- **329** THE DEVELOPMENT OF CHOLINERGIC CONTROL OF GROWTH HORMONE (GH) SECRETION. P.D. Gluckman (sponsored by M.M. Grumbach); Department of Paediatrics, University of Auckland, Auckland, New Zealand.
- As in man, acetylcholine stimulates GH release in adult sheep. The ontogeny of GH stimulation by cholinergic (muscarinic) agonists was studied in chronically catheterized fetal (term 147 days) and infant lambs. The cholinesterase inhibitor (CEI), physostigmine (PHY, 100ug/kg) given iv to 11 fetuses between 81 and 138 days gestation with a mean basal GH of 97.7±23.3 ng/ml did not affect fetal plasma GH. In contrast, in 6 infants (3-22 days), PHY stimulated GH from 10.3±1.7 ng/ml to 37.8±8.9 ng/ml (p<0.05) with the maximum response at 30 min. This response was abolished by pretreatment with atropine (AT, 100ug/kg iv), (n=3), demonstrating the muscarinic action of PHY on GH release. AT, alone had no effect on GH release (n=3). Neostigmine (120ug/kg iv), a CEI which does not pass the blood-brain barrier (BBB) also stimulated GH release in 3 infants and this effect was abolished by AT (n=3). This suggests that the site of action of cholinergic agonists on GH release is outside the BBB, probably at the pituitary. The late development of the GH response to PHY may represent either the development of cholinergic receptors or cholinesterase in the neonatal pituitary. The development of cholinergic stimulated GH release is in marked contrast to the regulation of GH by serotonin, dopamine, opiates and GABA which develop by 100 days gestation, and is surprising in view of the early appearance of acetylcholine in the fetal brain. Supported by the Medical Research Council of New Zealand.

- **330** INFLUENCE OF BIOLOGICAL MATURATION ON THE DETERMINANTS OF RESPONSE TO FUROSEMIDE. Thomas P. Green (Spon. by B.L. Mirkin), Dept. of Pediatrics, Div. Clin. Pharm., Univ. of Minn. Med. School, Minneapolis 55455.
- The effect of age on the determinants of the diuretic response to furosemide was evaluated in 22 infants (9 premature infants 2-6 days old and 13 infants 1 to 36 months old).
- The urinary excretion of furosemide, and therefore the presentation of the drug to its site of action, was significantly slower (p<0.005) in the premature infants ($t_{1/2}$ = 26.5±31.2 h) than in the older children ($t_{1/2}$ = 1.79±1.26 h). Despite the prolonged time of drug excretion, the younger infants excreted significantly less of the administered dose in the urine (20.7±17.5% vs. 49.2±22.9%; p<0.01).
- All but one infant in each group exhibited an increase in urine flow after furosemide administration and demonstrated a linear relationship between furosemide excretion rate and diuretic response. The increase in urine flow rate per unit furosemide excretion rate was significantly greater in the premature infants (p<0.001).
- The results suggest that the immaturity in at least two aspects of renal function contribute to the pattern of diuretic response seen in very young infants. Immaturity of organic ion transport limits the rate and amount of drug reaching its site of action. However, the inability of parts of the tubule not effected by the diuretic to compensate for the increased electrolyte and water loss leads to an enhanced sensitivity to the drug which is excreted.

- 331** THE INTERPRETATION OF DIURETIC RESPONSE FROM THE ANALYSIS OF URINARY DRUG EXCRETION DATA. Thomas P. Green (Spons. by B.L. Mirkin) Dept. of Pediatr., Div. Clin. Pharm., Univ. of Minn. Med. School, Minneapolis 55455.
- Studies were conducted in 13 infants receiving furosemide to test the validity of new methods for quantifying diuretic disposition and response. Drug elimination that occurs by a first order process should produce a time course of urinary drug excretion described by: $D_u = D_{u\infty} - 1/K \cdot dD_u/dt$ where D_u is the total amount of drug excreted at any time, dD_u/dt is the drug excretion rate at the same time, and K is the overall rate constant of elimination. Values of $D_{u\infty}$ (r=.998) and K (r=.938) computed from this equation both correlated strongly with the same values determined by conventional methods.
- Diuretic response was found to be linearly related to the simultaneous drug excretion rate for the duration of the study in each patient. Therefore, each patient's responsiveness (the increase in urinary flow rate per unit diuretic excretion rate) could be assessed. The product of diuretic responsiveness and total drug excreted in the urine was an excellent predictor of total diuretic response assessed by the usual method of increase in urinary flow rate over baseline values, integrated over the entire post-drug period (r=.951).
- The methods verified in this study will be especially useful in the study of young infants in whom complete urine collections and multiple serum samples and therefore, conventional means of assessing diuretic disposition and response are difficult.

- 332** REPRODUCTIVE DYSFUNCTION IN FEMALE OFFSPRING FOLLOWING PRENATAL EXPOSURE TO PHENOBARBITAL: CRITICAL PERIOD OF ACTION. Chhanda Gupta and Sumner J. Yaffe. Depts. of Pediatrics and Pharmacology, Univ. of Penn. School of Medicine and The Children's Hospital of Philadelphia, Philadelphia, PA 19104.
- Previously we demonstrated that prenatal administration of PB resulted in defects of sexual development. This study was designed to determine the precise time of action within the overall period of sexual differentiation, when PB produced its effect. PB was administered at 12-20, 14-20, 17-20 days of gestation (40 mg PB/kg/day) and postnatal days 1-8 (20 mg PB/kg/day). Female pffspring were tested for different reproductive functions:
	Onset of Puberty (days)	Normal Estrous Cycle (%animals)	Fertility (%animals)	Estradiol (pgm/ml plasma)	Estradiol Receptor (pMole/mg protein)
Control PB	34.6±1.2	91	100	59±12	.136±.037
12-20 days	38.4±1.1*	47*	58*	107±18	.265±.037*
14-20 days	37.2±1.1*	52*	63*	130±18*	.222±.026*
17-20 days	37.5±1.2*	46*	50*	155±25*	.242±.031*
1-8 post-natal	36.4±1.3*	70*	50*	268±16*	.202±.04*
- * p<.05 = significantly different from control animals
- These results indicate PB administration only during the period of neuroendocrine differentiation (17-20 days of pregnancy and 1-8 days postnatal) can produce all the effects attributed to PB.

- 333** REVERSAL OF PHENOBARBITAL (PB) INDUCED ALTERATION IN SEXUAL DIFFERENTIATION BY CYCLOHEXIMIDE AND HYDROXYUREA. Chhanda Gupta and Sumner J. Yaffe. Depts of Pediatrics and Pharmacology, Univ. of Penn. School of Medicine and The Children's Hospital of Philadelphia, Philadelphia, PA 19104.
- Previously we reported that PB administration during late pregnancy caused defects in sexual maturation of female offspring (delayed vaginal opening, irregular cycle and infertility). To elucidate the mechanism of these effects, inhibitors of protein RNA and DNA synthesis were studied. Time pregnant rats (S-D, CD strain) 5 per group, were injected, SC with different agents from 17-20 days of gestation.
- Results show that cycloheximide (CY), a protein synthesis inhibitor, and hydroxyurea (OH-u), a DNA synthesis inhibitor, when combined with PB significantly reduced reproductive dysfunction. However, α -amanitinin (α -Am), an RNA synthesis inhibitor, failed to protect the offspring from PB induced malformations. No inhibitor alone had any effect on reproductive function. Results suggest that prenatal PB produces its effect via new protein synthesis.
	Onset of Puberty (days)	Regular Cycle (%animals)	Fertility (%animals)	Estrogens in (pgm/ml)
Control	34±2.1	91	100	58±12
PB (40mg/kg/day)	37±1.9*	45*	60*	127±26*
PB+CY (60 μ g/kg/day)	35±2.0	67	70	79±11
PB+OH-u (160mg/kg/day)	35±2.1	80	83	80±28
PB+ α -Am (15 μ g/kg/day)	38±2.0*	0*	43*	157±37*
- (*p<.05 = significantly different from control animals).

- 334** METABOLISM OF THEOPHYLLINE TO CAFFEINE IN THE LOW BIRTHWEIGHT INFANT. Arthur F. Harralson, Jane Hoddging, Nasir Tejani (Spon. by Houchang Modanlou) Miller Children's Hospital, University of California, Irvine.
- Theophylline (T) and caffeine (C) serum concentrations were measured in 32 low birthweight premature infants using high performance liquid chromatography (HPLC). All infants were treated for apnea with T if clinically indicated with a loading dose of 4-6mg/kg followed by a maintenance dose of 2mg/kg Q12 hrs. None of the infants were receiving breast milk at the time the serum T and C concentrations were determined and there was no other known source of C intake. The mean birthweight of the infants studied was 0.92kg (range 0.65-1.20kg) and the mean gestational age was 27 wks (range 25-32wks). The mean weight of the infants was 0.86kg (range 0.46-1.05kg) after approximately 7 days of therapy when the serum concentrations were measured. Mean serum T concentration was 8mg/L (range 4-12mg/L) and the corresponding rate of T clearance was determined to be 18.6L/kg per hr. The mean serum concentration determined simultaneously was found to be 3.2mg/L (range 1.3-5.6mg/L). Caffeine serum concentrations were found to be approximately 40% of the corresponding serum T concentration. Since no infants received C from any known source, it is suggested the C detected resulted from T metabolism. The extent of the apparent metabolism of T to C is significantly greater than that noted in adults and children. Because of the known poor clearance of C in the neonate, clinically significant C accumulation may occur in infants being treated with T only.