547 FOLATE COENZYMES IN METHYLENE-H_4PTEGLU REDUCTASE (MR) DEFICIENT FIBROBLASTS. D.S. Rosenblatt, B.A. Cooper, S. Lue-Shing and N. Vera. (Spon. by C.R. Scriver). MRC Genetics Group, McGill Univ.-Montreal Children's Hospital; Hematology Division, Royal Victoria Hospital and Depts of Pediatrics, Medicine and Physiology, McGill Univ. Montreal, Quebec, Canada.

MR deficiency is the first inborn error of folate metabolism to be studied in cultured fibroblasts. Cells from patients with this disorder have between 14 and 20% of the normal enzyme activity. Because deficient fibroblasts fail to grow in a culture medium in which homocysteine replaces methionine, it was presumed that they lack methyl-H_4PteGlu, the product of MR and the methyl donor for the conversion of homocysteine to methionine. Folate concentration measured by Lactobacillus casei was not different in normal cells and in mutants, nor was the concentration of Pediococcus cerevisiae-active and Streptococcus faecalis-active folate in the cells. The deficient cells contained significantly less methyl-H_4PteGlu than did controls as determined by the ratio of P. cerevisiae or S. faecalis-active material to L. casei-active material. Analysis of folate levels before and after treatment with folate-depleted human serum reveals that in both normal and mutant cell lines most of the folate exists as folate polyglutamates.

548 POPULATION HETEROGENEITY IN HUMAN SPERM DNA CONTENT

Siddhartha Sarkar, C.W. Jones, W. Centerwall, E.T. Tyler, N. Shioura

The range of variability in the population distribution characteristics of sperm DNA content was surveyed in a selected group of donors. The donors included identical twins, carriers of chromosomal translocations and oligospermics. The DNA content of sperm was measured individually in an automated cytofluorimeter. In contrast to normal donors with high sperm counts and relatively constant modal values of sperm DNA, the balanced carriers of translocations were oligospermic and showed wide dispersion of modal values. This is an unexpected finding, since reciprocal events in the segregation of balanced translocations should not alter modal values of sperm DNA nor should they be associated with oligospermia. It appears that sperm DNA content in oligospermia, independent of its association with translocation, vary over a wide range. It is possible that genetic factor(s), unrelated to chromosome translocation control sperm DNA content.

549 DIFFERENCES IN MONOSACCHARIDE COMPOSITION OF MEMBRANE GLYCOPEPTIDES FROM CYSTIC FIBROSIS AND CONTROL SKIN FIBROBLASTS. Thomas F. Scanlin and Mary Catherine Glick. (Spon. by Stanton Segal). University of Pennsylvania School of Medicine, Children's Hospital of Philadelphia, Department of Pediatrics, Philadelphia.

Many of the abnormal secretory products found in cystic fibrosis (CF) can result from a defect in the cell surface membrane. Consequently, the monosaccharide units from the skin fibroblasts of four patients with CF and four age, sex and race matched controls were compared. The cells, grown under the same conditions, were removed from the monolayer by controlled trypsinization. The trypsin-sensitive glycopeptides and the cells from which the glycopeptides were removed were further processed and subjected to acid hydrolysis. The resulting monosaccharides were analyzed as their alditol acetate derivatives by gas liquid chromatography. Sialic acid content was measured separately by the thiobarbituric acid assay after mild acid hydrolysis and purification on Dowex 1-X8. The molar ratios of the monosaccharide units in the CF trypsin-sensitive glycopeptide fractions from the cell surface were different from those found in the controls. Specifically, the molar ratios of fucose: sialic acid: galactose: mannose: glucosamine were 1:3:4:2:10 for the CF fractions and 1:4:7:5:14 for the controls. Similar, although less pronounced, differences were found in the whole cell fractions. Thus, a refined analysis of the monosaccharide units reveals an alteration in the complex carbohydrates of the CF fibroblast cell surface when compared to controls. USPHS grants AM16859 and GM07025.

550 THE DEVELOPMENT OF A LAMBDA PHAGE VECTOR FOR RECOMBINATION CLONING OF MAMMALIAN GENES. Roy D. Schmickel, Golder N. Wilson, Barbara M. Hollar, The University of Michigan, Mott Children's Hospital, Department of Pediatrics, Ann Arbor.

The successful cloning of genes of higher organisms depends on the development of a simple and safe organism for recombination. In the Edinburgh Laboratory of Ken and Noreen Murray the Lambda phage was altered to permit the insertion of eucaryotic DNA. This phage has been modified to prevent lysogeny and recombination with the host E. coli. In addition, we have added three amber mutations to the phage (in genes for lysis, head formation, and capsid protein). This prevents growth of the phage in any wild-type, non-suppressor host. This phage is useful for cloning DNA because of the limited number of endonuclease restriction sites and the presence of a tryptophan gene. It has two targets for the endonuclease Hind III which are on each side of the tryptophan gene. The Hind III nuclease cuts the phage DNA into 3 separate fragments which can be rejoined with DNA ligase. The presence of the trp gene can be detected on a trp deficient E. coli host. The trp gene can also be replaced with a mammalian DNA of a molecular weight up to 15×10^6. In exchanging a new DNA for the trp DNA, the phage phenotype changes to permit easy selection of recombinant phage. We have recombined this phage with Xenopus ribosomal genes. This cloned DNA was labeled and used for mapping the human ribosomal genes and for in situ hybridization with human chromosomes.

551 NUCLEOSIDE PHOSPHORYLASE DEFICIENCY: IMMUNOLOGIC AND ELECTROPHORETIC EVIDENCE FOR ENZYME HETEROGENEITY.

Scott CR, Osborne W, Giblett E, Chen S-H, Amman A, and Biggar D: University of Washington; University of California; Hospital for Sick Children, Toronto.

An absence of purine nucleoside phosphorylase (NP) activity in humans is associated with T-cell dysfunction. NP catalyses inosine to hypoxanthine and guanosine to guanine in the purine salvage pathway. Two families with children affected with NP deficiency were studied by immunological and biochemical methods. Monospecific rabbit antibody was prepared against purified human NP to test for cross-reacting material (CRM). In studies using RBC hemolysates, one patient had no detectable enzyme activity and no CRM to the NP antibody. Her parents had 1/2 normal NP activity, less than normal CRM and an NP isozyme pattern with multiple slow-moving bands.

In a second family, two affected siblings had 0.5% residual enzyme, and 1/2 normal quantities of CRM. Each parent had an electrophoretic pattern for NP which was different from each other and different from the original family. The father had a normal amount of CRM and the mother 1/2 normal quantities of CRM.

Our studies document the existence of three separate mutations in two families which effect the structure of NP and which is associated with immune deficiency.

552 REDUCED PLASMA PROTEASE ACTIVITY IN PATIENTS WITH CYSTIC FIBROSIS. Thomas W. Seale, Owen M. Rennert. Dept. Pediatr., Univ. Fla. Coll. Med., Gainesville.

To elucidate the molecular basis of cystic fibrosis (CF), we proposed the following working hypothesis: 1) the CF mutation resides in the structural gene encoding a specific proteolytic enzyme, 2) a function of which is to catabolize one or more small biologically-active peptides. 3) Reduction of this enzyme activity as a consequence of mutational alteration results in accumulation of its peptide substrate(s) whose cholinergic-like target specific action results in pathophysiological changes of CF. The results we report support the first contention and confirm the observations of Rao and Nadler. Plasma samples from 44 individuals: 10 cystics, 10 obligate heterozygotes, 12 age-matched normal individuals and 12 contrast patients (e.g. asthmatics, α_1-antitrypsin deficients) were extracted with chloroform, activated with 1×10^{-4}M ellagic acid and assayed for protease activity at 37° using nitrophenylated protamine sulfate (5 mg/ml) in 0.1M phosphate buffer pH 7.6 with 0.15M NaCl. Cleavage products were quantitatively determined with fluorescamine. All but two cystics had substantially reduced plasma protease levels (mean value 2.1±.3meq arg/ml plasma/hr) compared to heterozygotes (4.8±0.5), normal individuals (5.8±.5) and contrast subjects (5.1±0.5). The exceptions had borderline normal activities. Isoelectric focusing of normal plasma revealed five discrete protease species with pIs between pH 4 and 6. In the CF plasmas, the same protease species is decreased by ≥ 70%; activity lost from this fraction corresponds to the observed decrease in total protease activity of unfractionated CF plasma.