410 SHUNTING OF PHENYTOIN IN VITRO METABOLIC ACTIVATION BY GLUTATHIONE TO LESS REACTIVE METABOLITES: ANIMAL AND HUMAN FETAL TISSUE.

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We previously reported the possible role of a reactive metabolite in phenytoin (DPH) teratogenic injury (Ped Res 16:131A, 1982). We now report data obtained to test the hypothesis that cellular thiols (T), including glutathione (GSH), may play a protective role in DPH-induced tissue injury. In vitro DPH covalent binding (CB) to fetal liver and placenta microsomes from AJ mice (a strain highly susceptible to DPH teratogenesis) was 91 and 73 pmols/mg protein, respectively. By contrast, DPH in vitro CB to liver microsomes from a single human male fetus (14 weeks gestation, weight 465 grams, spontaneous abortion) and to microsomes from two term placentas was 707 and 44 pmols/mg protein, respectively. Addition of T (GSH, cysteine) or T generator (methylthiazolidine) decreased DPH in vitro CB by 50%. Quantitative measurement by HPLC with on-line radiochemical detection of DPH metabolites (dihydrodiol, catechol, methoxycatechol, para-hydroxyDPH (p-HPPH), meta-hydroxyDPH) in the microsomal mixtures treated with GSH revealed increased dihydrodiol and p-HPPH.

These data demonstrate that: 1. human fetal liver microsomes activate DPH to a reactive intermediate, 2. the human fetus metabolizes DPH, and 3. cellular T decreases DPH in vitro CB. We conclude that the human fetus has the metabolic machinery to generate a reactive intermediate that may be responsible for DPH teratogenic injury.

411 UNDERSTANDING CO PRODUCTION BY SUCKLING RATS AFTER TIN-PROTOPORPHYRIN THERAPY: INTESTINAL SOURCES OF CO.

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In order to understand the lack of suppression of the suckling rat excretion rate of CO (VeCO) by tin-protoporphyrin (TP), except in association with broad-spectrum antibiotics [Posselt, et al. AJDC 140:147-150, 1986], we studied the role of intestinal CO sources in this model. The production of CO (VCOgut) by homogenates of Wistar rat intestine is highest in the suckling ileum, decreasing proximally; the gradient is reversed in the adult. After a single 25 umole TP/kg SQ dose, causing significant inhibition of hepatic and splenic heme oxygenase (HO), no difference in the VCOgut between saline- and TP-treated adults was observed. In sucklings, only the duodenal VCOgut was slightly reduced ($p < .05$).

	CO Production (x ± SD pmoles/min/mg protein)			
Suckling	(Adult)	Duodenum	Jejunum	Ileum
Saline n=18	(n=14) 47±14	(60±13) 83±26	(48±13) 107±41	(28±7)
TP n=15	(n=6) 38± 8	(60±12) 79±34	(49±14) 119±39	(25±9)

Treatment with ampicillin, kanamycin, and neomycin, with or without TP (up to 65 umole/kg), also did not suppress the VCOgut of adult or suckling rats. We conclude: 1) TP, at a dose which inhibits hepatic and splenic HO in adult and suckling rats, may not significantly decrease VCOgut; 2) the suppression of the suckling VeCO by TP and antibiotics cannot be explained by antibiotic-mediated inhibition of intestinal HO; 3) because heme is excreted into the intestine after TP treatment, the suppression of the VeCO by TP in adult rats suggests a comparatively decreased capacity of intestinal bacteria to produce CO.

●412 HOW DO FUROSEMIDE (F), INDOMETHACIN (I) AND AMINOPHYLLINE (A) AFFECT EXPERIMENTAL NECROTIZING ENTEROCOLITIS (NEC)?

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Drugs commonly used in the neonate have been implicated in the etiology of neonatal NEC. We assessed the effects of F, I and A in ischemia and reperfusion of the rabbit gut. Seven rabbits underwent laparotomy under anesthesia. In each, 8 intestinal loops (4 pairs), 5 cm long, were prepared. Each pair of loops was injected, respectively, with F (10 mg), I (0.5 mg), A (25 mg) and saline (S) (1 ml). One loop of each pair was rendered ischemic for 5 mins; the other loop with the same drug served as its non-ischemic control. The animals were sacrificed 4 hours after surgery. All intestinal loops were fixed and examined histologically by a single pathologist unaware of group assignment. Histologic changes were graded.

	Ischemia				No Ischemia			
	F	I	A	S	F	I	A	S
Necrosis	1	7	7	7	0	1	1	0
No Necrosis	6	0	0	0	7	6	6	7

The significant ($p<0.001$) findings that 6/7 ischemic loops containing F were normal and all ischemic loops with I & A were necrotic, suggest that intestinal reperfusion injury may be enhanced by I & A while F is protective, at these doses. These animal model results indicate the need for further studies in the use of these drugs in babies at risk for NEC.

●413 CARDIOVASCULAR RESPONSE TO ADENOSINE ANALOGS DIFFERS BETWEEN FETAL AND NEWBORN LAMBS.

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Hypoxia leads to an increase in endogenous adenosine, a compound which markedly alters blood pressure and heart rate via adenosine receptors. We hypothesized that the persistence of a fetal class of such receptors into the postnatal period might induce altered regulation of heart rate and blood pressure. To investigate whether the cardiovascular response to adenosine and its non-metabolized analogs differs in the pre- and postnatal period, heart rate (HR), arterial blood pressure (BP), breathing movements (BM), and blood gases (BG) were measured in the 8 fetuses (120-140 days gestation), and lambs (6-40 days) following IV injection of varying doses of phenyl-isopropyl-adenosine (L-PIA), N-5'-ethylcarboximide-adenosine or cyclohexyladenosine (CHA). In the fetus all analogs induced dose-dependent bradycardia (<70% of basal HR); potency order was PIA > NECA > CHA . D-PIA, $p < 0.01$. BP decreased ≤20%; BM were abolished. In the newborn lamb NECA induced significant tachycardia (≥125% of basal) but PIA and CHA decreased HR; potency order was NECA > PIA > CHA, $p < 0.01$, no apnea was observed. We conclude that ovine fetal lamb and newborn cardiovascular and respiratory responses to adenosine-like compounds differ significantly.

●414 DIRECT MEASUREMENT OF HEME OXYGENASE ACTIVITY BY GAS CHROMATOGRAPHY.

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Traditionally, heme oxygenase (HO) activity in tissue preparations is determined spectrophotometrically by measuring the rate of bilirubin production, a two-enzyme reaction sequence. The adequacy of this method depends upon the presence of excess biliverdin reductase and the transparency of samples. In order to study the effects of inhibitors (e.g. Sn-heme, 25 uM), and inducers (e.g. $CoCl_2$, 250 umoles/kg) of HO, the first enzyme in the sequence, independent of possible effects on other parts of this catabolic pathway or its end product (bilirubin), measurement of HO activity directly by gas chromatography (GC) through quantitation of carbon monoxide (CO) generated during catabolism of heme to biliverdin is desirable. HO activity [pmoles CO/mg protein/min, $\bar{x}\pm SD(n)$] was determined in 13,000xg supernatant of adult rat tissues.

	Liver	Spleen	Kidney	Intestine
Native activity	33±15 (26)	48±16 (26)	20±10 (19)	38±21 (8)
$CoCl_2$-induced	141±59 (4)	56±23 (3)	34± 5 (2)	40± 4 (2)
In vitro Sn-heme	20±11 (7)	19±11 (7)	14± 2 (4)	24±27 (3)

This method is sensitive, rapid and simple, and permits HO activity measurements in tissue preparations as well as intact cells. Tissue is homogenized with KPO_4 buffer, and typically 10 ul of the 13,000xg supernatant is used. The reaction (15 min) is performed in septum-sealed, CO-free reactors at 37°C in the presence of methemalbumin (0.8 mM) with and without NADPH (experimental, 1.6 mM and blank). The reaction is terminated by freezing to -80°C, and the head space gas is analyzed by GC.

▲415 CHOLINERGIC MECHANISM IN THE CEREBRAL CIRCULATION OF THE NEWBORN PIGLET.

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We have shown that, in newborn piglets, the vasodilatory response to 0.1 μM acetylcholine (ACh) is modest (Δ diam = +9±1% in 45% of the vessels, mean±SE), the predominant response is vasoconstriction to 100 μM ACh (Δ diam = -28±3% in 78% of the vessels), and is associated with release of vasoactive prostanoids. The present study investigates the mechanism of the vasoconstrictor response to ACh in 15 anesthetized, mechanically ventilated piglets by examining **a)** the cholinergic receptor subtype involved, and **b)** the potential role of prostanoid release. Using a closed cranial window, ACh (0.1 and 100 μM in CSF) was applied to the pial surface and arteriolar diameter measured with video dimension analysis system. Thirty vessels ranging in diameter from 44 to 204 μm were studied. In five piglets, pretreated with muscarinic antagonist atropine (0.5 mg/kg, I.V.), ACh had no effect on arteriolar diameter (Δ diam=+3±2 and 0±4% at respective concentrations, n=15). In 4 piglets, the muscarinic agonist, methacholine (100 μM), caused vasoconstriction (Δdiam = -15±6%, n=4) while nicotine had no effect. In 6 animals, administration of cycooxygenase inhibitor sodium indomethachin trihydrate (5 mg/kg, I.V.) also prevented the vasoconstrictor effect of 100 μM ACh (Δ diam = -3±2%, n=11). Indomethacin did not reveal a vasodilatory response to 0.1 μM ACh (Δ diam=+3±1%). The data suggest that, in the newborn piglet, the cerebral vasoconstrictor response to the parasympathetic neurotransmitter ACh results from muscarinic receptor activation and may be mediated by vasoactive product of the cyclooxygenase pathway. (NIH #RO1-20337 and #R23-19762-01)