whose cardiovascular variables were unchanged. It is concluded that hypoxemia induces a diuresis and natriuresis in piglets independent of changes in PaCO<sub>2</sub>, cardiac output or mean arterial blood pressure.

- 302** CYCLOHEXIMIDE INHIBITS LYSOSOMAL PROTEOLYSIS OF ENDOGENOUS BUT NOT EXOGENOUS PROTEINS. Jess G. Thoene and Rosemary Lemons. University of Michigan Dept. of Pediatrics, Ann Arbor.

Cystinotic fibroblasts accumulate lysosomal cystine from the degradation of endogenous and exogenous cystine-containing proteins. Lysosomal cystine accumulation can be modulated by the addition of cystine rich proteins to the culture medium or by labelling the endogenous protein pool with <sup>35</sup>(S)-cystine for varying periods. Cultures of cystinotic fibroblasts were incubated in <sup>35</sup>(S)-cystine-containing medium (40 mCi/mmol) for 4, 24, or 48 hr; treated with cysteamine for 30 min. to produce cystine depletion, and replaced in unlabelled medium with or without 100 μM cycloheximide (Chx). The plates were harvested after 24 hr in unlabelled medium and the amount of <sup>35</sup>(S)-cystine accumulation determined by high voltage electrophoresis. 100 μM Chx inhibited <sup>35</sup>(S)-cystine accumulation from all labelling periods. Results are expressed as CPM/10<sup>6</sup> cells of <sup>35</sup>(S)-cystine X 10<sup>4</sup>: 4 hrs-control 1.46 ± 50, Chx 0.34 ± 0.27 p < .01 n=4; 24 hrs-control 2.21 ± 1.36, Chx 0.56 ± 0.29 p < .05 n=3; 48 hrs-control 3.47 ± 2.93, Chx 1.00 ± 0.89 p < .10 n=3. Chx also increased the percent of radioactivity which was acid soluble (cells & medium) at 24 hr after all labelling periods (values in %): 4 hr control 49.5 ± 14.6, Chx 67 ± 3.2 p < .05; 24 hr control 41 ± 7.2, Chx 52.6 ± 5.5 p < .10; 48 hr control 37 ± 7.2, Chx 48.1 ± 1.7 p < .05. Chx had no effect on the accumulation of cystine from BSA added to the culture medium: control 0.45 nmol/10<sup>6</sup> cells, Chx 0.48 nmol/10<sup>6</sup> cells, p > 0.5 n=3. Chx, a known inhibitor of protein synthesis, also inhibits the lysosomal degradation of endogenous but not exogenous proteins, yet appears to enhance overall proteolysis.

- † **303** BIOCHEMICAL EVIDENCE OF CARDIOMYOPATHY IN GUINEA PIGS EXPOSED IN UTERO TO ALCOHOL. John D. Tobin, Jr., George R. Noren, James E. Surdy, Nancy A. Staley. University of Minnesota Medical School, Hennepin County Medical Center, and Veterans Administration Medical Center, Departments of Pediatrics and Pathology, Minneapolis.

We hypothesized that modest maternal ethanol intake would deleteriously affect developing fetal myocardium. Pregnant guinea pigs were given either water (C), n=13, or 2½% ethanol (EtOH), n=11, for the last ½ of pregnancy (12% of calories). At birth, progeny body and heart weights were measured, and cardiac sarcoplasmic reticulum (SR) was isolated for determination of Ca<sup>++</sup> uptake, Ca<sup>++</sup> binding, and Ca<sup>++</sup> stimulated ATPase.

Maternal mortality was not significantly different between groups: 0% in C, 18.2% in EtOH (p > 0.10). Litter sizes, proportion of progeny born alive, and progeny body weights in C and EtOH were similar (p > 0.10). Despite similar body weights, heart weight/body weight ratios of EtOH's were less than C's: 4.16 ± 0.65 (n=38) vs 4.61 ± 0.66 (n=29) gm/kg (p < 0.005). Ca<sup>++</sup> flux was also less in EtOH's than C's: Ca<sup>++</sup> uptake 132 ± 23 (4) vs 209 ± 4 (5) nM/mg Pr/min (p < 0.001); Ca binding 26 ± 8 (5) vs 53 ± 2 (5) nM/mg Pr/4 min (p < 0.001); Ca<sup>++</sup> stimulated ATPase 0.7 ± 0.3 (5) vs 1.2 ± 0.1 (5) μM/mg Pr/10 min (p < 0.01).

Modest consumption of ethanol during pregnancy significantly alters Ca<sup>++</sup> flux in isolated cardiac SR of the newborn guinea pig. These findings are similar to those observed in adults with alcoholic cardiomyopathy. These data suggest that altered cardiac excitation-contraction may contribute to the production of certain features of the fetal alcohol syndrome.

- 304** DIRECT PHARMACOLOGIC EFFECTS OF DHT ON THE ALVEOLAR TYPE II CELL IN VITRO. J.S. Torday, Department of Pediatrics (Physiology), Harvard Medical School, Boston, MA 02115

Previous studies from our laboratory have demonstrated that dihydrotestosterone (DHT) inhibits the production of pulmonary surfactant *in vivo* and its *de novo* synthesis in tissue culture (*J. Cell Biology*, 97, 86a, 1983). Studies in tissue culture suggest that DHT has no effect on the fibroblast-type II cell interaction which gives rise to surfactant, but blocks this mechanism in response to cortisol at very low doses (≤ 10<sup>-8</sup>M) comparable to the circulating level of androgens in the fetus and to the K<sub>d</sub> of the androgen receptor. More recent studies suggest that 10<sup>-7</sup>M to 10<sup>-5</sup>M DHT stimulates the growth of the fetal type II cell by 40-100% while reducing the synthesis of saturated phosphatidylcholine in a reciprocal manner. These results may explain the observed *in vivo* effects of DHT initially on the female fetuses (10<sup>-8</sup>M) and at pharmacologic levels on both sexes at serum androgen levels at an order of magnitude greater. We conclude that the native sex difference in pulmonary surfactant production is due to the effects of androgen on mesenchymal-epithelial interactions, but that DHT may have direct, pharmacologic effects on type II cell maturation as well. (Supported by NIH Grant #HL28315-02).

- 305** DIHYDROTTESTOSTERONE (DHT) INHIBITS SYNTHESIS OF SATURATED PHOSPHATIDYLCHOLINE. J.S. Torday, Department of Pediatrics (Physiology), Harvard Medical School, Boston, MA 02115

Male newborns are at a 2-fold greater risk of Respiratory Distress Syndrome than females. This risk is associated with delayed production of saturated phosphatidylcholine (SPC) in male human, rabbit, and rat fetuses. DHT administration to rabbit fetuses inhibits the production of SPC in an organ-specific manner and the anti-androgen Flutamide negates the native sex difference in SPC production. In the present study DHT was administered to pregnant rabbit does at 1 mg/kg from day 12 to day 27 of gestation daily and the fetuses were sacrificed on day 28. The fetal lungs were incubated with <sup>3</sup>H-choline chloride (10 μCi) for 1 hour and subsequently analyzed for radiolabelled PC and SPC content.

|            | Control      | n    | DHT         | n    | P            |
|------------|--------------|------|-------------|------|--------------|
| Female PC  | 2900 ± 1030* | (12) | 2483 ± 1133 | (9)  | NS           |
| Female SPC | 950 ± 366    |      | 866 ± 500   |      | NS           |
| Male PC    | 2883 ± 1166  | (14) | 1966 ± 416  | (11) | NS           |
| Male SPC   | 850 ± 250    |      | 583 ± 216   |      | < .01, Anova |

\* (dpm/mg protein/hr); mean ± SD

Male and female control lungs synthesized comparable amounts of both PC and SPC. DHT had no measurable effect on either PC or SPC synthesis by female lungs, or on PC synthesis by male lungs. However, DHT did have a significant inhibitory effect on SPC synthesis by male lungs. These data suggest that DHT inhibits the synthesis of pulmonary surfactant in a sex-specific fashion under these experimental conditions. The mechanism underlying this inhibition is currently under investigation. (Supported by NIH Grant #HL28315-02).