547 A METHOD FOR THE PRENATAL DIAGNOSIS OF CHRONIC GRANULOMATOUS DISEASE (CGD). Peter E. Newburger, Susan B. Rothchild, and Harvey J. Cohen. (Spon. by David G. Nathan). Harvard Medical School, Children's Hospital Medical Center and Boston Hospital for Women, Departments of Pediatrics and Obstetrics/Gynecology, Boston, Mass.

To provide a means for the prenatal diagnosis of CGD, we combined a modified nitroblue tetrazolium (NBT) slide test with quinacrine staining for Y chromosome fluorescence. Blood samples (10 µl) are applied to glass slides and the adherent cells incubated with NBT and phorbol myristate acetate, an activator of oxidative metabolism. Resultant metabolic activity produces microscopically observable formazan particles in 99-100% of adult and 95-100% of fetal granulocytes (PMN), in 0-1% of PMN from 4 CGD patients, and in 18-53% of PMN from 3 obligate CGD carriers. Since placental venipuncture samples are often contaminated with maternal blood, it is necessary to distinguish fetal and maternal cells in such mixtures. Quinacrine staining reveals a Y-body in 50-90% of male fetal PMN. Such identification of male PMN by Y-fluorescence allows detection and assessment of oxidative activity of CGD cells diluted 1:100 in maternal cells. Thus, the modified NBT slide test with quinacrine staining of blood obtained by placental venipuncture provides a method for the prenatal diagnosis of CGD in male fetuses at risk for the X-linked or autosomal recessive forms of the disease.

548 PRENATAL DIAGNOSIS OF CONGENITAL ERYTHROPOIETIC PORPHYRIA. Harold M. Nitowsky, Sigeru Sassa, Sachiko Nakagawa and Nasseem Jagani. Departments of Pediatrics and Gynecology-Obstetrics, Albert Einstein College of Medicine, and the Rockefeller University Hospital, New York.

Congenital erythropoietic porphyria (CEP) is a rare disorder of porphyrin metabolism in which uroporphyrinogen I (Uro I) and coproporphyrinogen I are found in great excess in tissues and excreta. This autosomal recessive disorder has been attributed to a partial deficiency of Uro III cosynthetase activity in cells of erythroid origin and other tissues. Studies of fibroblast cultures from skin of a 2 year old boy with CEP revealed an accumulation of protoporphyrin after incubation with Δ-aminolevulinic acid, but no increase in Uro I. A pregnancy of the mother of the patient with CEP was monitored by amniocentesis at 16 weeks gestation. The amniotic fluid had a pink-brown discoloration, and extraction of the porphyrins revealed a marked increase in fluorescence in comparison with control fluids, with characteristic absorption and emission spectra. The presence of marked excess of Uro I was confirmed by thin layer chromatography. Prostaglandin termination of pregnancy 4 days later confirmed the presence of excess Uro I in fetal tissues. Although the diagnosis of CEP can be confirmed by demonstration of reduced Uro III cosynthetase activity in amniotic cell cultures, the marked derangement of Uro I formation is evident in amniotic fluid thereby facilitating prenatal diagnosis of this disorder.

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549 VISUALIZATION OF HUMAN GLOBIN GENES BY RESTRICTION ENDONUCLEASE MAPPING: A NEW APPROACH TO THE PRENATAL DIAGNOSIS OF GLOBIN GENE DELETION. Stuart H. Orkin (Spon. by D.G. Nathan). Harvard Medical School, Children's Hospital Medical Center, Div. of Hematology-Oncology, Dept. of Pediatrics, Boston, Mass.

Analysis of the physical arrangement of globin genes in cellular DNA is now feasible. High molecular weight DNA is digested with site-specific restriction endonucleases, fractionated in agarose gels, and hybridized with radioactive complementary globin cDNA either in situ or after transfer to nitrocellulose filters. This technique permits direct, autoradiographic visualization of DNA fragments containing globin gene sequences in DNA isolated from normal individuals and those with hemoglobinopathies. Comparison of the hybridization patterns of different DNAs provides a convenient, sensitive system for the detection of globin gene deletions or rearrangements. Study of homozygous α-thalassemia (hydrops fetalis) DNA revealed complete absence of specific DNA fragments containing α globin sequences. The sensitivity and clarity of the visualization of this deletion indicate that this new approach will be especially useful in the prenatal detection of globin gene deletions or rearrangements in amniotic cell DNA of fetuses at risk for severe thalassemia syndromes.

550 A NEW FAMILIAL DYSMORPHIC SYNDROME Dustan C. Osborn & J. Philip Welch (Spon. by Richard B. Goldbloom), Dalhousie University, Department of Pediatrics, Halifax, N.S.

A family is presented, members of which show a constellation of features similar to, but distinct from, the so-called Noonan Syndrome. The proband was an eleven year old boy admitted to hospital for an unrelated condition. Physical examination revealed features similar to the Noonan Syndrome. Examination of the mother and six of seven sibs (2 female, 4 male) revealed some or all of the following in each: neck webbing, scleral telangiectasia, high arched palate, myopathic facies, low set ears, ptosis, mild mental retardation, pectus excavatum, short stature, unusual dermatoglyphics, and loose jointedness. All biochemical investigations to date have been normal. Cytogenetic studies have not shown an increase in the frequency of chromosome breakage. Typical features characteristically associated with the Noonan Syndrome (pulmonic stenosis and other heart lesions, cryptorchidism, or renal anomalies) were not present in any family member, and the scleral telangiectasia, sound in six of the eight individuals studied, has not been described in the Noonan Syndrome. The father did not show any of these features and the parents are not consanguineous. The pattern of inheritance is most likely autosomal dominant with variable expressivity.

551 CHROMOSOMAL MOSAICISM IN AMNIOTIC FLUID CELL CULTURES. David C. Peakman, Marilyn F. Moreton, Barbara J. Corn, Arthur Robinson. University of Colorado School of Medicine, Department of Biophysics and Genetics; National Jewish Hospital and Research Center, Denver.

The ability to differentiate true from pseudomosaicism is of major concern in prenatal diagnosis. Of 1000 amniotic fluids processed by in situ methods, a total of 26 demonstrated some degree of chromosomal mosaicism. Two, which had more than one colony possessing the same abnormality, were interpreted as true mosaicism. In both cases the same mosaicism (45,X/46,XX and 46,XX/47,XX+21) was shown to be present in the newborn or fetus. The remaining 24 cases, including 10 with trisomy 2, were interpreted as pseudomosaicism since only a single colony or partial colony demonstrated the aberrant chromosome complement. No phenotypic abnormalities have been noted in the babies delivered following a diagnosis of pseudomosaicism.

Chromosome Constitution	No. of Cases	Chromosome Constitution	No. of Cases
47,XX(or XY),+2	10	47,XX,+20	1
47,XY,+3	1	47,XY,t(1;13),+t(1;13)	1
47,XX,+4	1	46,XX,t(5;5)	1
47,XX,+6	1	46,XX,t(9;14)	1
47,XY,+11	1	46,Y,t(X;15)	1
48,XY,+14,+17	1	46,XX,i(Dq)	1
47,XX(or XY),+16	2	46,XY,-G,+mar	1

552 REACTION OF 4-METHYLUMBELLIFERYLGUANIDINOBENZOATE (MUGB) WITH SKIN FIBROBLASTS FROM PATIENTS WITH CYSTIC FIBROSIS (CF). M.Walsh Platt, Girimaji S. Rao, Stacy J. Simpson and Henry L. Nadler, Northwestern U. Med. Sch., Children's Mem. Hosp., Dept. of Ped., Chicago, Illinois.

Plasma of patients with CF has been shown to have reduced protease levels as measured by rate assays (Pediat.Res.9:739,1975) and by "titration" with MUGB (Am.J.Hum.Genet.29:111A,1977). In order to study whether the reduction in protease activity is a generalized phenomenon in CF, we have quantitated proteases in cultivated skin fibroblasts derived from patients with CF.

Fibroblasts were grown in MEM and fetal calf serum and harvested by scraping. The cells were homogenized in 0.01M veronal HCl and 0.15M NaCl, pH 8.3 and centrifuged at low speed. The supernatant was assayed for reactivity towards MUGB by following the liberation of 4-methylumbelliferone (4-MU). The activity was particulate, inhibited by benzamidine, and not inhibited by p-hydroxymecuribenzoate. The mean "titre" values (nmoles 4-MU released per mg protein) corrected for nonspecific hydrolysis of MUGB were:

Samples	n	Mean "titre" Level	Range
Controls (N)	8	1.27 ± 0.11	1.09-1.50
CF	14	0.66 ± 0.10	0.47-0.82
CF Heterozygotes (H)	8	0.82 ± 0.12	0.66-1.06

The differences were significant for N:H and N:CF ($p < 0.001$) and H:CF ($p < 0.01$). Cultivated normal human amniotic fluid cells gave values in the range of those observed for control skin fibroblasts.

These data suggest that the reduction in protease levels in patients with CF is a generalized phenomenon.