235 FETAL CEREBELLAR CELLS IN TISSUE CULTURE

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Fetal cerebellum, obtained between gestational ages 14-22 weeks, was dispersed by mild trypsinization and centrifuged once through 2% ficoll gradient; resuspended cells were inoculated in Eagle's minimal medium with 1% fetal calf serum to restrict the growth of fetal fibroblasts which usually require highly enriched medium. Growth of primary culture was dependent upon initial cell density. At higher concentrations, well separated colonies of multilayered cells and cell processes of a variety of morphology and size were formed within 3-4 days. Extensive outgrowth of processes connected the colonies and many nuclei appeared to migrate on them. Approximately 90% of cells showed positive immunofluorescence stain for the glial protein, S-100. A minor proportion of cells with uniform morphology, stained positively for the neuronal protein 14-3-3. The smallest class of cells did not require attachment for growth and stained positively both for S-100 and 14-3-3 proteins. All cell types appeared to be present in the cerebellum culture of a fetus (gestational age 21 weeks) with 18 trisomy. Although the intact brain appeared to be grossly abnormal, the only obvious difference was that the colonies were discrete and were not connected with extensive cell processes.

236 TRANSFER OF LEUCINE AND GLUTAMATE ACROSS HUMAN PLACENTA

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Previous studies in this laboratory using tissue slices demonstrated significant differences in placental uptake of leucine and glutamate. The transfer of these two amino acids across human placenta has now been studied with an *in vitro* perfusion technique. With both circulations open (not recirculated), the transfer rate for L-leucine from maternal (M) to fetal (F) circulation was 1.7 times that of the D-isomer. The latter rate was attributed to diffusion; the L:D ratio provided a measure of active transport. The L:D ratio F→M was 1, indicating polarity of the placental membrane with respect to active transport. In similar studies with glutamate, the D-isomer was more efficiently transferred in both directions (L:D ratio, 0.6 and 0.4 respectively). This unexpected result was explained by the rapid metabolism of the natural L-amino acid, only 15% of the placental uptake appearing in the opposite circulation. With H circuit open and F recirculated, an F→M gradient was rapidly achieved with L-leucine. In contrast, F levels of L-aspartate and L-glutamate progressively fell below M. A new peak appeared on the F aminogram consistent with glutamine-asparagine.

Rapid metabolism of the dicarboxylic acids by placenta serves as a barrier to transfer and may play a significant role in fetal H₂ metabolism.

237 NEONATAL TYROSINEMIA (NT) IN THE ESKIMO. RESULT OF A PROTEIN POLYMORPHISM? C.R.

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The QNGM performs genetic screening in the Eastern Arctic and has found a high prevalence of NT (14.8%, 1970-72; 11.0%, 1976) in the Eskimo compared to <0.5% in Caucasian controls. Three factors influence NT: ontogeny and the amount of hepatic apoenzyme (4-hydroxyphenylpyruvate dioxygenase); diet and tyrosine (protein) intake; diet and coenzyme (L-ascorbate) saturation of apoenzyme. When Nutrition Canada (1970-72) reported 47% of gravid Eskimo and 14.3% of the offspring at risk for ascorbate deficiency, the cause of NT seemed discovered. But our 1976 survey of plasma ascorbate (56 gravid women; 34 paired term and cord plasmas; 5 infants and 94 random subjects (7-71 yrs)) reveals no evidence for ascorbate deficiency now. Nor is a high intake of tyrosine a likely cause, since Eskimo infants are breast fed. Ontogeny is not a likely explanation: the frequency of prematurity is not increased among Eskimos. A protein polymorphism involving the dioxygenase apoenzyme deserves consideration to account for the 20-fold increase in NT among Eskimos. Treatment and follow-up is indicated because of the concern for mental impairment with persistent NT.

238 RESPONSE OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM TO FUROSEMIDE AND ACUTE SALT LOADING IN THE FETAL LAMB.

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Furosemide (FU) stimulates the renin-angiotensin-aldosterone system in the newborn lamb. This study was designed to assess whether FU or acute salt loading influences the renin-angiotensin-aldosterone system in the fetus. Twelve fetal lambs 102-131 days gestational age were studied (term=145 days). FU (2 mg/kg estimated fetal weight) was infused over 1-2 min. Blood samples were drawn at 8, 20, 35 and 65 min. post infusion. In a separate study hypertonic sodium chloride (2.5 mEq/ml) was infused at a rate of 10 mEq/kg over 2 min. Blood samples were drawn at 3, 7, 15 and 30 min. post infusion. Plasma renin activity (PRA) ng/ml/hr and plasma aldosterone (Aldo) ng/dl were measured by radioimmunoassay. Baseline PRA was 3.09±.37 (M and SEM) at 100-115 days and 8.37±.91 at 120-131 days gestation (p<.01). Baseline Aldo was similar in the two groups (5.6±.03<115 and 5.6±1.3>120 days). Following FU infusion in all fetal lambs, PRA increased from a baseline of 5.37±.71 to 8.56±1.24 at 8 min. (p<.01) and 20.36±5.8 at 35 min. post infusion (p<.05). Aldo, however, did not increase. Salt loading increased plasma osmolality from 297±1.8 to 318±5.6 mOsm/L at 3 min. post infusion (p<.001), with no change in PRA. Conclusions: between 102-131 days gestation in the lamb fetus, 1) baseline PRA increases progressively without a change in Aldo, 2) FU stimulates PRA but not Aldo, and 3) acute salt loading does not suppress PRA.

239 PLASMA PROSTAGLANDIN E LEVELS IN INFANTS AND CHILDREN.

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We have previously reported (Ped. Res. 9:379, 1975) that neonatal plasma prostaglandin E (PGE) levels are significantly lower than those of adults. We have since extended our observations through childhood, and by radioimmunoassay, have obtained the following results (mean ± SE).

	Plasma PGE (pg/ml)				
cord blood	2-3days age	3-12mo. age	1-6yrs. age	6-14yrs. age	adult
1480±390	110±50	140±70	181±52	280±90	530±60
n=8	n=8	n=10	n=11	n=10	n=11

PGE levels in cord blood were significantly higher (p<.05) than in adults. By 48-72 hours of age the plasma concentrations had fallen significantly (p<.001), suggesting that the neonatal lung is capable of PG inactivation. The PG concentrations increased with age, but continued to be significantly lower (p<.05) than those of adults. The reasons for these low levels are uncertain. But, the absence of the renomedullary body during the neonatal period, and the small size of the pre-pubertal reproductive tract, suggest that it is probably secondary to decreased production.

240 SLEEP PATTERNS OF NEWBORN INFANTS UNDER PHOTOTHERAPY.

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Sleep tracings were made of 24 full-term infants on the 2nd or 3rd day of life, using a Beckman Accutrace EEG. There were 9 control infants (Group I) and 15 infants undergoing phototherapy (Group II). EEG tracings were made 30-60 min. after feeding, for 1 hr. The mean sleep-time recorded for Group I was 24.4 min., and for Group II 24.3 min. Phototherapy lights and eye shields were kept on during EEG tracings of Group II infants.

Distinct, and statistically significant (p<.001), differences were observed in the sleep patterns of the two groups. The mean percent of REM sleep was greater under phototherapy (66.5%) than in controls (49.4%); conversely, non-REM sleep was less (33.5%) in the phototherapy group than in the control group (50.6%). Moreover, it was observed that periods of REM tended to be progressively longer, up to 300 sec., in Gp. II infants, whereas REM periods rarely exceeded 120 sec. in Gp. I infants. Active sleep predominated under phototherapy whether or not REM was present, but quiet sleep was present about 30% of the sleep-time tested in controls. Rather than inhibiting PFM sleep and consequent CNS protein synthesis, phototherapy appears to promote it.