● 390 ACETAMINOPHEN TOXICITY IN HUMAN KERATINOCYTES IN VITRO. Neil H. Shear, Stephen P. Spielberg, Daniel N. Sauder. U of Toronto, Hosp for Sick Children, Dept Pediat, Div Clin Pharmacol, Toronto, and McMaster U, Dept Med, Hamilton, Ontario.

The skin is a common target organ of adverse drug reactions. These reactions may be mediated by toxic drug metabolites. In vitro approximations of this process allow a non-invasive assessment of pathogenetic mechanisms. Keratinocytes (K) contain cytochrome P450 oxidases and detoxification enzymes. Acetaminophen (APAP) toxicity results from P450-generated metabolites which deplete cellular glutathione (GSH) and bind to macromolecules. Human K from a squamous cell carcinoma line (A431) were incubated with APAP (0, 1.0, 6.0, and 20 nM). Toxicity, expressed as percent of dead cells by trypan blue exclusion, revealed 6.4 ± 1.0%, 9.5 ± 2.4%, 39.1 ± 1.2%, and 45.3 ± 6.2% respectively. GSH was depleted to 56.8% of baseline at 20 nM APAP. After incubation with 20 nM APAP for 5 hr, cells were grown overnight in cysteine-free medium, 0.1 mM L-cysteine, or 0.25 mM N-acetyl cysteine, resulting in 55.0 ± 6.9%, 45.3 ± 6.2%, and 32.4 ± 3.9% dead cells respectively. K can activate APAP to toxic metabolites, and toxicity can be modified by cysteine availability. Fibroblasts showed no APAP toxicity without an exogenous microsomal metabolizing system. K obtained from skin biopsies of patients with cutaneous drug reactions may be useful in studying mechanisms of susceptibility to toxicity.

● 391 PHARMACOKINETICS OF PROPHYLACTIC INDOMETHACIN IN VERY-LOW-BIRTHWEIGHT PREMATURE INFANTS. Meindert Smith, Emmalee S. Setzer, Dyal C. Garg, Ronald N. Goldberg. Univ. of Miami, Jackson Memorial Hospital, Dept. of Pediatrics, and Clinical Pharmacology, Miami, FL. (Spons. by E. Bancalari).

The pharmacokinetics of indomethacin (I) was assessed in infants who received prophylactic (I) for patent ductus arteriosus (PDA). The first dose (0.2 mg/kg), given within 12 hours after birth, was followed by two q 12 hourly doses (0.1 mg/kg). The mean birth weight (BW) of these infants was 923 ± 134.0 gm (range:730-1070gm) with a mean gestational age of 28.1 ±2 wks (range:31-25 wks). None of the infants subsequently developed a significant PDA. Plasma (I) levels were measured by high performance liquid chromatography at 1, 13, 36, 72, 96, 168 and 216 hours after the administration of the first dose.

BW Group	n	t 1/2 (hours)	Steady State (μg/ml)	Clearance (ml/min)	Vol of Dist (ml)
<1000	5	94.0	0.92	0.30	410.8
≥1000	4	42.8	1.44	0.43	245.6
($\bar{X}$+SD)	9	71.3±39.1	1.15±0.92	0.362±0.002	373.4±197

The mean plasma t 1/2 of (I) in these infants (71.3±39.1 hrs) was significantly prolonged when compared with previously reported values. Furthermore the mean plasma t 1/2 of (I) was significantly longer in the <1000 gm infants ($p<0.05$). While the minimum efficacious dose for prophylactic (I) has not been established, all but one infant had a level > .250μg 7 days following (I). These data strongly suggest that previously recommended dosages of (I) for very low birth weight infants in the first 48 hrs. may result in very elevated and prolonged levels.

392 PHARMACOKINETICS OF NALOXONE IN PREMATURE INFANTS. Ina L. Stile, Maria Fort, Francoise Marotta, Robert Wurzburger, I. Mark Hiatt, Thomas Hegyi, UMDNJ-Rutgers Medical School and School of Pharmacy, St. Peter's Medical Center, Dept. of Pediatrics, New Brunswick, N. J.

Rapid disappearance of naloxone was observed in a group of very low birthweight infants examined for naloxone kinetics. Five infants (BW 1.20±0.25kg, GA 29±1wk) received 0.04 mg/per kg of naloxone intravenously, four within the first week of life and one on day 26. Serial serum samples were obtained at specific time intervals and frozen for subsequent analysis. Serum naloxone concentrations were measured by the radioimmunoassay method of Berkowitz (1975).

Serial naloxone concentration at 5 min. was 51.5±13.4pmole/ml, at 15 min. 36.7±4.0pmole/ml, at 30 min. 28.9±5.1pmole/ml, at 60 min. 20.4±5.9pmole/ml, at 120 min. 7.3±2.4pmole/ml, and 240 min. 1.5±0.4pmole/ml. No naloxone was detected at the next sample time (12hrs). The elimination rate constant (Ke) calculated from the decay portion of the elimination curve was 0.823± 0.130/hr. The calculated half life ($t_{1/2}$) was 51.8±9.2min. No correlations were found between Ke and $t_{1/2}$ and initial serum level, birthweight, gestational age, and postnatal age.

This group of infants demonstrated rapid elimination of intravenous naloxone, consistent with results obtained in adults.

393 TRANSIENT PSEUDOCHOLINESTERASE (PChE) DEFICIENCY IN PREMATURE INFANTS. A Strauss, HD Modanlou, Newborn Div, Miller Children's Hosp, Long Beach, UC Irvine.

Approximately 3% of the adult population have a genetic basis for decreased PChE enzyme activity. Levels of PChE activity in healthy term newborns and infants up to 4 months of age have been reported to range from 22-86% of normal adult values. The degree of decreased enzyme activity in premature infants is unknown. Levels of PChE were measured in 39 healthy premature infants (single determinations in 12 and serial weekly determinations in 27).Birth weight ranged from 1430-2070g and GA ranged from 28-37 wks. Ten adult donor and 20 term newborn blood samples were analyzed for comparison purposes. Levels of PChE were measured in duplicate by a kit dye reduction method (normal adult reference value 7-19U/ml). Abnormally low PChE levels were found in 10% of adult, 15% of term newborn and 20% of premature infant blood samples(NS). This incidence of low PChE activity is 3-5 times that reported for adults using a different method. In 6 premature infants with initially low PChE levels, the enzyme activity rose to normal adult range within 2 weeks. In 2 other premature infants PChE levels remained low and most likely represented congenital PChE deficiency. There was no difference in PChE activity on the basis of sex, race or GA. Hospitalized premature infants exhibit similar rates of quantitative PChE deficiency when compared to adults and term infants and are at potential risk for significant complications when exposed to depolarizing muscle relaxant agents. When succinylcholine use is considered in premature infants, measurement of PChE levels or use of alternative drugs may be indicated on the basis of transient PChE deficiency.

394 ANIMAL MODEL OF BENZOCAINE INDUCED METHEMOGLOBINEMIA Philip L. Townes, University of Massachusetts Med. Center, Department of Pediatrics, Worcester.

Methemoglobinemia has been reported in a small number of infants after topical application of benzocaine. Since it is a widely used medication, these rare occurrences have been considered to possibly represent idiosyncratic response. Varying amounts of benzocaine (1.5,3,6,12.5,25,50,100 mg) were administered by stomach tube, in a single 1.0 ml bolus, to 117 young (approx. 175 gm) Charles River CD male rats. Animals were sacrificed after 30 or 60 minutes and methemoglobin levels determined spectrophotometrically. Methemoglobin levels were negligible at the lowest dose of benzocaine (1.5 mg), but significant levels (20 - 70% of total hemoglobin) were found at the other dose levels. Pretreatment of animals with ascorbate (30 - 60 minutes before administering benzocaine) or simultaneous administration of varying amounts of ascorbate (up to 100 mg) failed to provide a protective effect during the period of observation (60 minutes) in animals receiving 3 or 6 mg benzocaine. Benzocaine does not induce methemoglobin formation in either incubated washed red cells or solutions of hemoglobin. Thus,the rapid formation of methemoglobin in intact animals suggests that the oxidant effect is caused by a metabolite of benzocaine. Washed red cells incubated in mixtures containing liver microsomes, benzocaine, and either NADH or NADPH were found to readily form methemoglobin, confirming notion that the oxidant is a biotransformation product of benzocaine. The relative rarity of benzocaine induced methemoglobinemia may be explained by overdosage rather than idiosyncratic response.

● 395 FETAL PULMONARY VASODILATION WITH HISTAMINE: MEDIATION BY H1 AND H2 RECEPTORS. Robert Truog, Frank J. Accurso, Randall B. Wilkening and Giacomo Meschia, (Spon. by Frederick C. Battaglia), Depts. of Pediatrics, OB/GYN, and Physiology, University of Colorado School of Medicine, Denver.

Histamine (H) is known to dilate the fetal pulmonary circulation. The receptor characteristics, however, have not been completely described. To do so, we determined dose response curves (DRC) to H and to H with infusion of H1 and/or H2 antagonists in 6 chronically prepared fetal sheep. A cuff electromagnetic flow probe measured blood flow to the left lung (QL). Catheters in the main pulmonary artery and aorta measured pressure. A left pulmonary artery (LPA) catheter allowed local infusion of H thereby minimizing systemic effects. QL increased logarithmically from H doses of 1/2 $ng \cdot kg^{-1}$ to 16 $ng \cdot kg^{-1}$. At H = 16 $ng \cdot kg^{-1}$, QL was 110% over baseline. The DRC plateaued at larger H doses. All H doses up to 125 $ng \cdot kg^{-1}$ had no effect on systemic or pulmonary pressure or on heart rate. Diphenhydramine (D), an H1 antagonist, given systemically, shifted the DRC to the right with a dose ratio of 26. Cimetidine (C), an H2 antagonist, had a similar effect with a dose ratio of 1.5. Simultaneous D and C resulted in a dose ratio of 55. H1 and H2 receptors were further confirmed by the use of specific H1 (2-pyridylethylamine) and H2 (Dimeprit) agonists. We conclude that the marked sensitivity of the fetal pulmonary circulation to H is mediated through both H1 and H2 receptors. These findings contrast with those of previous studies in the hypoxic adult pulmonary circulation where atypical H2 receptors may be present, but agree with findings in the hypoxic newborn lamb.