311 EFFECTS OF SERUM DILUTION AND OXIDATION OF ALBUMIN-BOUND BILIRUBIN ON THE DETERMINATION OF THE UNBOUND BILIRUBIN CONCENTRATION BY THE PEROXIDASE METHOD.

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The peroxidase test, although a sensitive and precise method for determining the unbound bilirubin concentration in serum from jaundiced neonates, has been reported to be inaccurate due to errors from sample dilution as well as oxidation of albumin-bound bilirubin. These potential errors were investigated in serum and defatted albumin solutions. A decrease in the peroxidase-determined apparent unbound bilirubin concentration (AUBC) with increasing serum dilution was found in bilirubin-enriched umbilical cord sera as well as sera from jaundiced infants, but was less marked in bilirubin-enriched defatted albumin solutions. The dilutional decrease in AUBC did not appear to be due to slow oxidation of albumin-bound bilirubin. Instead, the bilirubin-albumin complex was found to have a much lower dissociation rate constant in serum ($K_{-1} \pm SD = 3.3 \pm 0.2 \times 10^{-3} sec^{-1}$), than in defatted albumin solutions ($K_{-1} \pm SD = 1.7 \pm 0.6 \times 10^{-2} sec^{-1}$), causing the dissociation of the complex to be rate-limiting when serum is analyzed at the currently recommended dilution (1:40) and peroxidase concentration (5.0 µg/ml). In addition, there appears to be a dilutional enhancement of bilirubin binding by serum, but not by defatted albumin. Decreasing the serum dilution and peroxidase concentration should significantly improve the accuracy of the peroxidase test. Further studies are needed to clarify the effects of dilution on serum binding of bilirubin.

312 SERUM FREE TOCOPHEROL LEVELS IN PREMATURE INFANTS (PI) RECEIVING TOTAL PARENTERAL ALIMENTATION (TPN).

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Documentation (J Pediatr 95:869, 1979) that PI are biochemically deficient in Vitamin E (V-E) led to the recommendation of supplementing V-E during the first 2 mos. for PI less than 1500g. However, exact V-E requirements for PI have not been documented. The table shows the predicted level (mg/dl) of V-E on PI (n = 10) who were receiving TPN only without lipid source. The TPN contains 1 ml of M.V.I. (USV Pharmaceutical Corp.) providing about 1 IU/kg/day of V-E. The mean gestational age was 30.8 wks with a mean birth weight of 1401 g. Serum free tocopherol levels were measured using a fluorometric micro method (Amer J of Clin Path 36:133, 1966) on the PI's cord blood and on 8 weekly samples. The change in V-E levels over 8 wks was done by fitting a second degree polynomial curve through the points of plot of V-E versus time in wks. The curve was described by an equation: V-E level = 3.65 + 0.365 day - 0.00336 day. This shows that free tocopherol levels above 0.5 mg/dl could be attained after approximately 7 days of TPN administration.

TIME OF TEST	PREDICTED LEVEL
Cord Blood (CB)	0.365
1st wk	0.600
2nd wk	0.803
3rd wk	0.973
4th wk	1.109
5th wk	1.213
6th wk	1.284
7th wk	1.322
8th wk	1.327

313 EXCRETION OF THEOBROMINE IN HUMAN MILK AND SALIVA.

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Theobromine (TB) is a significant pharmacological ingredient for chocolate (C). 10 nursing mothers (ages 25 to 32) who were nursing 1-24 mos were studied. Other methylxanthines were avoided. Each mother ate 1 C bar (1.2 ozs) containing 60 mg TB and 5 mg caffeine (Caf). Simultaneous saliva and milk samples were collected at 0, 1, 2, 3, 5, 8, 12 and 15 hrs. Urine was collected on the infants for 3-6 hrs after maternal dosing. Samples were assayed for TB and Caf using HPLC with mobile phase 0.01 M Na acetate pH 4.0-acetronitrile (93:10) using theophylline as the internal standard. TB appeared in both milk and saliva by 1 hr and peaked in all but 1 pt by 3 hrs. Paired samples showed higher amounts of TB in milk in all but two pts. Saliva/milk ratios ranged from 0.7-1.3 during first 4 hours. Elimination phase $t_{\frac{1}{2}}$ ranged from 4.9-31.5 ($\bar{x}$ = 14.7±8.16) hrs for milk and 3.8-33.0 ($\bar{x}$ = 9.80±8.65) hrs for saliva. There was not agreement between milk and saliva values for 6/10 patients. Assuming each infant would ingest 90 ml milk every 3 hrs for 24 hrs after maternal ingestion of C, the amount of TB available for ingestion ranged from 0.44-1.68 ($\bar{x}$ = 1.06±0.47) mg or 0.73-2.80 ($\bar{x}$ = 1.77±0.79) % of maternal dose. In urine of nursing infants, levels of TB were below 0.6 µg/ml; no Caf was detected. There were no untoward symptoms in the infants. Usual intakes of chocolate does not appear to present significant doses of either TB nor Caf to the nursing infant.

314 CEPHAMANDOLE: MATERNAL-FETAL PHARMACOLOGY.

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To investigate the maternal-fetal transfer of Cephamandole (CMD) and its distribution in the fetus, we administered a single 1000 mg I.M. dose to 35 pregnant women (14-I trimester, 21-II trimester) 25 minutes to 17 hours prior to therapeutic abortion and sterilization by hysterectomy. CMD concentration was assayed microbiologically in maternal serum, myometrium, fetal tissues (placenta, brain, lung, liver, kidney) and fetal fluids (amniotic, CSF, urine and serum). Maternal serum half-life was 2.4 hours while peak serum concentration was 19 ug/ml at 1 hour and 1.5 ug/ml at 8 hours. Mean Fetal serum CMD concentrations were 25% of maternal serum at 1 hour, 30% at 2 hours, 44% at 4 hours and equal at 9.5 hours. There was no detectable CMD (<0.8 ug/ml or gm) level in fetal: brain (19 samples) and CSF (21 samples), liver (28 samples), lung (16 samples), urine (6 samples), amniotic fluid (29 samples). Of 10 fetal kidneys, only 2-II trimester (13 and 19 weeks' G.A.) had detectable levels of 1.4 and 1.35 ug at 1 3/4 and 3½ hours respectively. Placental concentrations of CMD ranged from 0.9 ug/gm to 3.6 ug/gm only between 1 and 4 hours of the study and otherwise was not detectable. CMD does not have a wide distribution in the fetus of the first-half of gestation following maternal dosing and would be useful for treatment of susceptible maternal infections.

315 INDIVIDUAL DIFFERENCES IN ARYL HYDROCARBON HYDROXYLASE (AHH) INDUCIBILITY IN CULTURED AMNIOTIC FLUID CELLS (AFC).

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AHH activity was measured in cultured AFC obtained from patients undergoing amniocentesis for advanced maternal age. Enzyme activity in cell homogenates was measured spectrofluorimetrically by the conversion of 3,4-benzpyrene to alkali-extractable products. Pooled samples were employed to characterize the enzymic activity. In most pooled samples AHH activity was induced from 2- to 5-fold after culture for 24 hours in the presence of 2,3-benzanthracene (BA) a polycyclic aromatic hydrocarbon (PAH). When a series of PAH's were compared as inducing agents 1,2-benzanthracene was found to be the most effective among 5 tested. Enzyme induction with all PAH's was maximal by 12 hours after exposure. Large differences in activity and inducibility were observed in cultures from 10 individual fetuses. Mean control AHH activity was 162±35 pmol/min/mg; mean induced activity was 329±72 pmol/min/mg. The ratio of induced to control activity for each fetus was determined. These ratios ranged from 0.98 to 4.48 and analysis by an iterative maximum likelihood procedure suggested the existence of two discrete populations. Thus AHH activity is present and inducible in fetal AFC as early as 14 wks. of gestation. Determination of AHH in AFC may provide a means for individualization of risk assessment for babies exposed to toxic xenobiotics in utero.

● 316 CAPTOPRIL IN THE TREATMENT OF UNCONTROLLABLE HIGH-RENIN HYPERTENSION IN CHILDREN.

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Captopril(C), an orally active inhibitor of angiotensin I converting enzyme, might be effective in the management of high-renin HTN. However, almost nothing is known about the effects of C in children. We have used C in 5 children (1.8 to 14.8 yrs) with uncontrollable high-renin HTN (PRA=21±6.3 ng/ml/hr, mean ± SEM). All patients (PT) had HTN secondary to severe parencymal or arterial renal disease. Vasodilator drugs were stopped in all 5 PT before starting C. C was started at 0.3 mg/kg/dose every 8-12 hours, and the final dosages ranged from 0.8 to 5.3 mg/kg/day. The 5 children have been on C from 4 to 15 months (mean=9 months). The blood pressure (BP;mmHg) response to C is shown in the following table.

	SYSTOLIC BP	DIASTOLIC BP
Before C	158 ± 8	106 ± 5
After C (1-4 wk)	126 ± 6*	74 ± 3*
After C (4-15 mos)	125 ± 6*	75 ± 5*

(* = different than before C at $p < 0.05$)

After starting C, other antihypertensive drugs were completely stopped in 1 PT and decreased in 3 PT. The only adverse reactions attributed to C were nausea and vomiting. It is concluded that C is effective for long-term control of BP in children with high-renin HTN uncontrolled by currently available drugs. Thus far, there have been a minimum of adverse reactions to C, and dosages of other antihypertensive drugs can often be decreased after control of HTN by C.