487 ABO BLOOD GROUPS AND SEX RATIO AT BIRTH. E. Bottini, E. Carapella, F. Gloria, N. Luccarini and M. Orzalesi (Spon. by C. D. Cook). Camerino Univ., Dept. of Genetics, Rome Univ., Dept. of Child Health, and Naples Univ., Dept. of Neonatology.

We have shown an interaction between ABO and placental alkaline phosphatase (PAP) polymorphisms (Am. J. Hum. Genet. 24, 495, 1972) suggesting that allele $P1^f1$ of PAP partially protects fetuses of blood group B from the damaging effects of antibodies produced by ABO incompatible mothers. On the contrary, ABO compatible fetuses are at a disadvantage, since "some" maternal immunological reaction is necessary for the implantation and maintenance of the zygote in utero. Since XY zygotes are antigenically more dissimilar from their mothers than XX zygotes, we expected a high M/F ratio in newborn infants of group B compatible with their mothers. The study of a consecutive series of 2296 newborns showed a M/F ratio of 1.86 among group B compatible infants. This deviation of the sex ratio from that of the general population ($\sim$ 1.05) is highly significant ($P < 0.01$) and further supports our hypothesis that PAP plays an important role in the maternal-fetal immunological relationship.

488 DECREASED OUABAIN BINDING IN CYSTIC FIBROSIS FIBROBLASTS. J.L. Breslow and J. Epstein, (Spon. by P.S. Gerald), Harvard Medical School and the Children's Hospital Medical Center, Dept. of Medicine, Boston.

We have compared the sensitivities of normal and cystic fibrosis (CF) skin fibroblasts to the cytotoxic effects of ouabain (OB), a cardiac glycoside known to inhibit the transport of ions across cell membranes. Cells from 9 unrelated CF patients when plated at low density for 24 hours in K+ deficient medium and then exposed for 24 hours to OB at concentrations from 1×10^{-10} to 1×10^{-6}M, show an increased survival when compared to normal cells. In agreement with these observations, studies of ^{3}H-OB binding show that CF cells bind less OB than normal cells in medium lacking K+. At low drug concentrations ($2\text{-}20 \times 10^{-9}$M), the first order rate constant for ^{3}H-OB binding in CF cells was approximately 70% of normal. Equilibrium binding experiments were performed in 3 sets of fibroblast strains from age-matched normal and CF individuals. In a Scatchard analysis the CF strains had 84 ± 2%, 56 ± 1%, and 88 ± 2% (mean ± S.E.M) of normal ^{3}H-OB binding sites per mg cell protein (for each strain the difference from normal is $p < .001$). Previous studies by others of OB resistant mammalian cells isolated in culture showed decreased OB binding as well as a decreased ability of OB to inhibit ion transport. We have studied ion flux in normal and CF cells using ^{86}Rb, a potassium analogue, and have shown identical inhibition of ion transport by OB in both cell types. Thus CF cells appear different from other previously described OB resistant cells. The relationship of these observations to the primary genetic defect in CF is not yet clear.

489 THE MECHANISM OF HYPERAMMONEMIA IN CITRULLINEMIA: Saul Brusilow, Mark Batshaw, Brian Robinson, Geoffrey Sherwood, Mackenzie Walser; Johns Hopkins Med. Inst. and Hosp. for Sick Children, Balto., Md. and Toronto, Ontario.

The hyperammonemia which occurs in patients with enzyme deficiencies of the urea cycle, apart from carbamyl phosphate synthetase (CPS) deficiency, is unexplained. In CPS deficiency, ammonium (NH_4+) is a substrate for the reaction and thus it accumulates. In argininosuccinic acid synthetase deficiency accumulation of citrulline (cit) is readily explained but accumulation of NH_4+ is not because although the ornithine transcarbamylase reaction is reversible the CPS reaction is irreversible and not product inhibited. The relationship between plasma levels of NH_4+ and other urea precursors was explored in a case of neonatal onset citrullinemia during 2-1/2 months of management with protein restriction and dietary supplements of nitrogen free analogues of essential amino acids. When plasma cit was 2 mM or less, hyperammonemia was not observed. At higher cit levels, NH_4+ rose with cit ($r=.65$, $p<.01$). Significant positive correlations were also seen between plasma NH_4+ and glutamine (gln) ($r=.71$, $p<.01$), NH_4+ and glutamate ($r=.58$, $p<.01$), and NH_4+ and alanine ($r=.37$, $p<.01$). The two amino acids giving the best prediction of NH_4+ concentration were gln and cit: $[NH_4]=-20+(.327\pm.52)$ [gln]+ $(.012\pm.001)$ [cit]; ($r=.71$, $F=85.47$, d.f.=71). Orotic aciduria occurred only when hyperammonemia was present. These findings suggest that hyperammonemia is a consequence of accumulation of cit, leading in turn to accumulation of carbamyl phosphate which constantly decomposes, releasing ammonium.

490 MONOAMINE AND AMINO ACID TURNOVER IN THE LESCH-NYHAN SYNDROME: EFFECTS OF L-5-HYDROXYTRYPTOPHAN (L-5-HTP) Salvador Castells, Chhaya Chakrabarti, Bertrand Winsberg, Maria Hurwic, James M. Perel, and William L. Nyhan, Downstate Med. Ctr., Dept. of Ped., Brooklyn, N.Y., New York State Psychiatric Inst., N.Y., and Univ. of Calif.,San Diego.

The oral administration of L-5-HTP and carbidopa to children with the Lesch-Nyhan syndrome has been reported to reduce the rate of self-mutilative behavior. In experimental animals, drugs which decrease brain serotonin induce aggressive behavior which is reversed by serotonin's immediate precursor L-5-HTP. A 6 m. old male with a complete deficiency of HGPRT was placed on L-5-HTP initially 24 mg twice a day and carbidopa 12.5 mg twice a day. Prior to therapy, CFS was obtained for 5-HIAA, HVA and amino acids, and repeated at 24 and 60 days of therapy. Thereafter, L-5-HTP was increased to 24 mg four times a day. CFS 5-HIAA, the metabolite of serotonin and HVA, the major metabolite of dopamine, were assayed by spectrophotofluorimetry, and amino acids by column chromatography. An increase in 5-HIAA was detected during therapy e.g., 45.9 ng/ml prior, and 170, and 123 ng/ml on therapy. After 12 m. of therapy, a one-day probenecid turnover study (100 mg/kg, divided into four doses) was done with the patient on and repeated off therapy. On therapy, CFS 5-HIAA pre-probenecid was 24.0 and after probenecid was 365.7 ng/ml,while off therapy after probenecid it was 77.6 ng/ml. CFS HVA and amino acids were not effected by therapy. This study indicated that L-5-HTP and carbidopa given orally specifically increase the turnover of serotonin.

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491 STUDIES OF ^{64}Cu AND ^{109}Cd EFFLUX AND UPTAKE IN MENKES KINKY HAIR (MKHS) FIBROBLASTS. Wai-Yee Chan, Adolfo D. Garnica, Owen M. Rennert. Dept. Pediatr. and Biochem., University of Florida College of Medicine, Gainesville.

Previous studies in our laboratory indicated decreased efflux of Cu and abnormal metallothionein (MT_1) in MKHS. To further identify the kinetics of these transport phenomena studies utilizing ^{64}Cu and ^{109}Cd were performed in MKHS and normal fibroblasts. MKHS and normal cells were grown in Cd-containing medium (Cd concentration = 1.5 µg/ml, ^{109}Cd concentration 0.15 µg/ml, spec. act. = 17.8 µCi/µg) for 20 hours, chased with non-radioactive medium and analyzed at 0, 5, 10, 20 hours.

	Hours (CHASE)			
[MKHS (dpm/mg protein):dpm/ml medium]	0	5	10	20
[normal (dpm/mg protein):dpm/ml medium]	1.2	1.8	0.5	0.6

^{64}Cu efflux studies employed a 20 hour pulse (Cu concentration = 10 µg/ml, ^{64}Cu concentration = 10 µg, sp. act. = 885 µCi/µg Cu) with chase intervals of 0, 5, 12, and 20 hours.

	Hours			
[MKHS (dpm/mg protein):dpm/ml medium]	0	5	12	20
[normal (dpm/mg protein):dpm/ml medium]	1.1	2.1	1.8	2.2

These data clearly indicate a pronounced defect in the efflux of Cu in MKHS fibroblasts as compared to normal fibroblasts. The cadmium results suggest some impairment of Cd-release by MKHS fibroblasts. Studies by Goka et al. (Pediatr. Res.10:365,1976) indicated increased uptake of ^{64}Cu by MKHS fibroblasts. Our studies of ^{109}Cd uptake suggest nearly equivalent uptake by MKHS and normal fibroblasts (MKHS/normal = 0.9-1.8). These data imply that the observed apparent increased uptake is not the primary phenomenon.

492 DEFECTIVE METALLOTHIONEIN IN KINKY HAIR SYNDROME FIBROBLASTS. W.Y. Chan, A. Garnica, O.M. Rennert. Dept. Pediatr. and Biochem., Univ. Fla., Gainesville.

Menke's Kinky Hair Syndrome (MKHS) is caused by defective copper (Cu) transport. Therapeutic trials with parenteral Cu indicate increased urinary Cu excretion, failure to increase hepatic Cu stores and subnormal ceruloplasmin response. These observations prompted study of Cu transport in cultured MKHS fibroblasts. In basal culture medium the intracellular Cu concentration is 300 ng/mg protein vs. 150 ng/mg in normal fibroblasts. Increasing the Cu concentration of the medium results in cell death at 15-20 µg/ml for MKHS vs. 30 µg/ml for normals. At Cu concentrations in the medium below 20 µg/ml, the Cu content of MKHS cells is twice that of normal cells, while from 30-45 µg/ml the intracellular Cu concentrations of MKHS and normal cells approach unity. These data indicate an inability of MKHS fibroblasts to handle Cu normally at all concentrations. The Cu induction profile of MKHS fibroblasts demonstrates a decrease in cadmium (Cd)-mercaptide absorption at 250 nm in the fraction corresponding to metallothionein (MT),which implies an increased affinity of MT for Cu. In basal medium decreased intracellular Cd levels exist in MKHS fibroblasts. Cd-induction (0.1-1.5 µg/ml) experiments indicate increased uptake by MKHS fibroblasts compared to normals. The ratio of MKHS [Cd]/normal [Cd] (ngm/mg protein or ng/µg DNA) ranged from 1.1-1.9 throughout the Cd-induction range. These results, together with the Zn, Cu, and Cd content of the metallothioneins as determined by atomic absorption spectroscopy, are compatible with an abnormal MT and defective Cu efflux for cultured MKHS fibroblasts.