354 **THE EFFECTS OF PREGNANCY (P) ON THE RESPONSE OF THE CYTOCHROME P-450-DEPENDENT MIXED-FUNCTION MONOOXYGENASE SYSTEM (P450) TO ETHANOL EXPOSURE AND DIET CHANGE.** George H. Lambert, Helen Leitz, and Alvin N. Kotake (Spon. John B. Paton). Pritzker Sch. Med., U. Chgo; Michael Reese Hosp., Depts. Peds., Pharmacol. & Physiol. Sci.

Many xenobiotics and endogenous substrates are metabolized by P450, an enzyme system induced by certain substrates. We and others have shown that P decreases P450 in animals fed lab chow; however, little is known of how P alters the interaction of xenobiotics, diet, and P450. Pregnant (Pg) and nonpregnant (Npg) female C57BL/6J mice were maintained on lab chow and H_2O ad lib before the study began. They were fed a diet containing 25% ethanol-derived calories (EDC). Controls were pair-fed a similar diet with 25% sucrose-derived calories (SDC). Day 6 of gestation was day 0 of the diet for Pg mice. The aminopyrine breath test (ABT) was done on diet days 0,2,4,8, and 10 by our previously described methods (Pediatr Res 17:150, 1983). The elimination rate constant was the ABT parameter used for comparison.

Diet	Day 0	Day 4	Day 8	Day 10
Npg EDC	52.8±7.7	49.2±11.7	68.3±11.5*	73.1±7.3*
Npg SDC	57.6±4.1	50.8±11.8	41.9±3.9	37.9±5.4
Pg EDC	49.0±6.3	43.4±6.0	41.4±6.9	41.0±4.5
Pg SDC	45.0±12.8	47.8±5.5	35.8±12.7	43.1±6.8

*Different from Npg SDC $p<0.005$

The change from lab chow to SDC caused a general decrease in P450 and prevented the decrease in P450, which we have previously shown to occur in pregnancy. Exposure of Npg mice to ethanol increased P450 activity, which was not seen in the Pg animals.

355 **ETHANOL (E) METABOLISM IN PREGNANCY (P) AS DETERMINED BY THE [^{14}C] ETHANOL BREATH TEST (EBT).** George H. Lambert, Helen Leitz, and Alvin N. Kotake (Spon. John B. Paton). Pritzker Sch. Med., U. Chicago; Michael Reese Hosp. & Med. Ctr., Depts. Ped., Pharm. & Physiol. Sci.; Chicago.

Maternal E consumption in P poses a known risk to the mother and fetus. The purpose of this study was to determine the effects of P on in vivo metabolism of E. The in vivo rate of E metabolism can be monitored by the EBT by measuring the rate of CO_2 derived from the metabolism of radiolabeled E (Toxicol Appl Pharmacol, 61:177, 1981). The EBT was conducted in 7 pregnant (Pg) and 5 nonpregnant (Npg) C57/B6J mice of similar ages. Day 0 was when vaginal plugs were found or when Npg mice were entered into the protocol. The EBT was conducted on days 6,8,10,14, and 16. [^{14}C] E ($5x10^5$ dpm) and unlabeled E (50 mg/kg) were administered i.p. The mice were immediately placed in air collection chambers, $^{14}CO_2$ was collected by bubbling exhaled air through an alcohol trapping solution and then a CO_2 trapping solution of ethanolamine methanol, and the rate of $^{14}CO_2$ exhaled was determined. The elimination rate constant (Kel) of labeled CO_2 was calculated and used as the measurement parameter of E clearance. The Kel did not change for Npg mice. In the Pg mice, the Kel did not differ from that of Npg mice on day 6 or 10 of gestation, but was increased on day 8 (Npg Kel=0.0592±0.002, Pg Day 8 Kel=0.0698±0.003; $p<.001$) and was decreased on days 14 and 16 (0.0525±0.002 and 0.0501±0.002, respectively; $p<.001$ vs. Npg). In summary, the rate of E metabolism in P changes biphasically. The rate of E metabolism as determined by the EBT increased significantly during organogenesis but decreased in late P.

356 INDOMETHACIN AND RENAL FUNCTION: THE EFFECTS OF ADDITION OF LOW-DOSE DOPAMINE. J.E. Larson, M.P. Leuschen, J.M. Baggett, R.M. Nelson. Univ. of Nebraska Medical center, Depts. of Pediatrics and Pharmacology, Omaha, NE.

The renal function changes induced by indomethacin during treatment of patent ductus arteriosus often preclude its use. To determine if concommitent low-dose dopamine improved renal function, we studied renal parameters in Beagle puppies. The animals were sedated; tracheostomy was performed and mechanical ventilation begun. The femoral artery and vein were catheterized and the right ureter isolated and catheterized for urine collection. Four groups were studied: controls, indomethacin treated, dopamine treated, and indomethacin and dopamine treated. Each animal was allowed to serve as its own control at time 0-120 min. At time 120 the treated animals received .2 mg/kg indomethacin intravenously and/or 4 mcg/kg/min dopamine by continuous intravenous infusion. Time 120-240 served as the next collection period. All animals received constant fluid intake. Blood pressure, heart rate and blood gases remained stable. Glomerular filtration rate (GFR), sodium para-aminohippurate (PAH) clearance, fractional sodium (Na) excretion, osmolar and free H_2O clearance were obtained. GFR was significantly decreased ($p<.05$) in animals that received indomethacin alone, and unchanged in the groups receiving dopamine, dopamine and indomethacin and controls. There was a trend ($p<.1$) indicating fractional Na excretion decreased only in the group receiving indomethacin. This data suggests that low-dose dopamine may improve renal parameters when used with indomethacin.

357 EFFECT OF POSTNATAL EXPOSURE OF CIMETIDINE ON BLOOD TESTOSTERONE LEVEL AND HEPATIC MICROSOMAL TESTOSTERONE HYDROXYLATION ACTIVITY IN THE RAT. Raul A. Lazarte[a], Nobuhiro Harada[b], and Masahiko Negishi[b], (Spon. by Daniel W. Nebert) [a]NICHD, NIH, Bethesda, MD and [b]NIEHS, Research Triangle Park, NC

Cimetidine (CIM), a potent anti-ulcer drug, is known to have an anti-androgenic effect, presumably through competitive binding to the dihydrotestosterone receptor. CIM given to men can cause hypoandrogenemia and sexual dysfunction. A single injection of CIM (160 mg/kg) to SD rats 3 days old significantly delayed the increase of body and testes weights. The blood testosterone levels were 30, 90, and 100 mg/dl at 4,6 and 8 weeks after treatment, respectively. These levels were 5 to 7 times lower than those in non-treated rats at corresponding times. Developmental expression of hepatic microsomal testosterone 16α-hydroxylase, which is known to be a male-specific activity and is imprinted by neonatal testosterone, was not significantly affected by this experiment. Treatment of adult male rats with CIM (160 mg/kg/day) for 5 days decreased testosterone levels to one-forth those of non-treated rats. The testosterone metabolites formed by hepatic microsomes from CIM treated adult rats were analyzed by thin layer chromatography; only formation of 16α-hydroxy-testosterone was inhibited. CIM in vitro was a better inhibitor of 16α-hydroxylase than of other hydroxylases. Direct binding of CIM or its metabolites to the cytochrome P-450 isozyme specific for 16α-hydroxylation is presumed to be the mechanism of inhibition.

● **358** DEVELOPMENT OF AN IMMOBILIZED ENZYME REACTOR FOR THE TREATMENT OF HYPERBILIRUBINEMIA. Arthur Lavin, Cynthia Sung, Alexander Klibanov, Robert Langer, (spon. by B.T. Smith); MIT, Dept. Nutrition and Food Sci., Boston.

Current treatment modalities for neonatal jaundice have limited effectiveness and serious toxicities. We have developed an extracorporeal blood filtering system capable of removing bilirubin, enzymatically. After screening a variety of cyclic amidases, proteases, and oxido-reductases, we found that bilirubin oxidase (BOx) from the fungus Myrethicium was uniquely capable of rapid degradation of bilirubin to a safe product. Using SDS gel electrophoresis, we established 80% purity for the enzyme. Oxygen electrodes and the guaiacol assay for H_2O_2 demonstrated the mechanism was oxidation by O_2 yielding water, not H_2O_2. Using NMR and electronic absorption spectra, TLC, and physical properties, biliverdin was identified as the sole initial product. Biliverdin, based on dose response curves, is 200 times less toxic than bilirubin in mitochondrial enzyme assays. Biliverdin is oxidized to the final product(s) only when all of the bilirubin in solution is oxidized. Free BOx was found to have a Km of 62 uM. BOx immobilized onto cyanogen bromide activated Sepharose 4B beads retained 20% of free activity levels. That activity was retained under physiologic conditions, unchanged for ten hours. At a reactor flow rate of 1 ml/min, an immobilized enzyme reactor (bed volume 15 cc) reduced bilirubin levels from 20 to 10 mg/dl within 90 minutes. Immobilized BOx is also active in human whole blood. Testing the reactor for in vivo efficacy and safety remains before clinical application begins.

359 DEVELOPMENTAL H^3-NITRENDIPINE BINDING IN IMMATURE SPINAL CORD NEURONS WITH INCIDENTAL TTX DISPLACEMENT Marcia J. Litzinger and Douglas E. Brenneman (Spon. J. Sidbury) Lab. of Dev. Neurobiol., NIH, NICHD, Bethesda,Md.

Ca^{++} may play a role in neurodevelopment through involvement in neurite extension, voltage-sensitive release of neurotransmitters and the expression of genes for enzymes involved in transmitter synthesis. Nitrendipine(NTP), a dihydropyridine, is believed to bind with the voltage-sensitive Ca^{++} channels. The development of voltage-sensitive Ca^{++} channels was studied using H^3-NTP.

The developmental curve of H^3-NTP in fetal mouse spinal cord cultures shows a multiphasic increase in binding as cultures matured. Day 19 was the point of maximal binding with a plateau phase on days 3-5. The amount of binding was greater (>5 fold) than that for Na^+ channel ligands at similar developmental periods. Scatchard analysis showed a non-linear relationship of H^3-NTP binding(.3-20nM). The two receptor sites showed increasing Kd's and Bmax's throughout development.

Tetrodotoxin (TTX), a Na^+ channel blocker, was shown to interfere with H^3-Nitrendipine (H^3-NTP) binding. TTX displacement was seen at 10^{-9}M concentration with maximal displacement at 10^{-6}M. The TTX effect was a developmental phenomenon. H^3-NTP binding showed 42%, 34% and 21% displacement on days 5, 8 and 23 respectively with 1 μM TTX. Mature cultures (Day 27) showed no significant displacement of H^3-NTP with 1 μM TTX. Both receptor sites were sensitive to TTX during early neurodevelopment. It is concluded that TTX and H^3-NTP have binding site similarity in early fetal mouse spinal cord cultures.