371 ACUTE STARVATION AND ACETAMINOPHEN TOXICITY IN YOUNG VS ADULT MICE. B. Sonawane, M. Sills, R. Schrager, and S. Yaffe, Dept. of Pediatrics and Pharmacology, Univ. of Penn. and The Children's Hospital of Philadelphia, Philadelphia, PA 19104.

Acute starvation, particularly in children, is often encountered due to infections. This is frequently accompanied by fever and requires antipyretic therapy. Since acetaminophen is widely used under these circumstances, we determined its toxicity (LD_{50}) in young (8 day old) and adult male mice (strain CD-1). Animals were deprived of food for 6 hours with free access to water. Mice of both ages were intubated orally with either a drug suspension (500 to 4000 mg/kg) in tragacanth (0.25%) or vehicle and twenty-four hour LD_{50} data were computed. In adults, food deprivation for 6 hrs decreased LD_{50} from 1500 mg (control) to 750 mg (starved). In yound pups, however, starvation did not change LD_{50} of acetaminophen. Furthermore, when we compared the half-life of acetaminophen (500 mg/kg, P.O., single dose) in the young vs adult control mice, the data show that the mean half-life was longer in the young (4.59 hrs) than in the adults (0.85 hrs). The fraction of the drug absorbed was also higher in the young (78%) compared to the adults (57%). Thus, these results indicate that acute starvation in young mice does not alter the toxicity of acetaminophen which is in contrast to adults. The reasons for this phenomenon are unknown. (Supported in part by NIH grant HD 10063).

372 SUSCEPTIBILITY TO PHENYTOIN-INDUCED CLEFT PALATE IN MICE CORRELATED WITH INHIBITION OF FETAL PALATAL RNA AND PROTEIN SYNTHESIS. Babasaheb R. Sonawane, Sumner J. Yaffe and Allen S. Goldman, Dept. of Pediatrics, The Children's Hospital of Philadelphia and Univ. of Penn., Philadelphia, PA 19104.

Phenytoin, an anticonvulsant which is commonly used in the treatment of epilepsy, is teratogenic in humans and produces cleft palate in susceptible rodents. We therefore investigated the effects of a teratogenic dose (50 mg/kg,S.C.) of phenytoin in the susceptible Ajax (A/J) and the resistant C57BL6 (B6) inbred strains of mice during the critical period of palatal differentiation (11-14 days). The drug produced 50% clefts in the A/J strain compared to 1.6% clefts in the B6 strain of mice. We further investigated whether phenytoin affects fetal palatal RNA and protein synthesis as do other clefting teratogens, glucocorticoids. Thus, a single dose of phenytoin (50 mg/kg) or vehicle was administered (S.C.) on the 12th day of pregnancy to both strains and RNA and protein synthesis was measured in fetal palates at various time intervals (3-72 hrs) by incorporation of ^{3}H-uridine (RNA) and ^{14}C leucine (protein). The results indicate that phenytoin produces a marked and persistent inhibition of RNA and protein synthesis in the fetal palates of the susceptible A/J strain. However, in the resistent strain, this drug produces only a slight and transient inhibition of palatal RNA synthesis, but does not affect protein synthesis. Thus, inhibition of palatal RNA and protein synthesis by phenytoin may be related to its teratogenic action on palatal differentiation at the critical period of development.

373 CONTENT AND ORGANIZATION OF SPERMATOZOAN DNA. Lester F. Soyka, Donald R. Mattison, Robert J. Kramer and Daniel W. Nebert, NICHD, NIH, Bethesda, MD.

The development of laser-based instrumentation has made possible the determination of individual cell DNA content at rates of 20,000/min and above. Forward light scatter, an index of cell shape and refractive index, can be concurrently measured. Moreover, subpopulations of cells can be sorted based on either of these parameters and collected for additional biochemical or morphological analysis. In order to determine changes occuring during epididymal maturation individual caput and cauda epididyma were obtained from C57BL/6N and AKR mice. Sperm were rinsed free in saline-EDTA, washed, fixed in ethanol and stained with propidiam iodide. A Coulter TPS-1 cell sorter was employed. In some studies freeze-thawing and sonication were employed to obtain a high yield of isolated sperm heads. A discrete homogeneous peak of fluorescent intensity corresponding to the haploid chromosome DNA content was defined and the purity confirmed by collection and examination by flourescent microscopy. Caput sperm heads had lesser flourescence than those from the cauda. Heating at 100 C for 10 min resulted in a shift toward greater flourescence. These changes indicate altered organization of DNA. Conclusions: The flourescent activated cell sorter can be used to measure DNA content and morphology of individual sperm with a precision and speed not previously possible. Condensation of DNA occurs during epididymal maturation. This new technology is immediately applicable to a number of new areas of developmental, pharmacologic and clinical studies of reproduction in the male.

● **374** PHARMACOGENETIC PREDISPOSITION TO PHENYTOIN HEPATOTOXICITY ASSESSED IN VITRO. S. P. Spielberg, G. B. Gordon, D. Goldstein, and D. A. Blake, Johns Hopkins University School of Medicine, Depts. of Pediat. and Pharmacol., Div. of Clin. Pharmacol., Baltimore, Maryland.

A small percent of patients treated with phenytoin (DPH) develop hepatotoxicity. Covalent binding of arene oxide metabolites (AOM) of the drug to cell macromolecules could result in cell death and secondary "hypersensitivity" reactions (fever, rash, eosinophilia). We tested the hypothesis that DPH hepatotoxicity results from decreased ability to detoxify AOM by exposing lymphocytes to AOM generated by a murine hepatic microsomal system. Cells from 17 controls (including 3 patients chronically treated with DPH without side effects) exhibited no toxicity (by trypan blue dye exclusion) over a DPH concentration range of 31-125 μM. When an inhibitor of epoxide hydrolase (TCPO) was added to the incubation, a dose-dependent increase in cell death was noted. AOM of DPH are thus cytotoxic in the system. Cells from 3 patients who experienced DPH hepatotoxicity manifested damage in the absence of TCPO. Cells from both parents of one patient, and the mother of a second showed an intermediate response. A sibling's cells had the same dose-response curve as one of the patients. All patients' cells showed enhanced toxicity from mephenytoin, but a normal response to acetaminophen (metabolites of the latter are not AOM). DPH hepatotoxicity may be based on decreased ability to detoxify AOM, and this appears to be a heritable trait.

● **375** SUSTAINED RELEASE THEOPHYLLINE (SRT): A SIGNIFICANT ADVANCE IN THE TREATMENT OF CHILDHOOD ASTHMA. E. Tabachnik, P. Scott, S. MacLeod, J. Correia, C. Newth, and H. Levison, Hosp. for Sick Children, Toronto.

The efficacy and safety of an SRT preparation (Theo-Dur®) was compared to regular release theophylline (RRT)(Theolair®) in the treatment of childhood asthma. We studied 34 children aged 6 - 12 years (mean 9.3 years) for 8 weeks. Both the SRT and RRT preparations were administered for 4 weeks in a randomized cross-over design. Individualized theophylline dosage was determined after pre-study measurement of a time concentration curve at steady state on SRT. Mean (+SD) dosage for SRT and RRT was 22.6 + 5.3 and 21.9 + 5.3 mg/kg/24 hours respectively. The SRT was administered 12 hourly; the RRT 6 hourly. Expiratory flow rate and lung volumes were assessed every 2 weeks. Peak flow rates were measured at home twice daily. A diary of symptomatology including wheezing, cough, appetite, activity and side effects was kept. No difference in pulmonary function or side effects was observed between the 2 treatments. However, breakthrough wheezing was significantly more frequent ($p < .01$) during RRT therapy. Pill counts and serum theophylline concentration monitoring showed better compliance with SRT treatment ($p < .01$). Subjectively, SRT therapy was preferred by 33 of 34 families. These results demonstrate conclusively that even in young children with rapid theophylline clearance, an SRT preparation administered twice daily is effective and safe. Because of decreased wheezing, improved compliance, and greater patient acceptance, SRT has definite clinical advantage over RRT in pediatrics.

376 POSTNATAL DEVELOPMENT OF THEOPHYLLINE METABOLISM IN PRETERM INFANTS. Fayez N. Takieddine, Kuo-Yi Tserng, Katherine C. King, (Spon. Satish C. Kalhan). Case Western Reserve Univ. at Cleve. Metro. Gen. Hosp., Cleveland, Ohio

Eight preterm infants, 26-34 wks gestation at birth, receiving aminophylline for apnea of prematurity were studied serially for theophylline (T) metabolism. Weekly urine & plasma samples were analyzed for T, caffeine (C), 1,3 dimethyluric acid (DMU), 1 methyluric acid (MU), 3-methylxanthene (MX) and theobromine (TB) by GC/MS. The sensitivity of this method is capable of detecting all metabolites in nanogram quantities.

Post Conceptional Age	Urine Metabolites % n	T	C	DMU	MU	Plasma Metabolites % n	T	C	DMU	MU
28-32 wks.	10	61±11*	10±3	20± 6	9±8	8	70±4	23±4	3±1.3	.9±1.0
33-37 wks.	12	53±12	10±4	25±12	11±7	13	72±7	25±7	2±0.6	.9±1.2
38-42 wks.	9	43±12	10±5	34±15	12±9	7	70±8	26±8	3±1.5	.7±0.7

*mean ± S.D.

Throughout the study, the plasma T levels achieved were 9.0±0.6 μg/ml. The major metabolites were T, C, DMU, & MU in plasma and urine. Urinary % of DMU increased with post-conceptional (PC) age and accounted for the decrease in % of T ($r=-0.82$, $p<0.001$). The fraction of C in plasma and urine remained constant. MX & TB were detectable in minute amounts. TB appeared only at later PC ages. Conclusion: 1) N-oxidation and C3-demethylation were active and progressively increased with post-conceptional age, and 2) The differential activities of N-oxidation/C3-demethylation with C1-demethylation implies multiple cytochrome P450 enzyme systems for theophylline metabolism.