MICROFLUOROMETRIC ANALYSIS OF SISTER CHROMATID EXCHANGES, INDICES OF CHROMOSOME FRAGILITY. Samuel A. Latt (Intr. by P.S. Gerald). Harvard Medical School, Children's Hospital Medical Center, Department of Pediatrics, Boston, Massachusetts.

Quenching of 33258 Hoechst fluorescence by 5-bromodeoxyuridine incorporated into chromosomal DNA (S.A. Latt, P.N.A.S. 70:3395, 1973) allows microfluorometric differentiation between sister chromatids, documenting the semiconservative distribution of newly replicated DNA. Sister chromatid exchanges are sharply demarcated and can be localized relative to quinacrine banding patterns. While the incidence of exchanges is approximately proportional to metaphase chromosome length, the majority occur either in interband regions or very near the band-interband junction. Sites of frequent exchange often compare with the locations of radiation-induced breaks and with break points of spontaneous chromosome translocations reported in the literature. Sister chromatid exchanges, analyzed microfluorometrically, are at least 200 times more frequent than breaks and appear to be very sensitive indicators of chromosome damage by the drug mitomycin C. These exchanges might similarly serve to gauge damage due to other chemical agents or radiation, as well as reflect increased susceptibility of chromosome regions to breakage as in Bloom's Syndrome or Fanconi's Anemia.

CONCOMITANT 'C' AND 'G' BANDS IN HUMAN CHROMOSOMES, C.L.Y. Lee, and J.P. Welch. (Intr. by Richard B. Goldbloom) Dept. of Pediatrics, Dalhousie Univ., Halifax, Nova Scotia, Canada

Constitutive heterochromatin in mammalian chromosomes was first demonstrated in 1970 by hybridisation of radio-labelled DNA with the DNA of cytological preparations. The technique has been modified subsequently to reveal either 'C' or 'G' bands; the latter may be produced by a variety of techniques utilising proteolytic enzymes, urea, detergents, and alkaline phosphate-buffered solutions. Thus far, however, 'C' banding techniques do not satisfactorily identify 'G' bands, and vice versa.

We have developed a technique utilising sequential 0.1 M NaOH and 0.05% trypsin treatments which consistently reveals both 'C' and 'G' bands in the same preparation. Furthermore, this method shows a clearer delineation of the 'C' bands than is usually seen by the standard 'C' band methods. Thus, we have observed (1) consistent differentiation of the 'C' band of the #1 chromosome into three distinct bands (2) frequent similar differentiation of the 'C' band of the #9 chromosome, (3) differentiation of the Yq12 band into at least two bands.

Investigation of a patient with 46,XX, inv(12)(p12q11) by this technique indicates that the functional cetromere does not occupy the whole of the #12 'C' band. A similar phenomenon probably accounts for the 'inactivated centromere' observed with some translocations.

FREE AMINO ACID CONCENTRATIONS IN FETAL SERUM. Harvey L. Levy, Aubrey Milunsky and Fredric D. Frigoletto. Harvard Med. Sch., Depts. of Neuro., Ped., and Obst., Mass. Gen. Hosp. E. K. Shriver Ctr., and Boston Hosp. for Women, Boston.

A number of inborn errors of metabolism (IEM) are not yet prenatally detectable. Perhaps most prominent among these is phenylketonuria. It is possible that in the presence of an IEM, the affected fetus will accumulate increased quantities of one or more of the metabolites and that such increases will be detectable by careful analyses of blood. In order to make such determinations, however, adequate methods and normal values must be available. We have quantitatively examined the serum free amino acids in seven fetuses of 12-20 weeks gestation. In most of the examinations 0.1 ml. serum was used although as little as 0.05 ml. was adequate. The serum was diluted and deproteinized in 1.0 ml. 3% sulfosalicylic acid, centrifuged, and the entire supernatant applied to an amino acid analyzer column. Qualitatively, amino acids were the same as those in postnatal blood or amniotic fluid. Amino acid concentrations were generally 2-3 times greater than corresponding concentrations as previously determined in amniotic fluid or maternal plasma of the same gestational time and of newborn plasma obtained on the first day of life. Specific values in u moles/ml. include Phe (.174 ± .049), Lys (.569 ± .138) and Ala (.580 ± .150), the latter two particularly concentrated. Within the gestational range examined, there were no consistent differences in the amino acid concentrations.

MATERNAL PHENYLKETONURIA AND HYPERPHENYLALANINEMIA. A PROSPECTIVE STUDY. Harvey L. Levy and Vivian E. Shih. State Lab Inst., Mass. Dept. Public Health, Harvard Med. Sch., and Mass. Gen. Hosp., Dept. of Neuro., Boston.

The possible effects of increased blood phenylalanine concentrations on the fetus are of considerable importance, particularly since many girls successfully treated for phenylketonuria (PKU) will shortly become of childbearing age. We have studied prospectively 17 offspring of 8 women who have had varying degrees of hyperphenylalaninemia (HPHE). These women have been discovered as a result of routine screening of an offspring, either of cord blood or of newborn blood. None has received any dietary therapy. Group I includes 3 women with mild HPHE (blood Phe 3-6 mg%) and Group II includes 2 women with intermediate HPHE (blood Phe >6-12 mg%; occasional mild phenylpyruvic aciduria). All 11 offspring are normal. Group III includes one woman with "atypical" PKU (blood Phe >12-15 mg%; moderate phenylpyruvic aciduria). Though she has a slightly higher IQ than her non-PKU sister (78 vs. 71), her 3 offspring have consistently lower IQs than her sister's 3 offspring. Group IV includes two women with "classical" PKU. In one, who is retarded (IQ 56), twin offspring are mentally retarded at age 4 yr. (IQs 67 and 61). In the other, who is dull normal, the infant offspring is developmentally slow at age 1 year. Neither microcephaly nor congenital anomalies has been noted. It would seem that the degree of fetal brain damage from maternal PKU and HPHE may be proportional to the maternal blood Phe level beginning at >12 mg/100 ml.

TRANSEPITHELIAL TRANSPORT OF L-PROLINE IN THE BLOCKED CATABOLIC MUTANT PRO/Re (HYPERPROLINEMIC) MOUSE. R.R. McInnes, F. Mohyuddin, C.R. Scriver. MRC Genetics Group, Montreal Children's Hospital and McGill University, Montreal, Canada.

PRO/Re mouse, a model of Type-I hyperprolinemia in man, provides the first occasion to examine the role of tissue oxidation in net reabsorption of an amino acid. Proline oxidase activity in PRO/Re kidney cortex is 2% of normal. Plasma pro is 0.7mM (10x normal) and the renal cortex pro is 1.3mM (4x normal). Net tubular reabsorption of pro is abnormally depressed in PRO/Re mice, but PRO/Re kidney takes up pro in vivo against a normal chemical gradient across the luminal membrane from urine & across peritubular membranes from blood. PRO/Re kidney cortex slices convert less L-pro at 1.2mM to CO_2 and retain 3x more in the form of pro vs normal slices. Concentration-dependent uptake of L-pro (0.02-10mM) across basilar membranes in slices is mediated by more than one system; affinity of these systems for L-pro is normal in PRO/Re kidney; however steady-state distribution ratios & net uptake rates are depressed in PRO/Re. These findings reveal that hyperprolinuria can result not only from saturation of absorption, but also from exaggerated efflux from cell to urine because of abnormal intrarenal pro accumulation.

GM_3 GANGLIOSIDOSIS: A NEW LIPID STORAGE DISEASE. Stephen R. Max, Noel K. Maclaren, Roscoe O. Brady, Roy M. Bradley and Marvin Cornblath, Depts. Ped. & Neurol., Univ. of Maryland Sch. of Med., Baltimore, and Developmental & Metabolic Neurology Branch, NINDS, NIH, Bethesda.

Inherited defects in sphingolipid metabolism are associated with a number of disorders. A one mo. old male (JH) was first seen because of poor physical and motor development, frequent seizures, and an unusual appearance. The first child of young, unrelated Jewish parents, JH weighed 7 lbs. 12 oz. at birth after a normal 8 mo. gestation. He was limp and unresponsive, with coarse facies, macroglossia, gum hypertrophy, squat hands and feet, flexor contractures of the fingers, thickened loose hirsute skin, large inguinal hernia, enlarged liver and spleen and normal fundi. Death at 3 1/2 mo. followed a series of bronchopneumonic episodes. These features suggested GM_1 gangliosidosis, which was ruled out by the finding of normal β-galactosidase activity in leukocytes and in a liver biopsy. The activities of acid phosphatase, β-glucosidase, β-N-acetylhexoseaminidase, α-fucosidase, α-mannosidase, and arylsulfatase A were greater than normal. The diagnosis of GM_3 gangliosidosis was established by thin-layer chromatographic analysis, which demonstrated the accumulation of GM_3 (N-acetylneuraminylgalactosylglucosylceramide) in post mortem samples of brain and liver. The enzymatic basis of the GM_3 storage is now under investigation. (Supported in part by the John A. Hartford Fndn. and the Tay-Sachs Assn. of Maryland.)