ABSTRACTS

alphabetically by senior author
● = paper presented

A POSSIBLE RELATIONSHIP BETWEEN DIETARY LYSINE AND UREAGENESIS. M. Abruzzo. California State U., Chico.

In an effort to better understand the relationship between lysine and urea production, sixteen adult rats were fed diets of two different lysine concentrations for eight days. Blood urea nitrogen, urine urea nitrogen, food and water consumption, and weight were measured over the eight day period. Also, urea cycle enzyme activities in liver, kidney, and brain were assayed. In addition, ornithine transcarbamylase, argininosuccinate synthetase, and arginase were assayed in vitro with different concentrations of added lysine.

The results of these experiments will be discussed in relationship to human urea cycle enzyme deficiency disorders, possible alternate mechanisms for urea synthesis, and in vivo control of ureagenesis.

●ENZYME THERAPY: RAT MODEL FOR β-GLUCURONIDASE REPLACEMENT STUDIES. D. T. Achord, H. Frankel, J. Glaser, F. Brot, and W. S. Sly. Washington U. School of Medicine St. Louis, Missouri.

An animal model for lysosomal enzyme replacement would be useful to provide answers about enzyme uptake, distribution, and fate in the whole animal that one could not get from human studies. Comparative studies of uptake of human β-glucuronidase by animal fibroblasts showed that fibroblasts established from a number of different species can distinghish "high" and "low" uptake forms of human β-glucuronidase. Because human β-glucuronidase was recognized by rat fibroblasts, was taken up with kinetics similar to those of human fibroblasts, and was stable in rat cells once taken up, the rat appeared a favorable model for in vivo studies. In addition, human β-glucuronidase can be recognized in rat organs following infusion because it survives heating which inactivates the rat enzyme.

Several points can be made from the studies to date using this animal model: 1) β-glucuronidase purified from human placenta (predominantly "low" uptake enzyme) is rapidly cleared from rat plasma following infusion. Thus, in vivo uptake is not "low", as was seen with fibroblasts. 2) At 24 hours after infusion, the placental enzyme is localized in liver (38%), spleen (3%), and lung (trace). None is seen in brain, heart, and skeletal muscle. 3) The human enzyme slowly disappears from liver and spleen with a half-life of 2.6 and 5.6 days respectively.

This model can now be used to study which modifications of the host or the enzyme influence uptake, distribution or fate of infused enzyme.

DETECTION OF CELL SURFACE ANTIGENS CODED FOR BY THE HUMAN C-7 CHROMOSOME IN HUMAN-MOUSE SOMATIC CELL HYBRIDS. D.P. Aden, and B.B. Knowles. Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania.

C57Bl/6 mice were hyperimmunized with somatic cell hybrids obtained by fusion of C57Bl/6 peritoneal macrophages with simian virus 40-transformed human cells. The only human chromosome present within the mouse-human hybrid cells at the time of immunization was the human C-7 chromosome. The antisera were examined by indirect immunofluoresence, indirect $^{125}I$ labeling and complement mediated cytotoxicity on a panel of human cells, human-mouse hybrid cells with and without the C-7 chromosome and on simian virus 40 transformed cells. The ability of the C-7 chromosome to code for cell surface antigens recognizable within this system will be discussed.

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TURNER'S SYNDROME ASSOCIATED WITH XO/XX p- q-. V. Agler, D. B. Evans, and P. A. Farber. Geisinger Medical Center, Danville, Pa., and Belvedere Medical Center, Carlisle, Pa.

The ASG banding technique revealed mosaicism associated with Turner's syndrome and the presence of short and long arm deletions on the same X chromosome. These deletions involved two-thirds of the short arm and one-half of the long arm.

The classical physical features for Turner's syndrome were present as well as primary amenorrhea, high gonadotropins, lack of estrogen production and a decrease in sex chromation on buccal smears.

HAPTOGLOBIN TYPES IN RHEUMATIC AND CONGENITAL HEART DISEASE. Y.R. Ahuja, R.R. Nair, J.S. Murty, Y.R. Reddi, G.D. Reddy, and V.S. Rao. Dept of Genetics, Osmania U., Hyderabad, and Institute of Child Health, Niloufer Hospital, Hyderabad, India.

Haptoglobin types (1-1, 2-1 and 2-2) of 47 patients of rheumatic heart disease (RHD) and 75 of congenital heart disease (CHD) were determined by electrophoresis, and their frequencies were compared with the expected values calculated from 181 control subjects. It was found that the distribution of frequencies of RHD patients in each of the Hp types was about the same as the expected values, but that in the CHD differed significantly. Moreover, it was noticed that in the CHD group the frequency in the Hp2-1 type was lower and the frequencies in the Hp1-1 type as well as in the Hp2-2 type were higher than the expected values. The results were consistent when the CHD group was split into septal defects and non-septal defects. There seems to be a hybrid effect of Hp2-1 expressed in the individual's physiology which makes him relatively less susceptible to CHD.

IMMUNOLOGICAL AND GENETICAL STUDIES ON FAMILIAL LYMPHORETICULAR MALIGNANCY IN NEWFOUNDLAND. P.W. Allderdice, J.M. Barnard, S.K., Buehler, J. Crumley, G.R. Fraser, E.W. Hudson, A. Jacquard, E. Knowling, D. Laing, B. Larsen, W.H. Marshall, A. Roberts, L.S. Salimonu, and other members of the West Coast Health Survey Group. Faculty of Medicine, Memorial U. of Nfld., St. John's, Nfld., Canada and Institut National d'Etudes Demographiques, 27, Rue du Commandeur, 75675, Paris, France.

Seven cases of Hodgkins disease have occurred in an extended, considerably inbred family in Newfoundland together with three cases of lymphosarcoma, two of thymoma, one of chronic lymphatic leukaemia and three of common variable immunodeficiency. Single cases of retinoblastoma, neuroblastoma, and rhabdomysarcoma have also occurred in close relatives. HL-A typing and haplotyping has been done on some 350 family members and the data will be presented in relation to possible association of cases with a single haplotype. In addition to overt clinical immunodeficiency, there appears to be a reduction in cell-mediated responsiveness in members of the extended family as assessed from records of conversion to BCG vaccination. Immunoglobin measurements on the community also show more abnormalities than do sera from a control population in St. John's. The pedigree, containing some 3,000 names is being analysed for the degree of inbreeding and for the degree of relationship between the patients; the preliminary results will be presented. At present the data are compatible with the idea that a mild immunodeficiency state, widespread in the extended family, is predisposing family members to the development of various lymphoreticular malignancies. Correlation of cytogenetic markers, genetic markers, viral antibodies, and epidemologic data is in process.

ON THE TIMING OF GROWTH OF 47,XYY-MALES. L. Alvesalo, and M. Kari. Institute of Dentistry, University of Turku, Turku, Finland.

Measurements of the deciduous teeth of 47,XYY-males strongly suggest that excess growth of these individuals occurs very early, most probably already in foetal life. The present results together with those of the earlier study (Alvesalo et al 1975) indicate that the influence of XYY-constitution on aberrant growth in quantity is continuous through the early stages of development and does not bring about any drastic changes in relative growth between and within two dentitions. The nature of the possible chromosomal influence on growth is discussed.

(Ref. Lassi Alvesalo, Richard H. Osborne and Markku Kari: The 47,XYY-male, Y-chromosome and tooth size. Am. J. Hum. Genet. 27: 53-61, 1975)

TAY SACHS SCREENING IN A FRENCH CANADIAN DEME: SUGGESTIVE EVIDENCE FOR POLYMORPHISM OF THE TAY SACHS GENE. E. Andermann, C.R. Scriver, R. Gold, L. Wolfe, G. Patry, R. Lafontaine, G. Geoffroy and F. Andermann, McGill U. and U. of Montreal, Montreal, and Laval U., Quebec, Quebec.

Twelve children in seven French Canadian families with no known Jewish ancestry were found to have the typical clinical features of Tay Sachs disease. In five families, the enzymatic features were indistinguishable from classical Tay Sachs disease, whereas in two families, they revealed Sandhoff's disease. The Tay Sachs families originate mainly from a deme on the north and south shores of the St.Lawrence River east of Quebec City. Certain ancestral couples are seen to recur in the pedigrees of the Tay Sachs families, suggesting a founder effect. We have undertaken a carrier detection program in the extended families of the Tay Sachs patients. The hexosaminidase determintations are performed by the automated method of Delvin et al (1974). Discrimination between carriers and normal homozygotes is achieved by a method devised by Gold et al (1973), based on Bayes' theorem. To date, 650 individuals from four families have been tested. For 242 individuals analyzed, excluding obligate heterozygotes, the observed heterozygote frequency was 16.5% as compared with an expected frequency of 14.6%, based on genetic hypothesis. The expected frequency of heterozygotes for the region tested was calculated to be 2.9%, similar to that found in Ashkenazi Jews, as compared to only 0.3% found in a large French Canadian control group from the Montreal region. The density distributions of hexosaminidase A vs B in normal homozygotes of the screened population were found to differ from the corresponding values in French Canadians from the Montreal region. These findings suggest the possibility that the gene(s) controlling hex A and B in the deme from which our families originate is (are) different from those in the control French Canadian population. Similarly the mutant allele in this deme might be different from that in Ashkenazi Jews.

A SYNDROME OF COLLAGEN VASCULAR DISEASE, SPASTICITY AND MENTAL RETARDATION IN AN INBRED KINDRED. F. Andermann, E. Andermann, I. Leppik, A. Eisen, G. Karpati, S. Carpenter and R.C. Elston, McGill U., Montreal, Quebec, and U. of N. Carolina, Chapel Hill.

Collagen vascular disease with spastic paraplegia and mental retardation in an individual is unusual; familial occurrence of this is unique. We have ascertained 13 individuals in 4 sibships who exhibit a spectrum of clinical manifestations consisting of various forms of collagen vascular disease (CV), including discoid lupus, polymyositis, dermatomyositis and juvenile rheumatoid arthritis; mild spastic paraplegia with mild mental retardation (SP); and severe spastic quadriplegia with severe mental retardation (SQ), alone or in combination: CV (2); probable CV (2); CV + SP (4); SP (2); SQ (3). All 13 affected individuals come from Ste. Thérèse de Gaspé, a small fishing village, and are offspring of consanguineous marriages. Their parents can all be traced to a common ancestral couple of Acadian descent who married in the Gaspé in 1816. The apparently random combination of manifestations might suggest that at least two autosomal recessive traits, those for CV and for SP/SQ, are segregating independently. However, the possibility of a single mutant gene with pleiotropic effects, perhaps determining a common immunologic defect responsible for both the collagen vascular and neurological manifestations, should be considered. HL-A typing in one sibship showed that all affected individuals inherited at least one W21 gene, but there was no good correlation between HL-A type and the clinical picture. Linkage studies using 23 independent systems resulted in informative matings for only 6 systems. Of the 40 possible combinations of clinical syndromes and markers tested, there was significant linkage at the 5% level for only two systems (Duffy and acid phosphatase). Further HL-A typing and linkage studies are being carried out.

COMMUNITY BASED GENETIC COUNSELING IN NORTH GEORGIA: AN INNOVATION IN SERVICE DELIVERY. L. G. Andrews, I. Benuck, D. Stansell and A. Falek. North Health District Genetic Counseling Program, Gainesville, Georgia; Georgia Mental Health Institute and Emory University, Atlanta.

Outreach genetic counseling in north Georgia has been developed to integrate with the existing regional public health service delivery system. In this report, we will present the genetic counseling program available to the more than 200,000 residents of predominantly rural communities located within a geographic region including part of southern Appalachia. The two genetic counselors in the program not only provide service from their main office in Gainesville, but also travel to homes, hospitals and other health facilities in the region. The program maximally utilizes previously established State and local professional resources for the purposes of referral, medical evaluation, diagnosis, laboratory studies and social services. Duplication of effort is, therefore, minimized. Local health professionals, particularly public health nurses and social workers, have been especially helpful in our outreach program with reticent rural and mountain families. The report will describe 1) the procedures within the program for intake, counseling, follow-up and evaluation, and 2) the interrelationships between the program and other agencies.

●COMPOUND LATERAL ASYMMETRY IN CONSTITUTIVE HETEROCHROMATIN: A NEW TYPE OF HUMAN CHROMOSOME POLYMORPHISM. R.R. Angell and P.A. Jacobs. U of Hawaii, Honolulu.

Human lymphocytes grown for one replication cycle in BrdU and stained by a Hoechst/Giemsa technique show differential staining in the constitutive heterochromatin of chromosomes 1, 9, 15, 16 and the Y. In chromosome no. 9 both sister chromatids stain darkly and symmetrically, while in 15, 16 and the Y simple lateral asymmetry is observed. No. 1 on the other hand shows a new type of chromosome polymorphism in which compound lateral asymmetry is present. The properties of this new type of polymorphism will be discussed in detail.

EVALUATION OF GENETIC COUNSELING IN DOWN'S SYNDROME. R.M. Antley. Indiana U., Indianapolis.

Evaluation studies on genetic counseling indicate that some families understand genetics and recurrence risk, while others do not. The purpose of this study was to evaluate the effectiveness of genetic counseling in terms of understanding of genetics, and to determine predictors of outcome. Thirty families were interviewed immediately before and after counseling to measure mothers' understanding of genetics, and their emotional states. Results indicated that mothers' educational level, anxiety, and desire for future children were significantly correlated with their genetic knowledge prior to counseling. For further analysis, mothers were dichotomized into high and low education groups, depending upon whether they had been educated beyond high school. The low education group (LEG) had a 36% correct score prior to counseling, compared with high education group (HEG), who had 66% correct. With counseling, LEG improved more (35%) than HEG (16%). After counseling, a perfect score on chromosome origin and recurrence risk of Down's was attained by 88% of HEG. Perfect scores were attained on the same two items by 47% and 63% of LEG. All mothers understood prenatal diagnosis to criterion. Overall, a perfect score was attained in all three areas by 57% of the mothers, and 71% had perfect scores on recurrence risk and prenatal diagnosis. Personal recurrence risk was known by 86%. Counselees with major flaws in their information were depressed mothers from LEG. These results suggest that LEG are most in need of genetic information, and that HEG come to genetic counseling to learn their recurrence risk and to verify and integrate information which they had learned. The latter suggestion emphasizes therapeutic counseling aspects of geneticist-patient encounters. Thus, there appear to be two different roles for the geneticist: 1)giving genetic information, and 2)therapeutic counseling. (Supported by Genetics Center Grant GM 2015401.)

●URINE ENZYME DIAGNOSIS IN ATYPICAL KERATAN SULFATE EXCRETING MUCOPOLYSACCHARIDOSIS. A. I. Arbisser, K. A. Donnelly, C. I. Scott, N. M. DiFerrante, R. E. Stevenson, A. S. Aylsworth. U of Texas Medical School at Houston and Baylor College of Medicine, Houston, Texas, Greenwood Genetics Center, South Carolina, U of North Carolina, Chapel Hill.

A 14 year old white female with odontoid hypoplasia, mild dysostosis multiplex, cloudy corneas and keratan sulfaturia was referred as having Morquio syndrome. Her clinical and roentgenographic findings were incompatible with this diagnosis.

Homogenates of the patient's cultured fibroblasts; concentrates of their culture media and of the urinary proteins failed to show any appreciable 6-sulfatase activity, either with polymeric keratan sulfate or with chondroitin-6-sulfate tetrasaccharides as substrates. Moreover, a specific product of 6-sulfatase activity, 3,6-anhydroglucosamine, was demonstrated in normal urine but not in the patient's urine or in those of a typical case of Morquio syndrome.

CRM-POSITIVE HEPATIC PYRUVATE CARBOXYLASE DEFICIENCY ASSOCIATED WITH RENAL TUBULAR ACIDOSIS (RTA). B. Atkin. N. Buist. A. Leiter, & M. Utter. U. of Ore. Health Sciences Ctr., Portland, and Case Western Reserve U., Cleveland Ohio.

A nine-month old male presented with failure to thrive and acute keto-acidosis. Lactic acidosis was confirmed by the following results: blood lactate 8.77 mM (n<1.0mM), pyruvate 0.79 mM (n<0.1mM), alanine 0.81 mM (n<0.30mM), beta-hydroxybutyrate 7.56 mM (n<0.08mM). When the plasma $HCO_3^-$ was 14 mEq/l and plasma pH was 7.22, the urine pH was 8.77 and $HCO_3^-$ was 55 mEq/l. These values confirmed the diagnosis of RTA. The urine was tested for the Leigh's inhibitor by J.R. Cooper and by J.V. Murphy; all tests were negative. There was no spontaneous hypoglycemia but following a fast of 24 hrs. the blood glucose was 34 mg%. Hepatic pyruvate dehydrogenase complex and citrate synthase activities were normal: pyruvate carboxylase (pc) activity was virtually absent but immunologically cross-reactive material to antibodies against chicken liver pc was present. Presumably pc is missing in brain and kidney accounting for the very severe retardation and for the proximal RTA. Normal tubular re-absorption of amino acids and glucose implies that the RTA is not due merely to a lack of renal ATP production. (Gruskin, et al., Ped. Res., 7, 832. (1973).

MAPPING LOW-MULTIPLICITY GENE SITED BY HYBRIDIZATION IN SITU: TESTS FOR FEASIBILITY REVIEWED. K.C. Atwood, E. Eicher and A.S. Henderson. Columbia U. College of Physicians and Surgeons, New York, and Jackson Laboratory, Bar Harbor, Maine.

Mouse translocation stocks permitting identification of the chromosomes known to contain the hemoglobin loci have been used to test the feasibility of mapping genes of low-multiplicity by means of hybridization in situ. We have demonstrated that the globin sites in the mouse can be mapped using available methodology. Analysis of data and appropriate feasibility tests will be discussed.

●PEROXIDASE DEFICIENCY IN NEURONAL CEROID-LIPOFUSCINOSIS. Y. C. Awasthi, H. H. Morris, S. S. Schochet, G. F. Powell and S. K. Srivastava, The U. of Tex. Med. Br., Galveston, Texas.

Neuronal ceroid-lipofuscinosis, Jansky-Bielschowsky type Batten's disease was confirmed in a 3 year old girl by the presence of osmiophilic curvilinear profiles in electron micrographs of skeletal muscle, and by neurological examination. An autopsy of a clinically affected sibling revealed intraneuronal PAS positive material. Peroxidase deficiency has been suggested to be the cause of this disease. Previously, the peroxidase deficiency was shown only with p-phenylene diamine as hydrogen donor whereas activity towards o-dianisidine and guaiacol was normal. Using all the three hydrogen donors, we find the enzyme activity in the leukocytes of the patient to be about 10 per cent of normal. On polyacrylamide disc electrophoresis, the peroxidase from normal leukocytes stained for enzyme activity using these three hydrogen donors, whereas patient's leukocytes showed no enzyme stain.

The patient was given daily doses of methionine (1 g), Vitamins 'C' (1 g), 'E' (400 i.u.) and '$B_{12}$' (100 units per week) for 5 weeks. At the end of this period the peroxidase activity in patients leukocytes towards all the three reagents increased about twenty fold. This regenerated activity was non-dialyzable, heat labile and behaved similar to normal enzyme on polyacrylamide disc electrophoresis. However, no definite clinical improvement was observed in the patient during 8 weeks of therapy. Although, we are unable to explain the deficiency and the rapid regeneration of patient's leukocyte peroxidase activity after therapy, the peroxidase deficiency appears to be a secondary manifestation rather than the primary cause of this disease.

LETHAL HYPERAMMONEMIA DUE TO PARTIAL ORNITHINE TRANSCARBAMYLASE (OTC) DEFICIENCY IN A SIX YEAR OLD MALE. A.S. Aylsworth, C.N. Swisher, and H.N. Kirkman. University of North Carolina, Chapel Hill.

A six year old Negro male developed hyperammonemia, vomiting, seizures, and coma and died with a diagnosis of the Reye syndrome after a rapid downhill course. He had been otherwise well except for one hospitalization with hyperammonemia two years prior to this admission. Microscopic examination of his liver revealed nuclear vacuolization as well as glycogen and lipid deposits in the hepatocytes. There was no hepatocellular necrosis or inflammation. Two half-brothers had previously died with similar symptoms at the ages of six and ten years. All three boys had different fathers. OTC activity in the patient's liver (1.2 U/mg prot.) was approximately 6% of the activity found in control livers (20.8 U/mg prot.). Activities of several other urea cycle enzymes were within the normal range. Analysis of cultured fibroblasts revealed a 46, XY chromosome complement. His clinical and biochemical findings are similar to those seen in females with a partial deficiency of ornithine transcarbamylase whose affected male relatives have a complete deficiency of enzyme activity and die in the first week of life. Because of the implications for genetic counseling of an X-linked trait, OTC deficiency should be considered as a possible diagnosis in patients who present clinically as cases of the Reye syndrome.

●VARIATION IN THE NUMBER OF ALPHA LOCI FOUND IN HETEROZYGOTES FOR HEMOGLOBIN G PHILADELPHIA (α68 ASN→LYS). R. M. Baine and D. L. Rucknagel. U. of Michigan Medical School, Ann Arbor.

There is considerable evidence that Caucasians have two α chain loci while Melanesians have only one. Bimodality in the proportion of Hb G in heterozygotes (Rucknagel and Winter, Ann. N. Y. Acad. Sci. 241: 80-92, 1974) suggests that the American Black population is heterogeneous with respect to the number of α chain loci, and that Black individuals may possess 2, 3, or 4 α chain genes. Further studies, now including 28 persons, show trimodality in the proportion of Hb G with modes at 22%, 30%, and 41% Hb G and support this hypothesis. Another possible explanation is the presence of some form of α thalassemia in the 30% and 41% classes.

To distinguish between these hypotheses, reticulocytes from persons with 30% (27% and 28%) and 41% (41% and 43%) Hb G were incubated with tritiated leucine and the specific radioactivity of each chain determined. The $\alpha^G$ chain specific radioactivity was lower than that of the $\alpha^A$ in all four cases, suggesting some defect in $\alpha^G$ synthesis. But approximately equal counts were incorporated into the α and β chains, implying no deficiency in total α chain synthesis. In the two persons with a high proportion of Hb G, $\alpha^A$ chain specific activity was greater than that of $\beta^A$; evidently the normal allele can compensate for defective $\alpha^G$ synthesis. Since $\alpha^A$ chain synthesis was unimpaired, α thalassemia seems an unlikely explanation for the trimodal distribution of Hb G. We conclude that the observed variability can best be explained by heterogeneity in the number of α loci in the American Black genome.

ATLANTA TAY SACHS DISEASE PREVENTION PROGRAM. W. R. Ballou, L. J. Elsas, D. J. Danner, R. P. Marion, and D. K. Wysowski. Div. of Med. Genetics., Dept. Ped., Emory U., Atlanta, Ga.

A Tay Sachs disease prevention program, initiated in May, 1975, was designed to mass screen a high risk population, assess the demography of the compliant individuals, confirm genotypes, and counsel heterozygotes. From an estimated Jewish population of 22,000, 2,335 individuals were screened in 23 contact hours. Data derived from questionnaires and fluorometric analysis of sera and WBC for hexoseaminidase A (Hex A) were stored on computer cards and analyzed using a Fortran IV SPSS5 program. Ninety eight and one-half percent of screenees professed Judaism and 72.5% were of Ashkenazi origin; 90% were between the ages of 18 and 50 years; 80% were married; 1.3% had relatives who were known carriers; and 6.2% of the males had pregnant wives. Their reasons for compliancy were as follows: 75% from direct contact through the sponsoring organization (NCJW); 21% from mass media; and 3% from rabbis. Only 1.0% came at the suggestion of their physician, but 55% requested that he be informed of the results. Sera were tested in triplicate for Hex A activity. Normal values were 57±7%. When Hex A activity was below 50%, patients were retested using both serum and WBC. Seventeen of 451 individuals whose tests are now complete were diagnosed as heterozygotes. An additional 26 have initial serum values of Hex A between 40 and 50%. Two sets of parents were heterozygotes and obtained prenatal monitoring. These data indicate that a high risk Ashkenazi population responds to directed advocacy by the Jewish Community; that the heterozygote frequency in this compliant Southern population will be between 1/30 and 1/10; and that mass screening programs must include retrieval, diagnosis, and treatment processes.

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UROPORPHYRINOGEN SYNTHASE (URO-S): STUDIES IN PORPHYRIC FAMILIES. R.M. Bannerman, M. Kreimer-Birnbaum and J.A. Edwards, Medical Genetic Unit, Department of Medicine, State University of New York at Buffalo, and the Buffalo General Hospital, Buffalo, New York.

In acute intermittent porphyria (AIP), there is a metabolic defect in the step catalysed by uroporphyrinogen synthase (Strand et al., Proc. Nat. Acad. Sci. 67:1315, 1970), which converts porphobilinogen (PBG) to uroporphyrinogen I.

We have adapted the blood URO-S assay of Granick et al. (Proc. Nat. Acad. Sci. 69:2381, 1972) to study normal individuals and families with different kinds of porphyria. Hemolysates were incubated with PBG for 1 hour at 37°C, and the porphyrins extracted and quantitated by fluorometry. URO-S activity was expressed as nanomoles of porphyrin formed per ml red blood cells per hour. Adult controls had a range of activity between 21.7 and 51.2 (mean: 36.5).

Patients with well-documented AIP show approximately half the normal level of activity of URO-S. In studies of their families similar low levels in hitherto symptomless individuals indicate carriers of the AIP gene. Normal levels of URO-S are found in patients with variegate porphyria and coproporphyria. The test is therefore of value in differential diagnosis, as well as in genetic counselling.

SWITCHING-OFF/SWITCHING-ON PHENOMENON IN LYSINE TRANSPORT BY MAMMALIAN INTESTINE. C.S. Bartsocas. U. of Athens, Athens, Greece.

L-lysine-$^{14}$C uptake was studied in vitro in intestinal segments of rats, in various ages, ranging between 15-day fetuses and adults. L-lysine in 0.065mM concentrations was accumulated against a chemical concentration gradient by processes which obeyed saturation kinetics. There appeared to be 2 peaks of lysine uptake values, one in the fetus, diminishing by the 17th fetal day and another the 2nd postnatal day. Fetal transport of lysine was not inhibited by anaerobic conditions and was not $Na^+$ dependent, in contrast with increasing $O_2$ and $Na^+$ dependence postnatally. A series of aminoacids including representatives of the neutral, iminoacid and dibasic groups failed to inhibit lysine uptake, with the exception of L-arginine, which was also antagonized by L-lysine. These findings suggest that in the rat intestine L-lysine is transported by at least 2 mechanisms, one fetal, not requiring $O_2$ and $Na^+$, and another developing postnatally with a peak during the 2nd day, which is Na-dependent and requires aerobic conditions.

(With the support of grants from the National Research Foundation and the American Philosophical Society).

●HETEROGENEITY OF DOMINANT MYOTONIA CONGENITA (THOMSEN). P. E. Becker.
Institut für Humangenetik der U. Göttingen, 34 Göttingen, Nikolausberger Weg 5a, West Germany.

Twenty-seven families with dominant myotonia came to my knowledge through an inquiry carried out in the German Federal Republic and West Berlin in the last years. One hundred twenty-six affected persons were available for investigation.

To all appearance dominant myotonias are heterogeneous. Three or probably four different types can be discriminated:

Type I: Thomsen's disease,
Type II: Myotonia congenita with muscle aches,
Type III: Myotonia congenita with severe cold-dependency,
Type IV: Myotonia with rare affection of facial muscles and intermittent course.

THE PREVALENCE OF DIVORCE AMONG FAMILIES OF CHILDREN WITH CYSTIC FIBROSIS. M. L. Begleiter, V. F. Burry and D. J. Harris. The Children's Mercy Hospital, Kansas City, MO.

Many members of the medical community share the opinion that genetic counseling couples have a higher prevalence of divorce than couples in the general population. The prevalence of divorce among families of children followed in the cystic fibrosis clinic of The Children's Mercy Hospital was 8% (2 of 25 couples). This figure is compatible with the reported prevalence of divorce for cystic fibrosis families of 11%. Pooled data from publications reviewing the experiences of genetic counseling clinics revealed a prevalence for marital breakdown of 3.1%. Data from spina bifida clinics in England and South Wales indicated 9 divorces among 312 couples (a 2.9% prevalence). For the purpose of comparison to a non-genetic, chronic disorder, the prevalence of divorce in families of children with leukemia was examined. Among 98 families followed in 4 different studies, there were 2 divorces and 2 separations, producing a prevalence for marital breakdown of 4.1%. The prevalence of divorce in the general population of the U. S. is approximately 14%. We conclude that there is no evidence to support the belief that families of children with genetic disease have a higher prevalence of divorce than couples in the general population. The published data indicate just the opposite; these families have a prevalence of divorce below the national average.

(This study was supported in part by the Carrie J. Loose Trust and the Victor Speas Trust and by funds from The Children's Mercy Hospital, Kansas City, MO.)

●CHARACTERISTICS OF NUCLEOSIDE PHOSPHORYLASE IN THE PARENTS OF A CHILD WITH DEFICIENCY OF THE ENZYME. C. Berglund, A. J. Ammann, E. R. Giblett. Puget Sound Blood Center, Seattle, Washington, U. of California, San Francisco, and U. of Washington, Seattle.

We have recently described a child with severe T-cell immunodeficiency, whose red cells lack purine nucleoside phosphorylase (NP) activity. The metabolic step catalyzed by NP is just beyond that catalyzed by adenosine deaminase (ADA). Since ADA deficiency causes severe immunodeficiency it is likely that the patient's NP deficiency is the basic cause of the defect in T-cell function.

The electrophoretic patterns of NP in red cell hemolysates from the consanguineous parents of the child showed three cathodal bands not present in the usual NP pattern. Extracts of cultured fibroblasts from the parents showed two extra cathodal bands. Fibroblasts from the child had no detectable NP. The red cell NP activity of 100 normal donors was 44.8 ± 5.5. Activity in the parents' red cells was below half of this value. Measurements of activity in normal and parental cultured fibroblasts were in similar proportion. These observations suggest that the parents are heterozygous for a variant allele with a slow-moving catalytically inactive product which is also less than normally able to form efficient heteromers with the normal gene product. Km values for NP from normal controls, the parents and heterozygotes for three other NP variants were quite similar while Vmax values ranged from less than half normal to normal.

●THE ABSENCE OF A MATERNAL EFFECT IN HUMAN CLEFT LIP AND PALATE. G. J. Bingle*1 and J.D. Niswander. N.I.H., Bethesda, Maryland. *1Pres. Address: Henry Ford Hospt., Detroit, MI.

Maternal and paternal age effects for cleft lip(CL) have been previously demonstrated in both man and mouse. Moreover, supportive evidence for the existence of developmental maternal effects which alter the frequency of CL or isolated cleft palate(CP) is quite convincing from genetic studies on mice; however, such evidence in humans is far less persuasive. Recently, the use of half-sibs has been proposed to test for the existance of maternal effects on congenital malformations. We hypothesized that if a major maternal effect exists in oral facial clefting and this effects is not sporadic, half-sibships ascertained through an affected cleft proband will have a higher frequency of clefting when the mother is the common parent. In an attempt to assemble a large enough population to test this hypothesis, over 8,000 pedigrees were screened and data were obtained from 14 CL and CP investigators. We gratefully acknowledge their generous cooperation, without which this study would have been impossible. After removal of known genetic or cytogenetic diagnoses from the cleft probands, a recurrence risk for CL based on 324 maternal half-sibs of .012 was obtained. This was nearly identical to a risk of .015 for paternal half-sibs. Frequencies of .004 for maternal half-sibs of isolated CP probands and .009 for their paternal counterparts were also found. These estimates when compared with concomitant sib risk data from the same populations would appear to be reasonable. They support previously reported data from twin studies and data from interracial crosses from Hawaii which fail to provide evidence of a major maternal effect in human cleft lip and cleft palate. Thus, there appears to be a discrepancy between human population data and animal models, in particular the mouse, that needs further exploration.

GENE DOSAGE EFFECT IN AN ESTABLISHED LYMPHOCYTE x LYMPHOCYTE HYBRID. A. D. Bloom, F. T. Nakamura, and K. Ohki. Depts. of Pediatrics and Human Genetics, College of Physicians and Surgeons, Columbia U., New York.

To study the regulation of gene expression in lines of human B cells, we have established and maintained in continuous culture a hybrid line derived from the fusion of the cells of two immunoglobulin-producing ($Ig^+$) human lymphoid lines (UM-1-6TG$^r$ and the citrullinemic UM-21-5). Both parental lines were pseudodiploid with marker chromosomes in each, and the hybrid (HLHL-1) has, after 18 months in continuous culture, 88.3% of its cells with chromosome numbers of 87 to 92, with random loss in the hypo-tetraploids and no detected diploids. While HLHL-1 was initially selected for in doubly selective HAT medium with citrulline replacing arginine, it has been maintained for the past 12 months in non-selective medium. The HL-A specificities of both parental lines are expressed in the hybrid, as determined by D. Pious, and the hybrid is $Ig^+$ by immuno-fluorescence and -diffusion. The hybrid has intermediate levels of HGPRT and argininosuccinic acid synthetase (AS), in which UM-1-6TG$^r$ and 21-5, respectively, are deficient. More recently, quantitative immunodiffusion has revealed a similar dosage effect for IgM: UM-1-6TG$^r$ secretes into the medium $3.2 \times 10^{-3}$ mg IgM per $10^6$ viable cells, while 21-5 may secrete up to $1.8 \times 10^{-3}$ mg IgM. HLHL-1, however, secretes 6.4 to 9.2 $\times 10^{-3}$ mg IgM, suggesting that the loci responsible for IgM synthesis in the hybrid are at least as active as in the parental lines. By fluorescence for cell-bound Ig, both the parental lines and the hybrid were positive for IgM and k chain production. It appears at present that in this human-human hybrid, the activity of genes responsible for synthesis of two enzymes (HGPRT and AS) and one Ig (IgM) is relatively stable, as is the karyotype.

●GENETIC CONTROL OF HEXOSAMINIDASE A AND B. H.J. Boedecker, C.M. Croce, C.J. Chern and W.J. Mellman. Wistar Institute of Anatomy and Biology, Dept. of Human Genetics, U. Pa. Sch. of Med., and U. of Pennsylvania Human Genetics Center, Philadelphia.

Cell hybrids and biochemical studies have thus far not completely explained the relation of human hex A and B. Chromosome 5 is certainly needed for the expression of hex B and 15 for hex A. The need for 5 to express hex A is contested.

A hybrid clone has been isolated (from the fusion of mouse peritoneal macrophages and an SV-40 transformed HPRT-deficient human fibroblast line, LN-SV) with 5 and 7 as the only recognizable human chromosomes. It expresses hex B. Heterokaryons between this clone and Sandhoff cells express both hex A and B; between Tay-Sachs cells only hex B.

A clone (from the fusion of an HPRT-deficient mouse line [IR] and human fibroblast line with an X/15 translocation selected in HAT medium) was found to have the translocation and chromosome 5. This clone treated with diphtheria toxin lost both the 5 and hex B activity. A "slow hex A" was found in the treated clones; the untreated clone had "slow" and "normal" hex A. We propose that the "slow hex A" is a heteropolymer of mouse hex and a human component (? $\alpha$ chain) whose determinant is on 15, and supports the theory that "normal hex A" requires a gene on 5 (? $\beta$ chain). Studies of these clones in progress with antisera to human A and B should further test this hypothesis.

●A MATRIX METHOD FOR CALCULATING RECURRENCE RISKS FOR GENETIC COUNSELING. D. R. Bolling, G.A. Chase, and E. A. Murphy. The Johns Hopkins University School of Medicine, Baltimore, Maryland.

The logic of calculating recurrence risks of Mendelian genetic traits by the Bayesian method is in many instances straightforward. But as the pedigree goes beyond two generations and the genotypes of phenotypically normal individuals are unknown, as in the X-linked recessive case, the logic becomes confusing even to the trained probabilist. Incorporation of mutation rates, incomplete penetrance, and other factors add to the confusion.

A method which simplifies the logic is presented. Four types of 2x2 matrices (A,T,G and W) are assigned to relevant members of the pedigree for X-linked recessive traits. Phenotypically normal males are given the T matrix and affected males the A matrix. Phenotypically normal females with offspring are assigned the G matrix. The matrices for a sibship are multiplied together. Information is transferred from one generation to another by premultiplying the sibship matrices by the G matrix and diagonalizing, which places the sums of the elements in each row on the diagonal. The elements of the matrices are conditional probabilities which may allow for mutation rates (forward and backward), incomplete penetrance and other factors. The W matrix is assigned to females with no offspring when mutations are considered. A complete expression for the conditional likelihood that a consultand is a carrier may be written down in matrix notation on inspection of the pedigree. It may then be systematically evaluated without further reference to the pedigree. The method is readily adapted to computers and modern electronic calculators for complete pedigree analysis. Illustrations of the use of the method in the X-linked case will be given.

●GROWTH KINETICS OF DIVIDING AND NONDIVIDING CYSTIC FIBROSIS SKIN FIBROBLASTS. W.E. Bolton, B.R. Haenelt, and S.C. Barranco. U. of Texas Medical Branch, Galveston.

The growth kinetics of dividing and nondividing human skin diploid fibroblasts derived from cystic fibrosis (CF) homozygotes, CF heterozygotes and normal individuals was investigated. All cell kinetic studies were performed on age, sex and passage matched cultures.

When replicate dishes (60 x 15mm) containing 3 x $10^5$ cells were plated in 5 mls of Ham's F-10 + 10% fetal calf serum, all cell lines entered lag phase for the first 18 - 24 hrs. Following lag phase the cell lines entered exponential growth within 10 hrs of each other. The exponential phase lasted 65 - 75 hrs. The cells reached plateau phase (nondividing) at a density of 8 - 10 x $10^5$ cells/dish. During the lag and plateau phases of growth, the biochemical activity of the cells was reduced when compared to cells in exponential growth.

The population doubling times increased with time in culture and no difference was observed between the three genotypes tested. The cell cycle times remained constant through the 10th subculture, while the growth fraction, or fraction of cells in the cell cycle, decreased with culture time; however, changes in the growth fraction and population doubling times appear to be related to cellular senescence in vitro rather than to cystic fibrosis. In addition the variability of the growth kinetics increased the longer the cells were in culture. These data suggest that knowledge of these cell kinetic parameters is essential for the accurate interpretation of results from cytological, biochemical and genetic studies.

FREQUENCY OF MONOGENIC FORMS OF HYPERLIPIDEMIA IN A 'NORMAL' POPULATION. H. Boman, W.R. Hazzard, J.J. Albers, M.N. Cooper, and A.G. Motulsky. U. of Washington, Seattle.

Blood samples were drawn from 1003 middle-aged males (range 30-59, mean 45.9 years) derived from a 'white collar' working population. Men whose lipid levels exceeded the 95th percentile for cholesterol or triglyceride were selected as index cases for family studies. Fasting lipid levels were determined on all available first and second degree relatives of these subjects. Using our criteria (JCI 52:1544, 1973) the majority (80%) of the hyperlipidemic subjects did not have a Mendelian disorder to explain hyperlipidemia. Only one kindred had familial hypercholesterolemia indicating a population frequency of 1/1000. Familial hypertriglyceridemia was inferred in 10% of the investigated families (1% of the total population). No single large pedigree of 'Combined Hyperlipidemia' was detected. However, about 10% of pedigrees (1% of the population) fitted the pattern of this disorder. Thus, approximately 2% of white, middle-class, middle-aged males may be heterozygous for one of the monogenic hyperlipidemias. These figures should be contrasted with the finding of 20% monogenic hyperlipidemias (4% Familial Hypercholesterolemia, 5% Familial Hypertriglyceridemia, 11% Combined Hyperlipidemia) found in a myocardial infarct population of similar age. These data suggest that the relative risk of myocardial infarction is highest for males with Familial Hypercholesterolemia (40 x), intermediate for Combined Hyperlipidemia (10 x), and lowest for Familial Hypertriglyceridemia (5 x). Risks for non-Mendelian hyperlipidemias appeared lower yet.

●UNEXPECTED HIGH FREQUENCY OF PATERNAL ORIGIN OF TRISOMY 21. C. E. Bott, G. S. Sekhon, and H. A. Lubs. Washington U. School of Medicine, St. Louis, Missouri, and U. of Colorado Medical Center, Denver.

Q and C band polymorphisms of chromosome 21 were studied in a group of 59 children with Down syndrome and their parents. This group included 26 families where the mother was 35 years of age or older. The purposes of the study were to determine 1) the overall frequency of Q and C polymorphisms of chromosome 21 among children with Down syndrome and their parents. 2) The frequency with which Q and C polymorphisms could be used to identify the source of extra chromosome. 3) The proportion of maternal and paternal non-disjunction. 4) The meiotic division (first or second) of non-disjunction giving rise to trisomy.

Several points can be made from this study: 1) overall frequency of Q polymorphisms is 42.9% in mothers, 28.6% in fathers, and 33.3% in Down syndrome patients. The frequency of Q polymorphisms in the normal population is approximately 7%. C band polymorphisms of chromosome 21 were found in only one of 59 families and were non-informative. 2) Paternal and maternal 21 chromosome could be distinguished on the basis of polymorphisms in 7/59 families (12% informative matings). 3) In this sample three instances of maternal non-disjunction (second meiotic division) and four instances of paternal non-disjunction (also second meiotic division) were found. These studies indicate a provocative association between Q band polymorphisms and trisomy 21. This also demonstrates that Q band but not C band polymorphisms are useful markers in determining the parental origin of extra chromosome in trisomy 21, and show that paternal origin of the extra 21 chromosome is not at all uncommon.

ZINC THERAPY SUPPRESSION OF ERYTHROCYTE MORPHOLOGICAL ABNORMALITIES IN SICKLE CELL ANEMIA (SCA). G.J. Brewer and L.F. Brewer, U. of Michigan, Ann Arbor.

We have used oral zinc therapy as a prophylactic drug against pain crisis in SCA in limited clinical trials. Zinc treatment has produced a marked reduction in overall crisis frequency although there is considerable patient variability in this improvement. Interpretation of subjective responses to a drug treatment in uncontrolled trials must be cautious because psychological effects may produce apparent results which are not related to antisickling effects. However, we have recently observed that oral zinc therapy reduces the number of morphologically abnormal erythrocytes (called irreversibly sickled cells, or ISC's) in SCA patients, which provides objective evidence that zinc is affecting the sickling process in vivo. ISC counts vary greatly among SCA patients (range 10-50%), but in contrast are relatively constant within a given patient (±5%). Coded slides are counted in a blinded manner. Counting error is small (S.D. = 2.4%). Three of 5 patients on our standard regimen of oral zinc sulfate (110 mg, 6 times/day) had a marked and statistically significant reduction in ISC counts after zinc treatment. In the other 2 patients ISC counts were not significantly reduced, again indicating heterogeneity in patient response to zinc. Zinc has a sound therapeutic rationale in SCA. Zinc improves sickle cell filterability in vitro and sickle cell survival in an animal model. It antagonizes some calcium effects in the cell membrane. Results presented here indicate objective evidence of an in vivo effect of zinc in SCA, and may be useful in predicting clinical benefit from zinc in specific patients. Finally, we point out that treatment in SCA may be aimed at the membrane lesion in sickling, since most evidence indicates that this is the point of zinc action.

SPECIFIC CHROMOSOMAL ABERRATION IN HUMAN NEUROBLASTOMA. G. M. Brodeur, G. S. Sekhon, and M. N. Goldstein. Washington U. School of Medicine, St. Louis, Missouri.

Analysis of chromosomes in human malignancies has been instructive in chronic myelogenous leukemia and in retinoblastoma, where specific abnormalities suggest a genetic mechanism for malignant transformation. We are examining human neuroblastoma for chromosomal alterations. Six human neuroblastomas have been analyzed to date by giemsa and fluorescence banding techniques. Two neuroblastomas were primary tumors from untreated children, and four were cell lines established from human neuroblastomas. Five of the six tumors studied were diploid or near diploid, and one was near tetraploid.

Several points can be made from the studies thus far. 1) A 1p- deletion was found in three of the neuroblastomas studied. It was found in both primaries, and in one primary it was the only abnormality detected. It was also found in one cell line, in addition to other abnormalities. 2) Giant marker chromosomes were present in three of the four cell lines at the time of initial study, and the fourth developed a giant marker in culture. However, three of these cell lines were from treated children and had been in culture for years. 3) Although double-minute chromosomes have been described in human neuroblastomas, none were found in the primaries or cell lines examined. Thus, a 1p- deletion was the most consistent abnormality found in three of the six human neuroblastomas studied. Additional studies of primary tumors should clarify whether this specific chromosomal abnormality is possibly related to the acquisition of malignant behavior in human neuroblastomas.

CHROMOSOME SEGREGATION PATTERNS IN MAN-RODENT AND MUNTJAC-RODENT SOMATIC CELL HYBRIDS. J.A. Brown and T.B. Shows. Dept. of Pediatrics, State U. of New York and Roswell Park Memorial Institute, Buffalo, N.Y.

Chromosome segregation patterns have been analyzed in 22 independent man-rodent hybrid clones isolated in HAT medium with counter selection in 8-azaguanine supplemented medium. Hybrids resulted from a fusion of RAG (HPRT$^-$) cells with human fibroblasts or leucocytes and data from 478 cells indicated the chromosome segregation patterns to be non-random. More human and mouse chromosomes were retained in fibroblast-RAG hybrids than in leucocyte-RAG hybrids. This observation could result from the formation of more 1S:2S hybrids in the fibroblast-RAG combinations. Fibroblast-RAG hybrids retained an average of 10.5 human chromosomes per cell compared to 5.5 chromosomes per cell in leucocyte-RAG hybrids, indicating that the human cell type may influence the retention or loss of human chromosomes. Exclusive of the X chromosome, nos. 6,7,10,11 and 12 were most frequently observed with chromosome 10 present in 73% of all clones. When the X chromosome was removed by counter selection, the numbers and types of autosomes retained was essentially unchanged, indicating no association between the X and autosomes in retention or loss of chromosomes in hybrids.

Muntjac chromosomes segregated in muntjac-RAG hybrids with extensive breakage of the muntjac complement. As in most mammals, these hybrids demonstrated a linkage of the genes coding for hypoxanthine phosphoribosyl transferase and glucose-6-phosphate dehydrogenase showing evolutionary stability of this chromosome segment. Cytologic evidence of chromosome breakage and thus instability of linkage groups in certain hybrids was confirmed by gel electrophoresis of 30 enzymes and found to be most prevalent in man-hamster and muntjac-RAG hybrids.

●FAMILIAL HYPERCHOLESTEROLEMIA (FH) AND CHOLESTERYL ESTER STORAGE DISEASE (CESD): TWO MUTATIONS AFFECTING SEQUENTIAL STEPS IN THE REGULATION OF CHOLESTEROL METABOLISM. M. S. Brown and J. L. Goldstein, U. of Texas Southwestern Medical School, Dallas.

Studies of cultured fibroblasts from patients with 2 different mutations in cholesterol(C) metabolism have delineated a specific process by which cells derive their membrane C from plasma low density lipoprotein(LDL). First,LDL binds to a cell surface receptor. The bound LDL is endocytized and transferred to lysosomes, where its protein is hydrolyzed to amino acids and its cholesteryl ester(CE) is hydrolyzed to free C. The C is transferred to cell membranes where it exerts 2 regulatory actions: 1)it suppresses C synthesis(↓HMG CoA reductase)and 2) it stimulates its own re-esterification(↑fatty acyl-CoA:cholesteryl acyltransferase). We now report measurements of the metabolism of LDL, radiolabeled both in its protein ($^{125}$I) and its CE ([$^{3}$H]cholesteryl linoleate). Mutant cells from FH homozygotes,which lack LDL receptors, failed to hydrolyze either the protein or CE of LDL,to suppress C synthesis,and to activate C esterification.Mutant cells from CESD patients bound and endocytized LDL normally and hydrolyzed its protein normally. However, because of their deficient lysosomal acid lipase, CESD cells showed reduced hydrolysis of CE of LDL. In normal cells incubated with LDL,sufficient free C was liberated from LDL CE within 6 hours to suppress C synthesis and to stimulate its re-esterification.In CESD cells,C synthesis and re-esterification were not affected until 24 hours,at which time the cells had accumulated unhydrolyzed LDL CE to levels 3-fold higher than normal cells. Thus, FH and CESD can be considered together as disorders of cellular C regulation involving mutations at 2 sequential steps in LDL metabolism -- FH involving the cell surface receptor that binds LDL and CESD involving the lysosomal acid lipase that renders the C of LDL available to the cell.

● Expression of Human Glyceraldehyde-3-Phosphate Dehydrogenase in Man-Rodent Somatic Cell Hybrids. G. Bruns and P.S. Gerald. The Children's Hospital Medical Center. Boston, Massachusetts.

The expression of human glyceraldehyde-3-phosphate dehydrogenase (G3PD) was analyzed in 23 primary man-hamster and 13 primary man-mouse hybrid clones derived from fusions of aneuploid rodent cells with WBC from 2 unrelated individuals carrying different X/19 translocation chromosomes. Twelve of 23 man-hamster and 8 of 13 man-mouse clones expressed human G3PD,manifested by the presence of 1 to 3 heteropolymeric isozymes in addition to the rodent enzyme. Concordant expression of human G3PD, TPI and LDH B was observed in all 23 man-hamster clones and concordant expression of G3PD and TPI was observed in all 13 man-mouse clones (LDH B was not analyzed in man-mouse clones). G3PD can presumptively be assigned, therefore, to the LDH B-TPI syntenic group and hence to chromosome 12. Pep-B, which has also been assigned to chromosome 12, was expressed concordantly with G3PD in 17 of 23 man-hamster clones and in 12 of 13 man-mouse clones. The expression of Pep-B was not concordant with G3PD (and with TPI or LDH B) in the remaining clones. The expression of G3PD was independent of 25 human enzymes which represent 17 syntenic groups in the man-hamster clones and of 22 human enzymes which represent 16 syntenic groups in the man-mouse clones. The possible juxtaposition of the loci for LDH B and G3PD is of interest in view of the similarity of the three dimensional structure of these enzymes. The association of 2 genes (TPI and G3PD) which specify sequential enzymes necessary for the metabolism of glucose is likewise of interest.

INCREASED INCIDENCE OF ABO ERYTHROBLASTOSIS IN BLACKS. K. A. Bucher, A. M. Patterson, C. A. Jones, and H. N. Kirkman. U. of North Carolina, Chapel Hill, N. C.

In a review of hospital charts for infants born at North Carolina Memorial Hospital during the period October 1965 to March 1973 we find that erythroblastosis due to ABO incompatibility is about 3 times as common in black infants as in white infants. Criteria used for diagnosis were: A or B infant of O mother with no Rh incompatibility and birth weight greater than 2500 grams, jaundice noted before 48 hours of age, maximum indirect serum bilirubin greater than 10 mg/100 ml, and a positive reaction to the direct Coombs test. With these criteria, incidence in blacks was 0.95% and in whites was 0.31%. This difference cannot be attributed to the differences in ABO gene frequencies between blacks and whites. As a cause of indirect serum bilirubin above 15 mg/100 ml, ABO erythroblastosis is as common in blacks as Rh erythroblastosis in whites.

●ABNORMAL CYCLIC AMP LEVELS IN ISOPROTERENOL-STIMULATED FIBROBLASTS FROM PATIENTS WITH CYSTIC FIBROSIS. M. Buchwald, Hospital for Sick Children, Toronto, Ontario, CANADA.

Cystic fibrosis (CF) is characterized by abnormalities in exocrine secretions. Since cAMP has been shown to be involved in the control of exocrine secretions we have examined the possibility that abnormalities of adenosine 3',5' cyclic monophosphate (cAMP) metabolism can be demonstrated in cells from CF patients. We have therefore used skin fibroblast strains derived from patients and from normal donors to study the interaction of these cells with isoproterenol, a β-adrenergic agonist known to increase intracellular cAMP levels. Five CF and four normal strains matched for age in culture were compared at various stages of the culture cycle. The cells were subcultured in parallel, grown with feeding, counted daily, and assayed for intracellular cAMP. At each assay point the cells were stimulated with $10^{-5}$M isoproterenol for 15' in the presence and absence of the phosphodiesterase inhibitor RO20 1724. cAMP was then measured using the Gilman protein binding assay. CF and normal cells grew at the same rate, unlike other published reports, and reached similar maximal cell and protein densities. The CF strains had higher intracellular cAMP levels than the normal strains at every assay point. At confluence the average intracellular cAMP level of the five CF strains was 660 (range 560-860) picomoles/mg protein and that of the four normal strains was 200 (140-260). The increased effect of isoproterenol on CF cells cannot be accounted for by different phosphodiesterase activities nor by different dose- or time- response curves and can be completely blocked by propranolol. Preliminary results suggest that this effect of isoproterenol is not due to an earlier senescence of CF strains. The increased sensitivity to catecholamines may reflect an intrinsic genetic property of CF cells. (Supported by MRC (Canada)).

A HISTOCOMPATIBILITY ANTIGEN ASSOCIATED IMMUNE RESPONSE GENE REGION IN MAN. C. E. Buckley, III M. L. Haysman, A. H. Johnson, D.B. Amos, Depts. Med. and Microb.-Immunol., Duke University Medical Center, Durham, N. C.

We HL-A typed and studied immune function in an informative 21 member family whose proband (39 WF) has bronchiectasis and hypogammaglobulinemia. A 35 year old sister also has hypogammaglobulinemia and chronic bronchitis. The proband's father and 17 year old daughter have low serum IgA. The proband's 15 year old son is an HL-A recombinant with low IgG and a Coombs positive hemolytic anemic. Four additional family members have symptomatic atopy. Measurements of scratch test responses to 68 pollen allergens and serum complement fixing antibody titers to 7 viruses were evaluated for HL-A associated responses in 16 family members. A least squares solution of simultaneous equations relating HL-A haplotypes and immune response was evaluated with an F test in order to assess HL-A associated antigen responses. HL-A associated responses ($p < 0.05$) were detected to 52/68 pollen allergens and 3/7 viruses (cytomegalovirus, influenza virus and parainfluenza virus). F ratios based on HL-A associated responsiveness to Kentucky Blue Grass, Red Oak, Mimosa and 32 other pollens were 2-fold greater when the HL-A recombinant chromosome was categorized with respect to the 1st locus. In contrast, F ratios derived from estimates of HL-A association of responses to 17 other allergens and the 3 viruses remained unchanged. This suggests the method used to detect HL-A haplotype associated responses was sensitive to changes associated with recombination between the 1st and 2nd HL-A loci. This family locates a possible immune response gene region proximate to genes controlling the 1st and 2nd locus HL-A antigens.

●INFORMATION ON AN UNTRANSLATED PORTION OF THE β CHAIN GENE PROVIDED BY A FRAMESHIFT HEMOGLOBIN MUTANT AND SEQUENCE STUDIES ON NORMAL β-mRNA. H.F.Bunn and B.G.Forget, (intr. by P.S.Gerald); Departments of Medicine and Pediatrics, Harvard Medical School, Boston, Ma. 02115

An asymptomatic woman and her son have a compensated hemolytic state due to an unstable hemoglobin variant, comprising 35% of the total. Hemoglobin Cranston was found to have an elongated β chain with a unique sequence of residues beginning at position 145:

β Cr (144→157):-Lys-Ser-Ile-Thr-Lys-Leu-Ala-Phe-Leu-Leu-Ser-Asn-Phe-Tyr-COOH

β A (144→146):-Lys-Tyr-His-COOH

It is likely that hemoglobin Cranston arose because of a frameshift mutation due to reduplication of the last two nucleotides (AG) of the codon for Lys 144. Such a frameshift could result from non-homologous crossing over of chromosomes or from other abnormalities of DNA replication or repair.

| | Lys | Tyr | His | Term | | |
|---|---|---|---|---|---|---|
| βA | AAG | UAU | CAC | UAA | GCN ... | |
| | | | ↓ | | | |
| βCr | AAG | AGU | AUC | ACU | AAG | CUN ... |
| | Lys | Ser | Ile | Thr | Lys | Leu |
| | 144 | 145 | 146 | 147 | 148 | 149 |

A $^{32}P$ labeled copy of normal β chain mRNA was fingerprinted as described by Forget et al (Ann N.Y.Acad. Sci. 241:290, 1974). Two oligonucleotide sequences were identified which could not be matched to normal β chain amino acid sequences, but which match perfectly the amino acid sequences of the final 9 residues of hemoglobin Cranston. One of these oligonucleotides contains a termination codon UAA immediately following the codon corresponding to the C-terminal tyrosine.

Hemoglobin Cranston provides new information on the structure of the β chain gene as well as an explanation of both the structure and genetic basis of hemoglobin Tak, the only other elongated β chain variant that has been described.

●FAMILIAL X-AUTOSOME TRANSLOCATION(X;21). H. M. Cann, S. Sakaguchi, J. Stone, E. Gold and L. Luzzatti. Stanford U., Stanford, California.

A newborn male with multiple congenital anomalies and 46 chromosomes was found to have 1 G group chromosome missing and an additional metacentric chromosome resembling E16. The karyotype of the proband's phenotypically normal, 27-year-old mother revealed 46 chromosomes with 15 C group and 3 G group chromosomes, the same metacentric chromosome as observed in the son, and, in addition, an acrocentric chromosome intermediate in size between the D and G groups. The G banding technique revealed only 1 normal X and 1 G21 chromosome. The metacentric chromosome represented an X deficient for the segment q22 or q23→qter. The banding pattern of the additional acrocentric chromosome was either 21pter→q21, proximally, and Xq22→Xqter, distally, or 21pter→q22, proximally, and Xq23→Xqter, distally. On the basis of her normal phenotype and her karyotype, we interpret these findings as a balanced X-autosome translocation involving exchange of segments of the long arms of the X chromosome and of chromosome G21: 46,X,rcp(X;21)(q22;q22). Her son, having inherited only one element of the balanced translocation, is 46,XY,-21,+derX,t(X:21)(q22;q22)mat. The proband is therefore monosomic for all but the most distal part of G21 and partially disomic for the X chromosome. Results of studies of the timing of X chromosome replication in mother and son using BUdR incorporation followed by Hoechst 33258 or acridine orange staining and tritiated thymidine labeling will be presented. Future studies include introduction of this translocation into an interspecific somatic cell hybrid to confirm the order of gene loci assigned to the long arm of the X chromosome and the assignment to G21 and localization of the locus for superoxide dismutase-1. Such somatic cell hybrids will also be studied for reactivation of an inactive X chromosome.

●PRIMARY NORMOFUNCTIONAL TESTICULAR HYPERPLASIA AND SEVERE PSYCHOMOTOR RETARDATION: A "NEW" INHERITED DISORDER. J.M.Cantú, M.Medina, H.Scaglia, T.Morato, M.E.Moreno, M.González and G.Pérez-Palacios, Instituto Mexicano del Seguro Social, Instituto Nacional de la Nutrición and Hospital Psiquiátrico Infantil "Dr.Juan N.Navarro", México D.F., México.

Four 46,XY male sibs (aged 22,15,12 and 9) were found to have severe psychomotor retardation (DQ range:28-44), congenital megalorchidia and macrogenitosomia. The testes were at least three times the size of age-matched normal males. Plasma levels of radioimmunoreactive LH,FSH,testosterone(T) and estradiol were normal. Adequate suppression of LH by exogenous T, significant increase of LH and FSH by LH-RH administration and normal T rise after HCG stimulation were observed in the three older patients. Adrenal and thyroid function tests were normal. Spermograms (postpubertal patients) were normal. Testicular histological studies revealed normal morphology and cell architecture in the prepubertal sibs, and a diminished number of Leydig cells with normal number and size of seminiferous tubules, though some of them with hypospermatogenesis, in the postpubertal sibs. The unrelated parents, two brothers and a sister were normal. The global evaluation leads to the conclusion that this distinct congenital disorder is probably caused by an X-linked recessive mutation. On the basis of this observation and of other instances of glandular hyperplasia, a new classification of hyperplasia is proposed.

FAMILIAL NAEVUS ANAEMICUS. H. Cardoso, R. Vignale and H. Abreu de Sastre. School of Medicine, U. de la República, Montevideo, Uruguay.

The naevus anemicus is a rare skin disease characterized by a patch of pale skin of normal texture usually seen on the trunk. The skin returns to normal aspect after sympathetic blocking. Although the incidence of the disease is higher in patients with neurofibrometosis, no familial cases have been described. We have studied a four generation family of 48 individuals in which 8 of them (5 females and 3 males) were affected. 40 members of the family were clinically checked. The affected individuals were also pharmacologically tested by blocking the sympathetic innervation of the zone. Though 2 males were dead at the time of the clinical examination, the male to male transmission of the character was probably confirmed by the familial reports. 5 members are carriers of the disease according to the genealogy. The value of penetrance estimated is rather low (0.35), therefore the hypothesis of a multifactorial type of inheritance could not be ruled out. But, considering that the frequency of the trait does not fall off sharply in first degree relatives and that the ratio population incidence to that in relatives is low, we suggest an autosomic dominant type of transmission with an incomplete penetrance in the family.

A STUDY OF SCHIZOPHRENIA IN HAWAII. Carter, C., McLaughlin, D. and Rashad, M.N.
U. of Hawaii and Dept. of Health, Honolulu.

A study of 1008 Caucasian, Filipino, Hawaiian, Japanese, and Chinese schizophrenics, admitted to the Hawaii Public Mental Health Services, was conducted for 1973-74. Prevalence rates per 100,000 were computed for each racial group. An analysis of the effect of age, sex and race was undertaken using the chi square method.

Schizophrenia was found to be most common in Chinese females and Caucasians and Filipinos of both sexes. Rates were lowest in Chinese males, and intermediate in Hawaiian females and Japanese of both sexes. Disease onset occured most often between 20-35 years of age for all races and was more frequent in males than females except in the Chinese. An analysis of length of hospital stay indicated that Japanese tend to remain for longer periods under treatment.

●INHERITED INS(5;6)(q33;q15q27) AND PARTIAL 6q SYNDROME

H. Chen, M. Tyrkus, P.V. Woolley, Jr., F. Cohen, and K. Mayeda.
Children's Hospital of Michigan and Wayne State University School of Medicine, Detroit, Michigan

Trisomies involving different C autosomes have been increasingly reported in the literature in the recent years and most of them well delineated. Trisomy of the chromosome No. 6 is, however, yet to be reported. We wish to describe a 16 month old Negro boy with partial trisomy 6q syndrome due to segregation of ins(5;6)(q33;q15q27) in three generations. The abnormal clinical features of this proband include: physical and mental retardation, extremely poor sucking, microcephaly, hypertelorism, flat nasal bridge, anteverted nares, long philtrum, hypoplastic around the mouth, constantly opened mouth, high arched palate, receded chin, low set and malformed ears, and short and stubby fingers. Dermatoglyphic findings include simian creases, single creases on both fifth fingers with clinodactyly,and three creases on both fourth fingers. The first cousin of his mother had growth and mental retardation and peculiar facies similar to the proband. Phenotypically normal mother and maternal grandmother of the proband are found to have ins(5;6)(q33;q15q27)by Q and G bandings. The patient has partial trisomy 6q for the segment q15q27. Studies on the segregations of red cell markers, gene loci on chromosome No. 6 and karyotypes of the members of this interesting family pedigree are currently in progress and will be presented.The characteristic chromosome aberrations and clinical manifestations of this patient may help in defining the partial 6q syndrome in the future.

●ENOLASE: HUMAN TISSUE DISTRIBUTION AND EVIDENCE FOR MULTIPLE GENE LOCI. S.-H. Chen, E.R. Giblett, and C.R. Scott. U. Washington and King County Central Blood Bank, Seattle, Washington.

Four different isozyme patterns of enolase were found in human tissue extracts by gel electrophoresis. Red cells had a three-banded pattern with a strong cathodal band (I) and 2 weak anodal bands (II and III). Brain had a similar three-banded pattern except that the anodal bands were much more intense. A third pattern consisting of only the cathodal band (I) was found in liver and in most other tissues. The fourth pattern, found only in muscle, also had a single band, but it moved somewhat faster than the cathodal band (I). The individual enolase isozymes were purified from human brain extract. In dissociation and reassociation experiments isozymes I and III can be generated from isozyme II, and mixtures of I and III produced isozyme II. The red cell electrophoretic pattern of enolase from a heterozygous individual consisted of six isozyme bands: 3 at region I; 2 at region II; and 1 at region III.

Based on the above findings, we propose that enolase in most human tissues is governed by two independent gene loci: $\underline{ENO_1}$ and $\underline{ENO_2}$, and that their polypeptide products form random dimers. A third locus may govern the structure of enolase in muscle.

●ASSIGNMENT OF THE STRUCTURAL GENE FOR CYTOPLASMIC GLUTAMIC-OXALOACETIC-TRANSAMINASE (GOT) TO REGION q24-qter OF HUMAN CHROMOSOME 10. C.J. Chern, W.J. Mellman and C.M. Croce. The Wistar Institute and Department of Human Genetics, U. of Pennsylvania, Philadelphia.

Human fibroblasts derived from a skin biopsy of a normal male with a balanced translocation [46, XY, t(10; 17) (10pter → 10q24::17p13 → 17pter; 17qter → 17p13::10q24 → 10qter)] were fused with a thymidine kinase (TK)-deficient cell line (IT-22) resistant to 30 μg/ml bromodeoxyuridine (BrdU), which was derived from a Swiss mouse. The fused cells were maintained in hypoxanthine-aminopterin-thymidine (HAT) medium which prohibits the proliferation of TK-deficient cells. Electrophoretic study and karyotype analysis of 18 hybrid clones revealed positive correlation between the expression of cytoplasmic GOT (EC 2.6.1.1) and the presence of the human chromosome 17 carrying the distal third of the long arm of chromosome 10. Six GOT-positive clones, which contained the human chromosome 17 plus 10q24-qter were back-selected in medium containing BrdU (90 μg/ml). They were found to lose concordantly GOT expression and the human chromosome 17 with the translocated distal third of chromosome 10. Since cytoplasmic GOT has been assigned to human chromosome 10, we conclude that the structural locus for cytoplasmic GOT is located in the distal third of the long arm (q24-qter) of human chromosome 10.

Studies of electrophoretic phenotypes of hexokinase in hybrids derived from the fusion between the human fibroblasts with translocation [t(10; 17)] and Chinese hamster are in progress.

●LOCATION OF MUTANT CARRIER IN EPITHELIAL CELLS OF PROXIMAL TUBULE IN HEREDITARY TAURINURIA. R.W. Chesney, C.R. Scriver, and F. Mohyuddin, McGill University, Montreal.

The mechanism of hypertaurinuria in homozygous mutant $tau^{t-}$ mice (C57BL strains) was examined and compared with homozygous normal ($tau^{t+}$) mice (A/J). Plasma taurine is similar in $tau^{t-}$ and $tau^{t+}$ strains. Urine taurine is 10 fold increased and net tubular reabsorption is decreased in $tau^{t-}$ ( 84% of filtered taurine vs >96% in $tau^{t+}$ mice). Intracellular taurine in renal epithelium is normal in $tau^{t-}$. Taurine is an inert solute during concentrative uptake by mouse kidney. Uptake by kidney cortex slices, which expose only basilar membranes to the medium is greater in $tau^{t-}$ than in $tau^{t+}$ tissue. Efflux from preloaded slices is identical in $tau^{t-}$ to $tau^{t+}$ slices. β-alanine is also transported on the taurine system; its loss into urine is greater in $tau^{t-}$ mice. β-alanine uptake by $tau^{t-}$ slices is greater than normal. β-alanine is vigorously oxidized in $tau^{t+}$ slices; its metabolism is greatly attenuated in $tau^{t-}$ slices but not in homogenates where tubule architecture is disrupted. Impaired permeation on the taurine carrier location in the proximal tubule luminal membrane causes taurine loss in vivo and retention in lumen lacunae of slices in vitro. We believe this to be the first topological assignment of a mutant amino acid carrier in the intact mammalian nephron.

PLASMA FREE AND ESTERIFIED CHOLESTEROL IN TWINS. Joe C. Christian, K. W. Kang, F. P. Harmath and D. J. Huntzinger. Indiana University School of Medicine, Indianapolis.

Variation of human plasma cholesterol has been studied in detail because of its association with coronary heart disease. Early twin studies revealed significant estimates of genetic variance for plasma cholesterol, but assumed the total variances of monozygotic (MZ) and dizygotic (DZ) twins were equal. More recently the National Heart and Lung Institute (NHLI) sponsored a study of 514 adult male caucasian twin sets from the NAS-NRC twin panel, and found that plasma cholesterol total variance was greater for DZ than MZ twins. When this difference in total variance was corrected for there was no significant genetic variance. Cholesterol was partitioned by thin-layer chromatography into the free and esterified fractions in the NHLI twins studied in Indianapolis. Analysis of variance for 68 MZ and 73 DZ twin sets revealed the following mean squares $(mg/100\ ml)^2$:

| | FREE CHOLESTEROL | | ESTERIFIED CHOLESTEROL | |
|---|---|---|---|---|
| | MZ | DZ | MZ | DZ |
| AMONG TWIN SETS | 230 | 196 | 1369 | 2230 |
| WITHIN TWIN SETS | 35 | 88 | 259 | 364 |

Plasma free cholesterol had no evidence for unequal total variance of MZ and DZ twins and greater total variance for DZ twins than MZ twins ($P < 0.05$). When this difference in total variance was adjusted for, there was no evidence for genetic variance of esterified cholesterol. This study suggests that free cholesterol levels are under genetic control but gave no evidence for a genetic component of variance for esterified cholesterol.

●ULTRAVIOLET LIGHT SENSITIVITY AND DEFECT IN DNA REPAIR IN FIBROBLASTS DERIVED FROM TWO PATIENTS WITH COCKAYNE SYNDROME. E. H. Y. Chu, R. D. Schmickel, M. H. Wade, C. C. Chang, and J. E. Trosko. U. of Michigan, Ann Arbor, and Michigan State U., East Lansing.

Cockayne syndrome is a well recognized inherited disorder consisting of cachectic dwarfism, prematurely aged appearance, mental retardation, microcephaly, retinitis pigmentosa, deafness, skin hypersensitivity to sunlight and early death. Fibroblasts in culture derived from two Cockayne patients (♂, age 2 1/2; ♀, age 4) exhibited increased sensitivity to ultraviolet light (UV) but not to X-rays, as measured by colony forming ability. Repair of UV-induced DNA damage in these cells are subnormal in terms of unscheduled DNA synthesis (autoradiography and measurement of radioactivity in the absence of DNA replication). Results from alkaline sucrose density gradient analysis indicate that in Cockayne cells single strand breaks of DNA occurred after UV irradiation but there was a marked reduction, as compared to normal fibroblasts, in the incorporation of $^3$H-thymidine into the small molecular weight single stranded DNA. Excision of UV-induced pyrimidine dimers in Cockayne cells was at the normal level. Complementation analysis of DNA repair in heterokaryons formed by cell fusion showed that the molecular defect in these two patients is probably identical but differs from those in various complementation groups of xeroderma pigmentosum.

FAMILIAL AGGREGATION, THE Pi SYSTEM, AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE B.H. Cohen, H.A. Menkes, D.A. Levy, P. Kreiss, G. Chase, E.L. Diamond and S.A. Brashears Johns Hopkins U., School of Hygiene and Public Health, Baltimore, Maryland

Despite the known causal relationship between certain environmental agents (e.g. cigarette smoking) and chronic obstructive pulmonary disease (COPD), differential response to their effects suggests the importance of host factors. The literature on familial COPD that has accumulated, especially since recognition of the association between genetically determined serum alpha-1-antitrypsin ($\alpha$-1-at) deficiency and COPD, reveals much still unexplained. Although $\alpha$-1-at variation is doubtless important, it is not a sine qua non for all COPD or even familial COPD. To obtain further insight into the etiology of COPD, it is necessary to examine both genetic and environmental factors per se and in combination, using a multifaceted approach. Such an investigation has been undertaken, and the preliminary observations are summarized. COPD patients have a higher frequency of Pi variant phenotypes than those without lung disease. Among other subjects, both smoking and Pi variants appear to be associated with increased pulmonary function abnormality. However, the familial aggregation of pulmonary impairment appears to involve a component that is not a direct function of Pi type or smoking, though an interaction of that component with smoking and Pi type is suggested.

EVALUATION OF GENETIC COUNSELING AS DELIVERED TO TWO DISPARATE POPULATIONS: RURAL AND SMALL CITY - UNIVERSITY. F. L. Cohen and W. L. Daniel. U. of Illinois, Peoria, and U. of Illinois, Urbana.

Recent reports to evaluate the effectiveness of genetic counseling clinics have come from primary hospital centers usually located in large metropolitan areas. Our genetic counseling service located within a university setting in a small city has patients coming from rural farm communities in Central Illinois as well as university students. More than 30% are from towns with populations below 5,000. The period of evaluation of our service covers the years 1969-1974. During these 5 years, 175 people received complete counseling services. Sources of referrals, primary reason for seeking counseling, understanding of the genetic disorder, understanding of the recurrence risk, family problems encountered, reaction to the counseling method and approach as well as over-all reaction is analyzed according to the type of genetic disorder presented.

●CHROMOSOME STUDIES IN FAMILIES WITH ATAXIA TELANGIECTASIA*. M.M. Cohen, J. Dagan, and M. Shaham. Department of Human Genetics, Hadassah-Hebrew U. Medical Center, Jerusalem, Israel.

Chromosomal studies were performed on peripheral blood lymphocytes and cultured skin fibroblasts from 5 Israeli-Morrocan families with ataxia telangiectasia. A total of 24 individuals, including 7 propositii was investigated. Among the probands, significantly elevated rates of chromosome damage were observed in both blood and skin. Skin fibroblasts of affected individuals showed several orders of magnitude more chromosome breakage than lymphocytes. Increased rates of chromosome damage were also observed in the fibroblasts of some phenotypically normal family members (obligate heterozygotes and sibs) when compared to normal controls.

An apparent abnormal clone of cells, possessing a large acrocentric marker chromosome ($14q^+$), was observed in varying proportions among cells of all the propositii (2-5% lymphocytes; 1-9% fibroblasts).

* Supported by grants from the Israeli Cancer Society and the Joint Research Fund of the Hadassah-Hebrew University Medical Center.

GENETIC ANALYSIS OF PEDIGREE DATA FROM A NATIONWIDE SURVEY OF DEAF CHILDREN. P.M. Conneally, S.P. Rose, P.B. Fox and W.E. Nance. Indiana U. School of Medicine, Indianapolis.

Information on hearing status was collected on 12,655 informative sibships as part of the Annual Survey of Hearing Impaired Children and Youth conducted by the Office of Demographic Studies at Gallaudet College, Washington, D.C. A total of 433 reporting sources, located throughout the U.S. were involved. In general, the probands were school age children ranging in age from 5 to 18 years and yielded an overall ascertainment probability ($\pi$) of 0.325. The data were studied for segregation, penetrance, frequency of sporadic, dominant and recessive deafness, and genetic risks. The sibships were partitioned into three classes depending on whether none (HxH), one (DxH) or both (DxD) parents were deaf. In 86 consanguineous HxH matings with a total of 298 offspring the segregation pattern was consistent with monomeric recessive inheritance with complete penetrance. In 11,900 non consanguineous HxH matings with 47,089 offspring, those with a negative family history included a high proportion (61%) of sporadic cases while among affecteds with a positive family history only 20% were sporadic. In both cases the segregation frequency was not significantly different from 0.25. The 254 DxH matings agreed with the hypothesis of dominant inheritance with a penetrance of 62% in those with a positive family history and 42% in those with a negative family history. In 421 DxD matings with 1356 offspring the segregation frequency was 0.27 (penetrance=0.54). An estimated 36% of these matings could not produce normal offspring and presumably reflected matings between parents who were homozygous for the same recessive genes. Overall estimates of sporadic, dominant and recessive deafness obtained were 49.3%, 7.6% and 43.1% respectively.

●VARIABILITY OF TOTAL CHOLESTEROL IN MONOCHORIONIC AND DICHORIONIC MZ TWINS. L.A. Corey, R.E. Harris, K.W. Kang, J.C. Christian, and W.E. Nance. Indiana U. School of Med., Indianapolis and U. of Wisconsin, Madison.

Total cholesterol (mg/ml) was measured in samples of cord blood from the individual members of 28 monochorionic and 23 dichorionic monozygotic (MZ) twin pairs. In each pair, the placental relationships were established by gross and microscopic examination of the fetal membranes and the zygosity was confirmed by typing for nine polymorphic blood group systems. The male:female ratio in the monochorionic group was 1:3, while the sex ratio in the dichorionic group showed no significant deviation from 1:1. Overall, the average total cholesterol level was significantly higher in females than in males (84 vs 70 mg/ml). Within chorion groups, variability was similar for males and females and corresponding mean squares from analyses of sums and differences in the two sexes were pooled. Estimates of the variability among twin pairs in each chorion group were homogeneous; however, the variation within dichorionic pairs was more than three times that of monochorionic pairs as evidenced by within mean squares of 911.8 and 278.9, respectively. Further, the average within-pair difference in the dichorionic group exceeded that of the monochorionic group (23 vs 16 mg/ml). This would seem to indicate that prenatal factors can influence cholesterol levels in the cord blood and that the intrauterine environment of monochorionic and dichorionic MZ twins are not entirely comparable. Apparently, the effects of prenatal environment on total cholesterol are more highly correlated in monochorionic than in dichorionic twin development. These observations emphasize the hazards of pooling data from monozygotic twin pairs of uncertain placenta type.

QUANTITATION OF CONTACT-FEEDING BETWEEN SOMATIC CELLS IN CULTURE. C.M. Corsaro and B.R. Migeon, Johns Hopkins U., Baltimore, Md.

Contact-mediated communication between cells has a role in regulation of embryogenesis, immune response, normal growth, and malignancy. Contact-feeding (cf) between $HGPRT^+$ and $HGPRT^-$ cells (metabolic cooperation) is a form of cell communication which can be studied in cell culture. We have developed an assay which reflects transfer of 6-thioguanylic acid from wild-type to mutant cells and which makes it possible to quantitate the degree of cf between a variety of cell types. Seeking genetic variation in human cells, we have screened fibroblasts from 9 Lesch-Nyhan males, 26 $HGPRT^+$ adults, and 15 different $HGPRT^+$ fetal tissues. These cells were remarkably similar in their degree of cf, indicating that this is a stable membrane trait. Using the assay as a potential indicator of mutations in membrane function, we noted no differences when fibroblasts from children with Menkes syndrome or cystic fibrosis were assayed. Studies of cf between $HGPRT^+$ and $HGPRT^-$ ouabain-resistant human cells showed that cf is not dependent on the ouabain-sensitive $Na^+K^+$ transport system. We also assayed combinations of $HGPRT^+$ and $HGPRT^-$ cells which differ in their cf ability (CHO, L, 3T3, and $SV_{40}$-transformed human). Results show that the extent of cf is determined by the cell less able to communicate and support Gilula's theory that gap junctions are the ultrastructural basis for cf.

●STUDIES OF THE MECHANISM OF PYRIDOXINE RESPONSIVENESS IN HOMOCYSTINURIA. D. R. Cox. U. of Washington, Seattle.

The mechanism of pyridoxine responsiveness in homocystinuria due to cystathionine synthase deficiency was studied in cultured skin fibroblasts from both pyridoxine responsive and non-responsive patients. All of the homocystinuric fibroblasts studied had 3-5% of wildtype cystathionine synthase activity when crude cell extracts were assayed. When the level of cystathionine synthase activity was assayed in intact cells by measuring the steady state accumulation of intracellular cystathionine synthesized from radioactive serine, there was no detectable activity in any of the homocystinuric cell lines, even though the assay could detect less than 1% of wildtype activity. There was a good correlation between cystathionine synthase activity measured in extracts and in intact cells when wildtype fibroblasts, or cells from an obligate heterozygote for homocystinuria were studied. Growth of homocystinuric cells in a 10-fold excess of pyridoxal HCl did not increase the cystathionine synthase activity as measured in extracts or in intact cells. These results show that residual cystathionine synthase activity plays no role in sulfur amino acid metabolism in the homocystinuric fibroblasts which were studied, and suggest that in these patients, the mechanism of pyridoxine responsiveness does not involve residual cystathionine synthase activity. It is possible however, that the amount of residual cystathionine synthase activity measured in intact homocystinuric fibroblasts does not accurately reflect the metabolic importance of residual enzyme activity in other homocystinuric tissues.

●DEFICIENCY ALLELE OF $\alpha_1$-ANTITRYPSIN: Pi $M_{Malton}$. D. W. Cox, Hospital for Sick Children and U. of Toronto, Toronto, Canada.

More than 20 alleles at the alpha$_1$-antitrypsin (AAT) Pi locus have been described. Pi $M_{Malton}$ is an allele resulting, like the Z allele, in a deficiency of AAT (about 15 to 20% of normal concentration). AAT of type $M_{Malton}$ is unlike Z in several respects. The mobility of $M_{Malton}$ AAT in acid starch gel followed by crossed immunoelectrophoresis appears to be identical to that of M AAT and not more cathodal as is Z AAT. Indirect evidence, using crossed immunoelectrophoresis, of neuraminidase-treated AAT, has indicated that M AAT may have 6.5 residues of sialic acid compared to 4.5 for Z AAT (Cox, Amer. J. Human Genet. 27, 165, 1975). $M_{Malton}$ AAT appears to have as many sialic acid residues as M AAT. $M_{Malton}$ AAT does not proceed to complete desialylation as readily as Z AAT.

In the kindred studied, two AAT deficient adult sibs are homozygous for Pi $M_{Malton}$. The 5-year old daughter of one and an adult cousin are of Pi type $M_{Malton}$ Z. All four have normal tests of liver function. The three tested have normal tests of lung function. All are less than 30 years of age.

Pi $M_{Malton}$ has been compared with M and Z by starch gel electrophoresis and crossed immunoelectrophoresis, agarose electrophoresis with immunofixation, and isoelectric focusing in acrylamide. It is probably a structurally different protein and not normal M present in an abnormally low amount.

There has been no indication for liver biopsy so we do not know if secretion of the $M_{Malton}$ AAT is impaired as it is for Z AAT.

●MULTIPLE ENZYME DEFICIENCY IN FAMILIAL HYPERLYSINEMIA. R. P. Cox, J. Hutzler, N. C. Woody*, and J. Dancis. New York U. Med. Center, New York, N.Y. and *Tulane Sch.of Med.New Orleans, La.

The major pathway for lysine degradation in humans is by condensation with ketoglutarate to form saccharopine which is then hydrolysed to α-aminoadipic-δ-semialdehyde and glutamic acid. Two inborn errors of metabolism involving these steps have been characterized, familial hyperlysinemia and saccharopinuria. The latter is due to a deficiency of the second enzyme in the pathway saccharopine dehydrogenase. A deficiency of the lysine ketoglutarate reductase, the first enzyme in the pathway, was previously shown in fibroblasts from patients with familial hyperlysinemia.

The sudden death of a child with familial hyperlysinemia allowed measurement of enzymes of lysine degradation in liver. As anticipated, lysine ketoglutarate reductase was absent in hyperlysinemic liver, but unexpectedly saccharopine dehydrogenase was also absent. Prompted by these findings the pathway for lysine degradation was examined in fibroblasts from two affected sibs and an unrelated patient with familial hyperlysinemia. Both lysine-ketoglutarate reductase and saccharopine dehydrogenase were reduced to less than 10% of normal activity. Attempts to induce saccharopine dehydrogenase in mutant cells by feeding fibroblast cultures saccharopine or to repress the enzyme in normal cells by limiting the lysine content of medium were unsuccessful. The deficiency of two enzymes sequentially involved in a metabolic pathway by a mutation is distinctly unusual, although it has been observed in orotic aciduria.

●AGE-DEPENDENT PENETRANCE IN MANIC-DEPRESSIVE ILLNESS AND ITS GENETIC IMPLICATIONS. R. R. Crowe and P. E. Smouse. U. Iowa College of Medicine, Iowa City and U. Michigan Medical School, Ann Arbor.

The age-dependent penetrance was described by a one-hit survival curve for manic depressive illness, using age of onset data from sixty-one affected probands. The model was corrected for normal demographic attrition. The utility of the penetrance function for pedigree analysis was illustrated, using the families of the sixty-one probands. A sex-linked dominant model of inheritance was about eighty-eight times more likely than an autosomal dominant model, and both were more likely than autosomal recessive, sex-linked recessive, or polygenic models. The sex-linked dominant and autosomal dominant models were also contrasted, by means of the age-specific morbidity risks for sibs and children of probands. Both models provided a fairly close fit of expectation to observation, but the sex linked model was preferable. Other possible uses for the penetrance function are indicated.

MONILETHRIX: VARIABLE EXPRESSION OF A DOMINANT GENE IN HAIR. R. K. Curtis. Redken Laboratories, inc., Van Nuys, California.

Exemplified by a characteristic node–internode patern along the hair shaft, together with a flattening-degradation of the nails and keratosis pilaris, monilethrix is transmitted as an autosomal dominant trait. As such, it appears to exhibit a wide variation in its phenotypic expression among carrier individuals spanning the spectrum from non presence, to pronounced—visible with the unaided eye.

Through the use of polarization microscopy, some 60,000 hair samples scanned over a period of 18 months during a routine cosmetic hair analysis, have produced 11 definite proband individuals of European descent. By taking into consideration biases in both directions, the estimated phenotype frequency of $\sim$1/5500 is an order of magnitude greater than previously reported.

Presented are the results of an investigation into eight independent families, exemplifying monilethrix in as many as five generations, with emphasis on the variation of the trait, and difficulties encountered during this kind of research.

GENE EXPRESSION IN MEIOTIC AND POST-MEIOTIC GERM CELLS: DNA POLYMERASE AND THYMIDINE KINASE ACTIVITIES. D. L. Daentl, R. P. Erickson and C. Betlach, U. of California, San Francisco.

Studies of DNA polymerase activity during mouse spermatogenesis have resulted in the following observations: 1)pre-meiotic and meiotic stages (primary and secondary spermatocytes) have the highest levels of activity; 2) post- meiotic stages are associated with a decline in activity to 1/14 of the maximal level by the time that testicular maturation has been completed (30 days); 3) no further reduction of activity is observed in mature sperm from the vas deferens. The magnitude of sperm activity is unexpectedly substantial (25.3 $\pm$ 6.7 pmoles $H^3$dTTP/mg protein/hr.) since others have found that repair DNA synthesis does not occur at this stage. Both activated natural and synthetic templates can be utilized and, in addition, high levels of thymidine kinase are also present. (370 pmoles $^3$H-dT/mg protein/hr.)

These findings raise the possibility that the male gamete can contribute enzymes for replication of the embryonic genome.

A CYTOGENETIC EVALUATION OF JERUSALEM NEWBORNS CLASSIFIED ACCORDING TO 7-GEOETHNIC SUBGROUPS*. S. Dahan, M.M. Cohen, and R. Cohen. Department of Human Genetics, Hadassah-Hebrew U. Medical Center, Jerusalem, Israel.

Cytogenetic investigations were carried out on cord blood lymphocytes of 500 normal healthy newborns. This population was divided into 7 geoethnic groups based on birthplace of the 4 grandparents of the infant. No numerical chromosomal aberrations were observed but 10% of the individuals manifested cytogenetic variants. Six inherited structural abnormalities (4 inversions and 2 deletions) were observed while the remainder were classified as "minor variants". The chromosomal variants were distributed randomly among the population, so that no particular variant was characteristic of a given subgroup.

* Supported by grants from the National Foundation - March of Dimes and the Israeli Ministry of Health.

●POLYMORPHIC ELECTROPHORETIC VARIANTS OF VITAMIN $B_{12}$ BINDING PROTEINS IN HUMAN PLASMA. S.P. Daiger, M.L. Labowe, L.L. Cavalli-Sforza. Stanford University, Stanford, California.

Polyacrylamide gel electrophoresis followed by autoradiography was used to observe the electrophoretic pattern of human plasma proteins binding vitamin $B_{12}$. Samples were from approximately 100 Caucasians. Binding proteins were labeled with $^{57}$Co-vitamin $B_{12}$ at physiologic concentrations; electrophoresis was conducted at 3°C in an alkaline buffer system. At least seven distinct electrophoretic patterns were observed, showing from two to four major bands each. Samples retaken at intervals of up to one year from several of these individuals, representing each common phenotype, yielded patterns identical to those originally observed.

Preliminary family studies produced results consistent with an autosomal mode of inheritance of four alleles at one locus. Each homozygote exhibits a pair of bands with a mobility characteristic of that type but with the same relative distance between bands. Heterozygotes have the pattern expected from the sum of the corresponding homozygotes. Numbering from the fastest to the slowest allele, tentative gene frequencies are $Tc^1$-.005, $Tc^2$-.034, $Tc^3$-.494, $Tc^4$-.467 (*Tc* for "transcobalamin").

Samples taken as plasma/EDTA, plasma/Na-citrate or serum showed essentially the same phenotypic pattern, but heparinized plasma produced highly distorted patterns. We speculate that heparin combines with vitamin $B_{12}$ binding proteins. Immunologic evidence indicates that these proteins are not haptoglobin, ceruloplasmin, transferrin, hemopexin or glycine-rich β-glycoprotein. Activation of the complement system with zymosan does not change the electrophoretic patterns. Also, these proteins are electrophoretically distinct from a protein which binds free cobalt. Supported by NIH Grant GM 20832-01A1.

BIOCHEMICAL AND GENETIC CHARACTERIZATION OF MURINE ARYLSULFATASE. W.L. Daniel. U. of Illinois, Urbana-Champaign.

At least two forms of p-nitrocatechol-$SO_4$-arylsulfatase exist in murine tissues. One form (arylsulfatase A) has a $K_m$ of 0.73 mM, a pH optimum of 5.3-5.4, a molecular weight of 180,000±5000, a relative electrophoretic mobility of 0.35 at pH 8.3 in disc acrylamide gels, and is not inhibited by 0.2M NaCl. Arylsulfatase B has an apparent $K_m$ of 1.3 mM, a pH optimum of 5.9, a molecular weight of 44,000±2000, a relative electrophoretic mobility of 0.25 in disc acrylamide gels, and is partially inhibited by 0.2M NaCl.

C57BL/6J liver contains twofold higher arylsulfatase activity than either A/HeJ or C3H/HeJ mouse liver. SWR/J mouse kidney possesses two- to threefold greater arylsulfatase activity compared to A/HeJ kidney. These strain differences primarily reflect variation in arylsulfatase B and appear to result from structural enzyme differences. Kidney arylsulfatase B levels are increased by androgens and may be subject to unidentified female humoral factors since kidneys of lactating females have enzyme activities equalling or surpassing those of mature males.

MAJOR CONGENITAL MALFORMATIONS IN THE OFFSPRING OF EPILEPTIC WOMEN. L. Dansky, E. Andermann, F. Andermann and A. Sherwin, McGill U., Montreal, Quebec.

In recent years, a number of reports have suggested an increased frequency of major congenital malformations such as cleft lip and/or cleft palate, cardiac anomalies and microcephaly in the offspring of epileptic mothers receiving anticonvulsant medications during pregnancy. We have initiated both retrospective and prospective studies in an attempt to elucidate the teratogenic risks of anticonvulsant drugs. Detailed family and reproductive histories were obtained from 54 randomly selected epileptic women who had 165 pregnancies, 114 on anticonvulsant drugs. Spontaneous abortions tended to be higher in the medication group (22.8% vs 10.0%) but the difference was not significant. Perinatal mortality was low in the medication group, in contrast to most reports in the literature. 14 of 88 viable offspring (15.9%) of mothers on anticonvulsant medication were born with the following malformations: 6 congenital heart disease, 3 cleft lip, 2 ventral hernia, 1 hypospadias, 1 tracheo-eophageal fistula and 1 microcephaly. The anticonvulsant drugs administered to these mothers included various combinations of diphenylhydantoin, phenobarbital, primidone, ethosuximide and tridione. 3 of 46 offspring (6.5%) of mothers not on medication had Sturge Weber syndrome, congenital dislocation of the hip, and congenital heart disease respectively. There was no significant difference in seizure frequency and other maternal complications during pregnancy, or in family history of major congenital malformations, between offspring with and without malformations. Over 20 pregnant epileptic women have been studied prospectively. Monthly anticonvulsant drug levels were recorded during pregnancy. No significant differences were found in mean anticonvulsant levels between mothers of normal and malformed offspring.

THE MENTAL RETARDATION SYNDROM G 22. K.-H. Degenhardt, M. Geisler, K. Weisse, and A. Grubisic. Institut für Humangenetik and Zentrum der Kinderheilkunde, Klinikum der Universität Frankfurt/Main. Fed.Rep.Germany.

Recently two male siblings, aged 9 and 11 were seen in our genetic clinic both severe mentally retarded with microcephaly (-2 SD), short stature, tetramelic spasticity and multiple congenital anomalies of similar types, i.e. strabism, irregularities of teeth positions, enamel defects, uvula bifida, coxa valga both sides (b.s.), hypogenitalism (micropenis, cryptorchidism), anal stenosis resp. anal atresia operated. Furthermore the I$^{st}$ had: large external ears b.s., preauricular papillomas b.s., left hip joint subluxation, agenesis of kidney left. The II$^{nd}$had: large ear right with preauricular papillomas, severe hypoplastic external ear left, auditory canal atresia left, cleft soft palate, coarctation (?), anomalous position of kidney left, syndactylia of toes II-III left. Banding analysis of chromosomes yielded trisomy 22 in both cases, but the one of three chromosomes 22 had a pericentric inversion, which also was present in one of two chromosomes 22 of the mother. About 16 cases with similar clinical symptoms and a supernumerary chromosome G have been described in the international literature; in two cases also siblings were involved; but chromosome identification by banding analysis is yet missing in all these cases.

A NEW ECTODERMAL DYSPLASIA WITH DISACCHARIDE INTOLERANCE
Dodd, A, Johnston,M, Reed, W.B., and Lowry, R.B.
University of British Columbia, University of California, Irvine

A new genetic disorder with hyperpigmentation.
Thin blonde partial alopecia and dystrophic nails. Severe malnutrition from disaccharide intolerance with severe diarrhia treated by hyperalimentation was noted in two boys nearly indentical in appearance and an unrelated girl. Patients seen by multiple genetists and declared a new genetic dermatological disorder, probably autosomal recessive.

The following disorders were considered and ruled out: Dyskeratosis Congenita, Naegeli Syndrome, Hidrotic Ectodermal Dysplasia, Progeria, Rothmund-Thompson Syndrome. Immunological studies at Sloan Kettering by Dr. Robert Good were negative because both siblings and the girl once had persistent respiratory illnesses.

GENEALOGICAL STUDIES OF BLACK FAMILIES OF MARYLAND. K.R. Dronamraju. Maryland Afro-American and Indian Study Center, Baltimore.

Extensive genealogical studies of Black families of Maryland, have been initiated under the auspices of the State of Maryland Commission on Afro-American and Indian History and Culture. Pedigrees are being compiled for those families residing in Maryland for at least three generations. The study, planned as a contribution to the appreciation of Black heritage, is expected to yield meaningful data on inbreeding and incest, if any, and genetic admixture with other ethnic groups.

INCREASED MATERNAL TRANSMISSION AND ALTERED SEX RATIO IN EARLY-ONSET HEREDITARY SPHEROCYTOSIS. P. A. Duncan. New York Medical College, and Division of Pediatrics, Westchester County Medical Center, Valhalla.

Hereditary Spherocytosis (HS) is an autosomal dominant disease known to exhibit marked variability in its clinical expression. Forty-five infants in 42 families were ascertained from the world literature as early-onset HS on the basis of hematologic data documented in the first month of life.

Although analyses of most perinatal and genetic factors for early-onset HS were normal or revealed the anticipated ratio for an autosomal dominant disease, there is a statistically significant (2.5:1) increase in the maternal:paternal transmission ratio.

Since there are more early-onset HS cases with maternal transmission, an additional factor, possibly intrauterine, may be responsible for this disparity. This factor, in addition, may be responsible for the significantly (3.5:1) increased number of affected males observed in maternally transmitted early-onset HS.

Sib data suggests this postulated intrauterine environmental factor of the affected mother produces symptomatic perinatal HS without affecting the overall transmission ratio anticipated for an autosomal dominant disease.

●PROBABLE LINKAGE BETWEEN ESSENTIAL FAMILIAL HYPERCHOLESTEREMIA AND C'3. R. C. Elston, K. K. Namboodiri, R. C. P. Go, R. M. Siervogel, and C. J. Glueck. U. of North Carolina at Chapel Hill and U. of Cincinnati, Cincinnati, Ohio.

Essential familial hypercholesteremia and 19 polymorphic autosomal blood markers (ABO, MNS, P, Rh, Jk, Fy, Lu, 6-PGD, ADA, AK, AP, PGM1, GPT, C'3, Hp, Gc, Gm, Inv and GBG) were found to be segregating in a large complex pedigree comprising 195 individuals. Initially hypercholesteremia was determined by an estimated cut off value of total cholesterol such that the probability of misclassification should be minimized; the computer program LIPED (Ott, 1974) was used, assuming the complex pedigree to be made up of two independent branches, to calculate lod scores for linkage between hypercholesteremia and the 19 markers at ten pairs of values of the male and female recombination fractions: $(\lambda_m,\lambda_f) = (0,0)$, (.1,.1), (.2,.2), (.3,.3), (.4,.4), (.06,.1), (.12,.2), (.18,.3), (.24,.4) and (.3,.5). The largest lod score found by this initial screen was 1.74, with C'3. This linkage was then further examined, but taking hypercholesteremia to be a quantitative trait, measured as (1) total age-adjusted log cholesterol and (2) a linear function of age-adjusted log cholesterol and age-adjusted log triglycerides; both these traits have been shown to fit autosomal dominant transmission in this pedigree (Elston et al., 1975). For this analysis the more comprehensive program GENPED (Kaplan, 1975) was used, allowing for the dependence between the two branches of the pedigree. The linkage was found to be significant at the 1% level, and confirms a similar result tentatively found by Ott et al. (1974). Now, however, the maximum likelihood estimate of $\lambda_m$ is found, as expected, to be smaller than that of $\lambda_f$.

●QUANTITATIVE IMMUNOLOGICAL STUDIES ON CYTOPLASMIC SUPEROXIDE DISMUTASE: HIGH CONCENTRATION IN RED CELLS OF DOWN SYNDROME. A.W. Eriksson, R.R. Frants and P.H. Jongbloet. The Free U. of Amsterdam, Institute of Human Genetics, The Netherlands.

The controversy over the relation of enzyme activity to gene action in trisomies was reopened in a Lancet editorial (2, 1554, 1974), concluding that a convincing gene dosage effect in Down syndrome has never been reported. Sinet et al. (Lancet 1, 276, 1975) found erythrocyte cytoplasmic superoxide dismutase (SOD-A) to be 1.4 times higher in trisomy 21, than in normal children, and interpreted the results as a gene dosage effect. We have investigated the SOD-A protein concentration in red cells with an immunological technique in 1) Down syndrome patients (4-29 years) 2) age, sex and ward matched mentally retarded, but chromosomally normal patients, and 3) staff mempers from the same institute. No overlapping was found between staff and Down syndrome patients, but an overlapping between the control group of mentally retarded was observed in four cases. Whether the increased SOD-A in Down syndrome is due to a gene dosage effect has to be proven more definitely, as there are a number of reports on enzymes with an enhanced activity in trisomy 21. Most of these elevations seem to be unspecific, being found only in certain cell types, in trisomies, influenced by infections, cell age, etc. SOD-A, however, is the only enzyme for which the locus has been assigned to chromosome 21. This fact strongly supports the hypothesis that the high SOD-A level in Down syndrome is a consequence of gene dosage effect.

| Group | N | $\bar{x}$ |
|---|---|---|
| Down syndrome | 33 | 143 |
| Mentally retarded | 33 | 103 |
| Staff | 33 | 100 |

$\bar{x}$ = average relative SOD-A concentration
N = number tested

A CYTOGENETIC SURVEY OF RESIDENTS AT THE LAURELTON STATE SCHOOL IN PENNSYLVANIA. P. A. Farber, V. Agler, J. Scott and R. Fleckenstine. Geisinger Medical Center, Danville, Pennsylvania, U. of Texas Med. Branch, Galveston, and Laurelton State School, Laurelton, Pa.

Residents with Down's syndrome and mental retardation associated with various congenital malformations were selected for karyotype analysis. Conventional and G-banding techniques for lymphocyte chromosomes were used.

Among 52 individuals, 40 were classified as Down's syndrome by physical diagnosis. Among these 40 residents, there were observed 38 cases of trisomy 21, 1 case of 14/21 translocation, and 1 case of trisomy 21 mosaicism. Normal karyotypes were observed in the 12 individuals without Down's syndrome.

Supported in part by Grant #74-3 from the Institute for Medical Education and Research, Geisinger Medical Center.

COMPARISON OF HISTORICAL, QUALITATIVE, AND QUANTITATIVE METHODS TO ASSESS LATERALITY. C. C. Faust and R. C. Juberg. L.S.U. at Eunice, Louisiana, and L.S.U. School of Medicine in Shreveport, Louisiana.

We proposed to show that laterality is not a qualitative difference of right and left handedness but a quantitative characteristic estimable by history of side preferred in common functions, by observation of preference in certain habits, and by quantitative expression of comparative ability in tests of skill.

Our 232 subjects were 54 white undergraduates, their 108 parents, and 70 of their siblings. Each indicated hand or foot use for 22 different functions (handwriting...kicking a ball) and estimated her (128) or his (104) degree of handedness. Qualitative preference for upper extremity functions included pointing, clapping, sweeping, tying, folding arms, and clasping hands, and for the lower extremity stepping, hopping, and crossing legs. We timed or graded 5 tests of hand ability (grip strength, finger tapping, stereognosis, grooved pegboard, and dowel driving), 1 test of foot ability (tapping), and writing with each hand.

We computed a proficiency index for each subject by averaging 4 of 5 hand functions (difference in right and left values/inferior value). History of hand use correlated closely with this quantitative estimate, but qualitative preference in habitual functions did not. History of foot use correlated with the proficiency index, and qualitative preference correlated less.

We concluded that these tests estimate quantitative degree of laterality, that history of use is less reliable, and that preference in some simple habitual functions is least reliable.

MEASUREMENT OF LYSYL OXIDASE ACTIVITY IN CELL CULTURE. N. Di Ferrante, P. V. Donnelly and D. Tavella. Baylor Coll. of Med., Houston, Texas.

Skin fibroblasts of two patients affected respectively by X-linked Ehlers-Danlos and cutis laxa secreted low levels of lysyl oxidase as measured with labeled elastin and collagen substrates. Thus, this assay may be relevant for the study of unclear syndromes involving collagen and/or elastin. The low levels of enzyme in culture medium require concentration by lyophilization, $(NH_4)_2SO_4$ precipitation or ultrafiltration, procedures which may cause losses or its denaturation. Since the insoluble substrates used bind the endogenous lysyl oxidase, we thought of adsorbing the enzyme present in the culture medium on previously inactivated substrate by mixing them together for 1 hr at $4^{o}C$. Thereafter, the pelleted substrate-enzyme complex, suspended in 2 ml of 0.1 M borate buffer pH 8.0, containing 0.15 M NaCl, is incubated at $37^{o}C$ for 4-8 hrs. Water is distilled from each incubation mixture and from BAPN-containing controls; 1 ml aliquots are counted for released $^{3}H$. The results may be expressed per number of cells or per mg cell protein/plate. The amount of radioactivity released is proportional to length of incubation up to 8 hrs and to volume of culture medium employed. With this method, the previously described case of Ehlers-Danlos Type V shows 30% and 14% of the normal levels of lysyl oxidase activity when tested respectively with elastin and collagen substrates. Supported by National Foundation-March of Dimes, USPH AM-10811, GM-00081, GM-19513 and HL-05435.

●PARTIAL TRISOMY 1q. W.H. Finley, J.H. Garrett, and S.C.Finley. U.of Alabama in Birmingham

No specific clinical syndrome has been established for complete or partial trisomy of chromosome 1. The propositus was a 1710 gram male infant who was born to phenotypically normal Black parents. The infant was referred for cytogenetic studies because of multiple congenital abnormalities which included hirsutism, cleft palate, low-set malformed ears, micrognathia, lateral bowing of both arms with clutched overlapping fingers, penile cordae, undescended testes and a partial syndactyly of the right 2nd and 3rd toes. The infant expired at age 2 days. Pathological studies revealed a small thymus, asymmetry of the thyroid,cerebellar hypoplasia, subependymal hemorrhages, biventricular cardiac dilatation, duodenal atresia, a patient urachus, hydronephrosis and intraabdominal testes. Analysis of chromosomes from cultured fibroblasts showed the infant to have an abnormally long B group chromosome. G-banding studies on the family of the propositus identified his mother and a phenotypically normal male sibling to have a balanced translocation interpreted as t(1;4)(1pter→1q25::4q35→4qter;4pter→4q35::1q25→1qter).His father and four phenotypically normal siblings were found to have normal karyotypes. The karyotype of the propositus is interpreted as having the segment 1q25→1qter present in triplicate.

The abnormal phenotype in association with partial trisomy 1q in this patient will be compared to 3 previously reported cases.

RING CHROMOSOME #21 MOSAICISM IN A CHILD WITH CNS AND SKELETAL DEFECTS. H.K. Fischman, A.D. Bloom, A. Moorthy, and J.D. Rainer. Department of Medical Genetics, N.Y.S. Psychiatric Institute and Departments of Human Genetics and Development, Pediatrics, and Psychiatry, Columbia U. College of Physicians and Surgeons.

A white male child with multiple congenital anomalies was referred to us for chromosome evaluation. Detailed physical examination at the age of one year revealed microcephaly, hypotonia, hypoplastic nails, partial blindness and deafness, bilateral optic nerve and choreoretinal colobomata, nystagmus, unilateral megalocornea, antimongoloid slant and a unilateral simian crease. Two chromosome analyses of peripheral blood cells were done seven months apart and involved a total of 98 karyotypes. The initial analysis at five months of age, revealed three main cell lines: 46,XY(21%)/46,XY, r or del 21(45%)/47,XY+r or del 21(34%). G-banding positively identified the ring chromosome as a #21, that was invariably small and centromere-bearing. The repeat analysis, at the age of one year, showed no significant change in proportion of cell types, but the ring chromosomes varied in morphology, and included double size rings, figure eight forms and interlocked rings. This heterogeneity probably originated from sister chromatid exchanges, resulting in bridge-breakage-fusion cycles. The duplication-deficiencies thus produced by this unstable ring chromosome are variable. Thus, two types of mosaicism are present, one of cell lines, and the other in the heterogeneity of the ring itself. This variability is reflected in the irregularity of clinical symptoms described in the literature for the ring 21 condition. A reasonable explanation for the production of this mosaic is non-disjunction of chromosome 21 early in embryogenesis, followed by ring formation, and subsequent loss of a normal #21.

●A QUALITATIVE ABNORMALITY OF MUTANT HUMAN CYSTATHIONINE SYNTHASE WHICH IS MODIFIED BY VITAMIN $B_6$ THERAPY. L. Fleisher, R. Longhi, H. Tallan and G. Gaull, Dept. Ped. Res., N.Y. State Inst. Res. Ment. Retard., Staten Is., N.Y., and Dept. Ped. and Clin. Genet. Ctr., Mt. Sinai Med. Sch. of CUNY.

The thermostability of human cystathionine synthase (CS) and the effects of *in vitro* addition of pyridoxal phosphate (PLP) and *in vivo* $B_6$ therapy were investigated in samples of cultured cells and liver biopsy material. After 3 minutes of preincubation at 55°, an increase of enzyme activity was consistently observed in extracts of 6 fibroblast cultures (59% activation), 2 long-term lymphoid cell lines (25%), and 6 liver specimens (133%) from normal individuals. No activation was observed in enzyme from fibroblasts of 3 patients with CS deficiency or in liver extracts from 2 untreated ($B_6$-responsive) patients. However, liver extracts from the same 2 patients (and a third affected sib) showed activation (74%) while receiving $B_6$. Enzyme activation (177%) was observed in extracts of liver biopsy material from 2 obligate heterozygotes, although some variability in activation was present in extracts of fibroblasts from 3 other heterozygotes. This "activation phase" was followed by inactivation with time of preincubation at 55°. Addition of PLP prior to preincubation of normal and heterozygous fibroblast extracts doubled the half-life of the enzyme, although smaller effects were seen in patients. Addition of PLP to the preincubation mixture of liver extracts prevented inactivation in normals, heterozygotes and patients. The demonstration of a qualitative abnormality in CS of affected individuals, which is normalized by therapy with $B_6$, and the observation of a protective effect of PLP against heat inactivation of the enzyme may shed light on the mechanism of action of $B_6$ therapy in CS deficiency.

●SUBREGIONAL MAPPING OF THE GENE FOR NUCLEOSIDE PHOSPHORYLASE ON CHROMOSOME 14 BY INTERSPECIFIC HYBRIDIZATION OF HUMAN CELLS WITH A t(X;14) TRANSLOCATION. U. Francke. U. of California, San Diego.

Purine-nucleoside phosphorylase (NP) is a trimeric enzyme which is detectable as a single isozyme in electrophoresis of lysates from cultured human cells. The enzyme activity has recently been found to be absent in a case of isolated T cell defect (Giblett et al, Lancet I, 1010,1975). Rare autosomal codominantly inherited variants exist, but no linkage relationships have as yet been established. The NP locus has been assigned to chromosome 14 by somatic cell hybridization (Ricciuti and Ruddle, Nature NB 241: 180, 1973).

We have confirmed this assignment and have more precisely defined the intrachromosomal location of this gene by somatic cell hybridization experiments using male and female cells containing the balanced translocation t(X;14)(p22;q22). Peripheral lymphocytes were fused to a pseudodiploid HGPRT deficient established Chinese hamster cell line. 26 primary hybrid clones were isolated and maintained in HAT selective medium. Parallel subcultures from generations 16, 24 and 40 after clonal isolation were fully karyotyped and analysed electrophoretically for the presence of human NP and HGPRT. The human NP phenotype segregated discordantly with each human chromosome except for chromosome 14 and the der (14), t(X;14) translocation chromosome. Eight clones which had retained the der(X), t(X;14) translocation chromosome under HAT selective pressure had lost the human NP phenotype. The HGPRT present in all clones was of human electrophoretic type. These results indicate localization of the NP gene in region 14pter →14q22, which includes the short arm and proximal half of the long arm of chromosome 14.

●ETIOLOGICAL RELATIONSHIPS BETWEEN CATEGORIES OF CONGENITAL HEART MALFORMATIONS. F.C. Fraser and A.D.W. Hunter. The Montreal Children's Hospital and McGill University, Montreal, Quebec, Canada.

Pairs of siblings with congenital heart malformations of different types were analyzed for evidence of non-random association of defects within families, that might suggest a genetic predisposition common to two or more kinds of malformation. An excess of pairs were noted for tetralogy of Fallot and pulmonic stenosis, tetralogy of Fallot and transposition of the great vessels, and tetralogy of Fallot and ventricular septal defect, suggesting that there may be a developmental relationship between these lesions. This finding is supported by a recent study in the Keeshond (Patterson, Am. J. Card. 34: 187, 1974) demonstrating a genetic predisposition common to tetralogy of Fallot, pulmonic stenosis and ventricular septal defect. Thus the method does seem capable of revealing etiological relationships, probably genetic, between different types of cardiac lesion. Recurrence risks for sibs of children with these defects will now have to be refined to take into account the possible recurrence of related types.

●AUTOSOMAL RECESSIVE HIDROTIC ECTODERMAL DYSPLASIA. K. Fried. U. Dept. of Genetics, Asaf Harofe Hospital, Zerifin, Israel.

The present report deals with an apparently new type of autosomal recessive hidrotic ectodermal dysplasia. The propositus was a three year old boy with a repaired right cleft lip. He was the product of a first cousin marriage and the only other individual with ectodermal dysplasia in the family was a double first and double second cousin of the propositus who herself was the product of a first cousin marriage. The family belonged to the Egyptian Karaite isolate in Israel. Both affected children had partial adontia, conical peg shaped teeth, delicate and sparse hair (that did not require cutting), a tendency to profuse sweating and marked facial similarity. Sweat pores were noted in normal abundance on dermatoglyphic examination. General development, hearing, vision and nails were considered normal.

GENETIC COMPARISON OF MAJOR AND MINOR PROLINE-RICH COMPONENTS OF MAJOR SALIVARY PHENOTYPES. R.D. Friedman and R.C. Karn Temple U. School of Dentistry, Philadelphia, Penna. and Indiana U. Medical Center, Indianapolis.

Aside from their biochemical characterization, the major acidic proline-rich (Pr) salivary proteins have been genetically interpreted thru family and population studies. Other more minor salivary Pr components have been studied and partially characterized by several investigators. In this study, isoelectric focusing (IEF) of parotid saliva in the ampholyte range, pH 3-5, was used to separate the major and minor Pr proteins from one another and from other salivary proteins. Several major Pr phenotypic samples were selected for IEF on the basis of their combined representation of all the known Pr alleles and gene products. Following IEF, fractions containing various major and minor Pr components were electrophoresed on polyacrylamide gels and the probable association between specific major Pr proteins and minor components was indicated. Additionally, IEF of certain phenotypes in the above range resulted in partial purification of major Pr proteins useful for immunologic comparisons. Cross-reactivity and immunologic identity was observed between every major Pr protein fraction in double immunodiffusion and immunoabsorption experiments.

●FAMILIAL CARRIER MANIFESTATIONS IN FAMILIES WITH X-LINKED DUCHENNE MUSCULAR DYSTROPHY. Z. R. Frouhar, R. Spiro, and M. L. Lubs. U. of Colorado Medical Center, Denver.

The purpose of this report is to present evidence that carrier manifestations in families with X-linked Duchenne muscular dystrophy (DMD) is non-random.

Three families with DMD in which carrier manifestations have been familial will be reported. These families were among 47 families, representing an almost total ascertainment in Colorado. In these three families 13 out of 23 known carriers had symptoms in the form of pronounced calf enlargement, moderate muscle weakness and/or CPK levels exceeding 20 times the upper limit of normal. In one of the families 4 carriers have been diagnosed as having pseudo-hypertrophic muscular dystrophy. The severity of the clinical symptoms in these four females were similar to those of limb girdle muscular dystrophy. In all of the above cases, the family history was compatible with X-linked inheritance. The affected males have had a clinical course typical for DMD. Death occurred between the ages of 18 and 24. Very few manifesting carriers were found in the other 44 families, and no other female was diagnosed as having muscular dystrophy.

Several possible mechanisms for the observed familial nature of carrier manifestations such as a modifier gene, selective X-chromosome activitation or inactivation, and multifactorial influences will be discussed.

The study shows that the carrier state for X-linked DMD has to be considered in women with mild to moderate muscular dystrophy in order to give correct genetic counseling.

SEVERE ORNITHINE TRANSCARBAMYLASE DEFICIENCY IN A FEMALE INFANT. A. Fujimoto, L.J. Raijman and M.G. Wilson. U. Southern California, School of Medicine and LAC-USC Medical Center, Los Angeles.

Ornithine transcarbamylase deficiency is an inherited X-linked dominant disorder leading to lethal neonatal hyperammonemia in affected hemizygous males. Heterozygous females may be completely asymptomatic or show a variable degree of protein intolerance, which is usually manageable by a low protein diet. Prenatal fetal sexing has been offered in pregnancies which are at risk for producing affected males.

We have studied a female infant with severe hyperammonemia who died at 11 months of age. She did not tolerate more than 0.3 gm. protein per kg body weight per day and failed to gain weight on 150 cal./kg diet consisting of Controlyte$^{R}$ (Doyle), freamine, glucose-electrolyte solution, vitamins and infant formula to provide 0.3 gm. per kg of natural protein. Analyses of the liver tissue obtained within 45 minutes after death showed a marked deficiency of ornithine transcarbamylase: 5% of normal in contrast to the usual 12-25% found in symptomatic heterozygous females. Chromosome analysis showed 46,XX, normal female karyotype. The severe deficiency of ornithine transcarbamylase seen in our patient may be due to the result of unfavorable lyonization or a metabolic disorder distinct from the X-linked dominant form.

EMPIRICAL RISK IN A BREAST CANCER KINDRED, E.J. Gardner, M.H. Skolnick and S. Hanks, Utah State U., Logan, and U. of Utah, Salt Lake City.

The breast cancer kindred (Number 107) originally described by E.J. Gardner and F.E. Stephens in 1950 now has more than 1,300 members representing seven generations, many of whom are still below the breast cancer susceptible age. The two generations descended from the original sibship, many of whom are now deceased, have been divided into two groups: (1) those whose progenitors in the sibship had cancer and (2) those whose progenitors in the sibship did not have cancer. The first group included 28 women with breast cancer and five with cancer in other sites among 91 women. The other group had one member with breast cancer and one with uterine cancer among 37 women. The risk of breast cancer compared with 1960 incidence data is 10.04 times higher in the cancer-prone families, even though they contain some apparently normal branches. Therefore, the risk is even higher in the cancer-prone branches. The highest incidence of breast cancer stems from a sib whose husband's line had a high incidence of urinary and uterine cancer, indicating the possibility of a complex etiology.

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●NORMAL RENAL CELLS IN CULTURE: BIOCHEMICAL IDENTIFICATION OF D-VAL-SELECTED CELLS AND EVIDENCE FOR INTRACLONAL HETEROGENEITY. S.F. Gilbert and B.R. Migeon, Johns Hopkins U., Baltimore, Md.

Study of normal differentiation in cells in vitro is impeded by rapid proliferation of less differentiated cells in culture. We have devised a nutrient medium (D-val) to select for the growth of certain differentiated epithelial cells based on the ability of these cells to metabolize D-valine to its essential L-enantiomer (Cell 5: 11-17, 1975). Epithelial cells derived from human and rodent kidneys proliferate in D-val, whereas fibroblasts of similar origin cannot. Studies of "differentiated" renal enzymes are in progress to determine if the D-val-selected cells are actually derived from renal parenchyma. Preliminary results suggest that gulonic acid dehydrogenase and carbonic anhydrase are found in significant amounts in the D-val-selected renal cells in contrast to fibroblast controls.

Furthermore, we have observed kidney-specific morphological patterns develop within individual clones of human fetal kidney cells. Clones (identified as such by G6PD variants) which initially consist entirely of epithelioid cells evolve centers of "fibroblast-like" cells as clonal density increases. These "fibroblast-like" cells stream out from the center to encircle and subdivide areas of the epithelioid cells, yet give rise to subclones entirely of the epithelioid type. This indicates that cell morphology is, at least in part, cell density dependent, and that a single renal cell has the ability to produce progeny of two morphological types.

●"NORMAL" CHILDREN, "ABNORMAL" RESULTS. J. P. Girves, G. A. Chase, and E. A. Murphy. Johns Hopkins U., Baltimore, Maryland.

The advent of multiple laboratory testing to determine whether a patient has any one of a large number of abnormalities raises the question of false positives. Misclassification of a healthy subject as "abnormal" may occur with appreciable frequency if tests are very sensitive and not highly specific. It can be shown that for certain kinds of screening procedures even quite reasonable values of sensitivity and specificity yield absurdly large numbers of false positive results.

The ratios of false positive to true positive, and of false negative to true negative, results for various screening methodologies are presented. These ratios depend upon sensitivity, specificity, and frequency of the abnormal states as well as on the distribution of the test criterion in healthy and diseased groups. Contour graphs are constructed showing these relationships in detail.

Implications of these results for public health programs involving genetic screening are discussed using screening for urinary amino acids as an illustration.

●A STUDY OF THE DEMOGRAPHIC AND PSYCHOSOCIAL FACTORS THAT AFFECT THE COMPREHENSION AND UTILIZATION OF GENETIC COUNSELING IN A CYSTIC FIBROSIS CLINIC POPULATION. M. M. Gluckson, P. Piccio and C. R. Denning. College of Physicians and Surgeons of Columbia U. New York, N.Y.

Because genetic counseling is such an integral part of the comprehensive care of Cystic Fibrosis (CF), a study of 100 families currently attending the CF clinic was conducted to identify the factors that affect the transmission and perception of genetic information. Complete demographic background information was recorded as to family composition, marital status, race, religion, income level, years of education, occupation and countries of origin. In addition, the attitudes of parents toward their marriage, family size, abortion, and use of contraception both before and after the diagnosis of CF were studied. Selective data from the questionnaire is summarized in the table below:

| CHILDREN | | RACE | | RELIGION | | MARITAL STATUS | | ATTITUDE TOWARDS FAMILY PLANNING | | |
|---|---|---|---|---|---|---|---|---|---|---|
| CF | 138 | Cauc | 91% | RC | 66% | Mar. | 79% | Contraception | Before Diagnosis | After Diagnosis |
| Normal | | H-S | 6% | P | 19% | Sep. | 6% | | | |
| Sibs | 149 | Blk | 3% | J | 12% | Div. | 11% | Used | 45 (48%) | 69 (73%) |
| | | | | Other | 3% | Single | 4% | Not used | 49 (52%) | 25 (27%) |

It was found that each family's psychosocial functioning, level of education, religion, degree of stress and style of coping with the burden of a chronic illness does affect their comprehension and application of genetic information.

●THE EFFICIENCY AND ROBUSTNESS OF PEDIGREE ANALYSIS. R. C. P. Go, E. B. Kaplan and R. C. Elston. U. of North Carolina at Chapel Hill.

To test the efficiency of using pedigrees over nuclear families, sets of 20 complex pedigrees in which a major locus accounted for about 15% and 50% of the total variability of a quantitative trait, additive and dominant, were simulated. Each pedigree consisted of four children, two parents with three siblings each, and both pairs of grandparents. Five different maximum likelihoods were calculated, depending on how many individuals in each pedigree were used. The root mean squared errors for seven parameters of interest were calculated, on the basis of 30 replicates for each model and likelihood, and the five likelihoods compared. The general conclusion was that, except to estimate gene frequency, complex pedigrees are more informative per individual than nuclear families.

To test the robustness of the Elston-Stewart method of pedigree analysis, data arising from three different mechanisms were generated: an entirely environmental one, the phenotypic distribution being a mixture of two normal distributions; an additive polygenic one, but with varying amounts of sibling environmental correlation added; and the same one again, except that the data were transformed to produce skewness. Sets of 50 nuclear families with four siblings each were simulated and tests performed to detect the presence of a major gene. Two criteria were used to establish the presence of a major gene: (1) a mixture of two normal distributions fits the data significantly better than a single normal distribution, and (2) the transmission probabilities in the single gene model do not depart significantly from Mendelian values. The method of analysis was found to be quite robust when appropriate precautions were taken.

PREVENTION OF GENETIC DISEASES: HETEROZYGOTE DETECTION BY TEAR β-HEXOSAMINIDASE A. J.D. Goldberg, J.H. Truex, and R.J. Desnick. University of Minnesota, Minneapolis.

Mass-screening programs designed for the prevention of recessive diseases require conveniently obtained enzyme sources which can be assayed by automated procedures. Tears, easily collected on absorbent Schirmer strips, provide a non-invasive source of stable lysosomal hydrolases with high specific activities. Therefore, tears have been evaluated as a source of β-hexosaminidase A activity (Hex A) for the detection of heterozygous carriers of the Tay-Sachs gene. A fully automated, fluorometric procedure has been developed to determine the total and heat stable β-hexosaminidase activities in tears (60 assays/hr); optimal assay conditions which inactivated only Hex A were: buffer=0.03M citrate-0.04M phosphate, containing HSA (50μg/ml), pH 4.1, μ=0.05; substrate=3.0mM 4-MU-2-deoxy-2-acetoamido-β-D-glucopyranoside; and thermal inactivation=52°C for 3.5 min. The mean (and range) % Hex A in tears, leukocytes, and plasma from various individuals are compared below.

| | Tears(automated) | Leukocytes(manual) | Plasma(manual) |
|---|---|---|---|
| Normal Controls (n=225,50,50): | 46.1 (34-67) | 73.3 (60-83) | 66.4 (52-83) |
| Normal Pregnant Women (n=28): | 44.8 (34-55) | 69.8 (55-98) | 45.5 (34-60) |
| Obligate Heterozygotes (n=15): | 26.4 (18-32) | 49.9 (38.5-59) | 42.5 (26-50) |

These data indicate that pregnancy does not effect the % Hex A in tears, comparable to the findings in isolated leukocytes. Thus, tears provide a more reliable source of Hex A than plasma or serum for heterozygote discrimination; the ease of collection and the minimal preparation required for automated analysis make tears particularily convenient for large scale screening for carriers of Tay-Sachs disease and potentially other inherited enzyme deficiencies.

A CASE OF 46,XX,inv(7q) WITH DEVELOPMENTAL ANOMALIES. D.M. Goldenberg, L. Smith, K. Schlich, and C.C. Mabry. Depts. Path. and Ped., U. Kentucky, Lexington.

The report of a 22-month-old female child with 46,XX,inv(7q) is presented. The inversion of the long arms of chromosome no. 7 was identified in blood lymphocytes and skin fibroblasts of the proposita, which were banded by the trypsin-Giemsa method. Similar leucocyte cultures from the peripheral blood of the parents appeared normal. The proposita has a malformation syndrome characterized by bilateral cleft lip and palate, abnormal facies, bilateral cup-shaped ears, developmental retardation, and failure to thrive. The child also suffers from persistent, extensive, seborrheic dermatitis. Bilateral cup-shaped congenital deformity of the ears has been described as transmitted by a single dominant gene. Ear and palate anomalies have been reported in 3/5 cases of partial trisomy 7q. This particular case suggests that an inversion of the long arms of chromosome 7 without any apparent gain or loss of genetic material may nevertheless be associated with congenital anomalies. It is of interest that the abnormal chromosome no. 7 would not have been identified without the use of chromosome banding.

ALPHA FETO PROTEIN (AFP) IN THE ANTENATAL DIAGNOSIS OF CONGENITAL ANOMALIES. B. Goldman, E. Stern, G. Barkai and S. Mashiah, Sheba Medical Center (Tel-Hashomer), Israel

In an attempt to correlate AFP levels in amniotic fluid with congenital anomalies 40 pregnancies were monitored for the following indications: previous neural tube defects (17 cases);suspected neural tube defect in the present pregnancy (12 cases); evident chromosomal aberrations (6 cases) and in miscellaneous congenital malformations (5 cases).

In line with previous observations high levels of AFP were found in anencephaly (2 cases), hydrocephaly (1 case), Potters syndrome (1 case) and in sacrococcygeal teratoma (1 case). It is of interest that of three different chromosomal aberrations, diagnosed intrautero, only in D-trisomy (2 cases) high levels of AFP were found, while in G-trisomy (3 cases) and X-trisomy (1 case) normal values of AFP were recorded.

●SOME OBSERVATIONS ON THE CORRELATION OF W-27 AND ANKYLOSING SPONDYLITIS (AS) AND REITER'S DISEASE (RD) IN CAUCASIANS AND BLACKS. A. E. Good, J. S. Schultz, and J. J. Kapur. VA Hospital and U. of Michigan, Ann Arbor.

The correlation between the histocompatibility antigen W-27 and AS and RD is now well established. Experiences such as Graham's in a back pain clinic have pointed out the importance of histocompatibility typing as an aid in diagnosis of these syndromes. In the latter study 410 out of 459 patients were W-27 negative. Of these, only one had AS. Our material confirms the remarkable association of W-27 with AS (29 W-27+/32) and RD (32 W-27+/34) in Caucasians. Studies of families of several of these patients show a very high incidence of the 2;27 haplotype in both AS and RD. Increased frequency of this haplotype has been reported by Mickey for AS but not RD. Other family members with definite AS or RD symptoms are invariably W-27 positive but many W-27 positive family members are asymptomatic.

In Blacks only 3 of 10 patients with AS are W-27 positive. These three positive patients and one additional deceased patient, in whose family W-27 is segregating, are, or have been, severely crippled and bedridden. The remaining patients are less severely affected "walking" spondylitics. None of the 4 Black RD patients studied thus far are W-27 positive.

Caution should therefore be exercised in using W-27 typing as a basis for excluding AS or RD in Black patients. There may be another antigen associated with spondylitic syndromes, the incidence of which is limited largely to Blacks. The association with this unknown antigen may be due to the effect of the antigen itself or to linkage disequilibrium with the same antigen which is in linkage disequilibrium with W-27 in Caucasians. Technical problems also exist in the interpretation of sacroiliac x-rays due to augmented bone density in this racial group.

●GENETIC COMPLEMENTATION OF PROPIONYL-CoA CARBOXYLASE DEFICIENCY IN HETEROKARYONS OF HUMAN FIBROBLASTS. R.A. Gravel, K.F. Kam, K.J. Scully, and Y.E. Hsia, Hospital for Sick Children Toronto and Yale U., New Haven.

Propionicacidemia in man results from a deficiency of propionyl-CoA carbolxylase (PCC) activity. We have examined genetic complementation of PCC deficiency in heterokaryons produced by fusing mutant fibroblasts in the presence of inactivated Sendai virus. Restoration of PCC activity was monitored in individual multinucleate cells in situ using a radioautographic procedure which detects the incorporation of $^{14}$C-propionate into TCA-precipitable material. Each mutant incorporates negligible amounts of radioactivity compared to control cells. Activity is not restored when different mutants are mixed without virus or when homokaryons are produced by self-fusion. Five mutant lines were fused in all pairwise combinations and examined for the recovery of PCC activity after radioautography. Three complementation groups were revealed: one of a single mutant and the others of two mutants each. Fusions between members of different groups produced multinucleate cells with silver grain patterns of considerably greater density than observed for the parental self-fusions. Fusion between members of the same group failed to show an increase in isotope incorporation. Two mutants, showing excellent complementation by radioautography, were examined for complementation by the direct assay of PCC after large scale fusion. PCC activity of the virus-treated mixture with 23% multinucleate cells was 183 picomoles/min/mg protein compared to 16 units for the untreated mixture (normal range: 800-1200 units). We conclude that PCC deficiency results from mutations of heterogeneous origin. The results suggest the possibility that more than one gene is involved. Supported by MRC MA-5698, Canada.

ESTIMATES OF HERITABILITY AND THEIR SAMPLING VARIANCES FROM COMBINED MZ AND DZ TWIN DATA. M. Grossman. U. of Illinois at Urbana-Champaign , Urbana.

Christian et al. (Am. J. Hum. Genet. 26:154-161, 1974) compared estimates of genetic variance, from combined monozygotic (MZ) and dizygotic (DZ) twin data, based on their accuracy and precision of estimation. In quantitative genetics, interest is not only on the estimate of the genetic variance, but also on the ratio of the genetic variance to the phenotypic variance, the heritability.

Three methods of estimating the heritability and their sampling variances have been derived based on combined MZ and DZ twin data where the genetic variance is estimated using: (I) only the among-twin-sets mean squares, (II) only the within-twin-sets mean squares, or (III) the weighted average of the heritabilities estimated by methods I and II, the weights being reciprocals of their variances minus their covariance.

Method III has minimum variance and is therefore the best (most precise) estimate. The extent to which the method III estimate is better than the unweighted (or equally weighted) average of the heritabilities based on methods I and II, depends in part on the number of MZ and DZ twin sets.

FAMILIAL RISK AND CANCER CONTROL. H. A. Guirgis, H. T. Lynch, F. D. Brodkey, J. Lynch, P. Lynch C. Kraft, K. Maloney, L. Rankin, T. Westercamp & M. Schwartz. Creighton U., Omaha, Nebraska

The cost, limited professional manpower, limited facilities, and motivational problems of patients make it impractical to screen the entire adult population for early signs of cancer on a regular basis. Needed are programs which can be geared to those individuals who are at the highest risk for cancer. Family histories obtained through detailed medical-genetic questionnaires, coupled with follow-up personal interviews were performed on 1,254 consecutive adult patients undergoing multiphasic cancer screening in a mobile cancer detection unit in rural Nebraska. Of these patients, 78 (6.2%) gave a history of having had cancer. This figure is comparable to expectations from the New York State Tumor Registry. The size of family and the average age of the relatives account for only 6.3% of the observed variation, but are significantly correlated with family history of cancer. As more first degree relatives were affected with cancer, a greater percentage of probands themselves manifested cancer. Findings showed that 8.9% of the probands developed cancer when there was one cancer in a single first degree relative; 16.2% had cancer with two family members affected and 27.4% had cancer with three or more affected family members. These data show clearly the nonuniformity of the distribution of cancer in this population; they also indicate that family history of cancer could be used profitably as a cancer risk index in cancer screening programs. A risk profile should also employ epidemiologic information including habit patterns, occupation and environmental carcinogenic exposures. Collectively, their utilization should enhance cancer control programs. Projection of our data to the 1970 U.S. Census suggests that 3,109,000 U.S. citizens have three or more first degree relatives with cancer. Supported by Nebraska Division of the Amer. Cancer Society.

GENETICS OF HUMAN PAROTID SALIVA PROTEINS. S. Guttormsen and L. Weitkamp, U. of Rochester Medical School, Rochester, N.Y. Using 3 quite different electrophoretic techniques, six systems of genetically determined variation have been previously described in human parotid saliva: Pb (Azen, 1972), Pa (Friedman et al., 1972, 1975), Pr (Azen and Oppenheim, 1973), Sal I and Sal II (Balakrishnan and Ashton, 1974) and Db (Azen Denniston, 1974). We have typed parotid saliva on over 250 family members, most of them in each of the three major electrophoretic systems, and propose on the basis of the correspondences among the multiple protein bands in each system that at most three loci, Pb, Pr and possibly Db, are required. The Pr and Sal systems refer to the same phenotypes. In parotid saliva these can be attributed to four principal alleles at a single locus. The superior resolution of the Sal electrophoretic system permits us to modify the interpretation of Azen and Denniston, however, to the effect that $Pr^1(S)$ and $Pr^2(F)$ do not have an analogous relationship to $Pr^{1'}$ and $Pr^{2'}$. We suggest that $Pr^{1'}$(our T) is a third allele, common in blacks and not whites, which produces a shift in the primary band (to the x region) analogous to the difference between F and S. In contrast $Pr^{2'}$($F^+$ pattern) produces a band electrophoretically indistinguishable from F plus a minor band in the cathodal area of region V (also x region), clearly different from T. The $F^+$ minor band (= $Pa^+$) disappears on treatment with mercaptoethanol and need not be attributed to an allele at a locus different from Pr. The Db bands, on the other hand, have been found in both S and $F^+$ phenotypes, although they are more frequently associated with S. No recombinants between Db and Pr have been observed. Thus, Db is either part of or in linkage disequilibrium with Pr.

●BIOSYNTHESIS OF HB WAYNE IN RETICULOCYTES. S. M. Hanash and D. L. Rucknagel. U. of Michigan Medical School, Ann Arbor.

All of the known hemoglobin variants containing elongated α chains are present in markedly reduced quantities relative to hemoglobin A. Hb Wayne is the result of a frame-shift mutation at or near position 138 yielding α chains elongated by 5 amino acids. The phenotype consists of Hb A plus two abnormal Wayne components, one having an asparagine residue at position 138 and the other an aspartic acid at the same position. Together, they comprise approximately 3% of the total hemoglobin. The aspartic acid form is believed to be derived from the asparagine form by deamidation. The biosynthesis of hemoglobin Wayne was studied using reticulocyte rich fractions of peripheral blood cells from a heterozygote. Cells were incubated in the presence of $^3H$ arginine for 4', 8', 12', 60' and 3 1/2 hours. The different hemoglobins were separated on carboxymethyl cellulose chromatography. The aspartic acid form of Hb Wayne was further purified by preparative isoelectric focusing. The globin chains were separated on carboxymethyl cellulose chromatography in 8M urea. The aspartic acid Wayne chains were rapidly labelled and the specific activity was lower than that of normal α chains, suggesting that similar to hemoglobin Constant Spring, decreased synthesis rather than increased degradation was responsible for the reduced quantities of hemoglobin Wayne present in heterozygotes. Preliminary evidence suggests that a similar pattern exists for the asparagine form.

From the reduced amounts of abnormal hemoglobin present in the various chain elongation mutants it appears likely that the untranslated portion of the alpha chain mRNA might have an important function with respect to its translation or stability.

●THE ISOLATION OF A NEW PROTEIN COFACTOR REQUIRED FOR THE IN VITRO HYDROLYSIS OF TAY-SACHS GANGLIOSIDE. P. Hechtman & D. LeBlanc. MRC Genetics Group, Montreal Children's Hospital and Dept. of Biology, McGill U., Montreal, Quebec. Intr. by R.J.M. Gold.

Crude extracts of normal human liver containing both hexosaminidases A & B catalyze the hydrolysis of the terminal galNAc residue of Tay-Sachs ganglioside.

When crude liver extract is chromatographed on DEAE-Sephadex, Hex A and B are separated and recovery of both isozymes is >80% when artificial substrates are used. Recovery of activity toward the Tay-Sachs ganglioside ($GM_2$) substrate, however, is <20% and this activity is entirely associated with the Hex A peak.

The rate of $GM_2$ hydrolysis by the Hex A containing fraction can be enhanced at least ten-fold in the presence of a factor that cochromatographs with Hex B. The stimulating factor, however, is not Hex B. The factor can be completely resolved from Hex B by gel filtration on Sephadex G-100. Using calibrated G-100 columns the molecular weight of the factor is about 49,000.

The stimulating factor is labile to heat, precipitable by $(NH_4)_2SO_4$ and non-dialyzable. Factor activity cannot be replaced by human serum albumin.

Purification of the stimulating protein and study of the mechanism of its enhancement of the $GM_2$ hydrolytic activity of Hex A is continuing in order that the sites of action of various human mutations leading to $GM_2$ gangliosidoses may be better understood.

GENETIC ANALYSIS OF HUMAN VISUAL PARAMETERS. J. P. Hegmann, A. J. Mash and B. E. Spivey. Pacific Medical Center / University of the Pacific, San Francisco, California.

Strabismus is a clinical term denoting conditions in which malalignment of the visual axis interferes with binocular single vision. Although the common form of congenital or early onset strabismus is clearly familial, it is not transmitted in the human population in a pattern consistent with a single-gene model. As the condition is considered clinically, it typifies a threshold character; however, its expression depends on underlying motor and sensory characteristics which do show continuous variability.

Measures indexing various motor and sensory functions of the visual system were obtained from three populations (composed of family units) with differing incidences of diagnosed strabismus and were subjected to genetic analysis in order to delineate the nature of gene differences associated with these indices. To the extent that defining populations according to the incidence of strabismus defines populations with different gene frequencies (or allelic forms) genetic variance for key variables in the abnormality should differ among these populations.

SPONTANEOUS ABORTION FOLLOWING AMNIOCENTESIS IN 267 WOMEN UNDERGOING PRENATAL DIAGNOSIS. R. H. Heller, K. J. Winn, T. A. Baramki, J. M. Rary, I. J. Park. Johns Hopkins University, Prenatal Diagnostic Center, Baltimore, Maryland.

Between 8/1/69 and 12/31/74 283 women requested amniocentesis following prenatal genetic counseling. Of these, 16 women spontaneously aborted between the time of counseling and the scheduled date of amniocentesis. Among the remaining 267 women, 8 miscarried between 1 day and 13 weeks after amniocentesis. Obstetrical abnormalities were identified in 4 patients; infection in 2 patients; and no apparent cause uncovered in 2 patients. Discolored amniotic fluid noted at the time of amniocentesis often signified subsequent fetal loss.

●DELAYED MUTATION AS A CAUSE OF HUMAN DISEASE. J. Herrmann. U of Wisconsin, Madison.

Delayed mutation implies the presence of an unstable premutated allele which like the wild and the fully mutant alleles at the same locus may be heritable as such. The changes from the wild to the premutant allele (premutation) and from the premutant to the mutant allele (telomutation) may depend on various exogenous or endogenous mutagens or mutation promoting conditions and genetic constitutions. In man, the model of delayed mutation was previously considered for achondroplasia, the Wiedemann-Beckwith syndrome and retinoblastoma. Investigation of familial cases of leukemia, familial hemophagocytic reticulosis, the prune belly syndrome, carotid body tumors and Wilms' tumor suggests that the model of delayed mutation may also be pertinent to these five conditions. The proportion of pre- vs telomutant alleles at the different loci responsible for the genetic forms of these disorders appears to relate to the type of cell division (meiotic vs mitotic) and to the sex of the carrier of the premutant allele.

PSYCHOLOGICAL EVALUATION OF FAMILIES WITH GENETIC DISORDERS. V.L. Herzberg and J.N. Richie. U. of Arkansas Medical School, Little Rock, and L.S.U. School of Medicine, Shreveport, LA.

Psychological aspects of genetic disease were investigated by subjective, open-ended interviews with affected and unaffected family members. Sixteen individuals from twelve families selected to compare varying patterns of inheritance agreed to participate in the study. Interviews were conducted by a psychiatrist and questions from medical students enrolled for elective credit were permitted. The interviewer and students were provided with the family history, pedigree and pertinent medical and genetic references a week in advance of each interview. Each session was recorded on tape with the permission of the participant.

The course of the interview was guided by the individual's responses to general introductory questions. This nondirective approach frequently revealed sensitive areas previously undisclosed during genetic counseling sessions. It was noted that the final decision regarding family planning did not necessarily reflect the expected rationality involved in reaching that decision. Profiles of the participants reveal that the capacity to utilize the information obtained in genetic counseling is dependent upon the stability and maturity of the individual personality. Comparisons between families and psychological assessments of relationships within families lends evidence to the highly individual nature of the counseling and decision making processes.

Techniques for revealing psychological mechanisms which interfere with the rational decision making process will be evaluated. Recognition of abnormal psychological mechanisms requiring referral to other sources will also be discussed.

CHEMICAL MUTAGEN HYPERSENSITIVITY IN ATAXIA TELANGIECTASIA. D. I. Hoar and P. Sargent U. of Toronto, Toronto, Ontario.

Ataxia telangiectasia (AT) is an autosomal recessive disorder characterized by defects of the immune system, an increased risk of malignancy and enhanced spontaneous chromosomal breakage. Because of the latter characteristic, this syndrome has been classified along with Bloom's Syndrome (BS) and Fanconi's Anaemia (FA) as a Chromosomal Breakage Syndrome.

The observed hypersensitivity of FA cultured cells to mitomycin C and the suggestion of a defect in excession repair along with the observed defect in DNA elongation in BS suggested that a relationship between chromosomal breakage and altered DNA metabolism exists. For this reason, we chose to examine parameters associated with altered DNA metabolism in AT cells.

Three different chemical mutagens have been tested with cultured fibroblasts from AT patients. All eight cell lines tested show significantly increased sensitivity to Actinomycin D while there appears to be heterogenity with respect to methyl methane sulfonate and mitomycin C sensitivity.

The results of these studies and others designed to delineate more precisely the site of the defect in AT will be discussed. (Supported by MRC of Canada grant MA 4998).

●ISOLATION OF PROLIFERATING HYBRIDS BETWEEN HUMAN DIPLOID FIBROBLASTS IN THE ABSENCE OF SELECTIVE SYSTEMS. H. Hoehn, E.M. Bryant, P. Johnston, T.H. Norwood and G.M. Martin. U. of Washington, Seattle.

The only convincing evidence to date for the formation and viability of somatic hybrids between euploid human cells has been reported by Migeon et al. (PNAS 71: 937, 1974). The lack of the availability of a greater variety of selective systems has hampered further progress in the isolation of such hybrids. We have employed the technique of Sprague et al. (JCB 70: 781, 1974) to identify tetraploid clones emerging from dilute platings (in standard culture media) of G6PD A and G6PD B skin fibroblast mixtures derived from different individuals. Of 3,203 colonies screened, 108 were isolated with the stainless steel cylinder technique. 21 of these were 100% tetraploid as evidenced by counts of over 50 metaphases. 3 tetraploid clones were proven to be hybrids on the basis of a triple band electrophoretic pattern. The fusion protocol was also applied to a mass culture of fibroblasts from a G6PD A/B heterozygote. Of 6,944 colonies, 136 were isolated, 22 of which were 100% tetraploid. Only 1 of these showed the heteropolymer isozyme pattern. In both types of fusions, there were a total of 5 additional isolates with a heteropolymer component but not a symmetrical 1:2:1 distribution. These were found to be mixoploids on cytogenetic analysis. We are in the process of continuously passaging and retesting all hybrid isolates. In the 100% tetraploid hybrid clones, no chromosomal or electrophoretic changes have been noted at up to nine passages in culture. This work demonstrates the feasibility of isolation of proliferating hybrids from any given combination of parental fibroblast strains. Supported by NIH grants GM 15253 & AM 04826.

HIGH IN VIVO RATES OF METHIONINE BIOSYNTHESIS IN TRANSFORMED HUMAN AND RAT CELLS AUXOTROPHIC FOR METHIONINE. R. M. Hoffman and R. W. Erbe. Genetics Unit, Mass. General Hosp., Boston.

Recently it was reported that 3 oncogenically transformed rat, mouse and human lines can neither grow nor survive in $B_{12}$- and folate-supplemented media in which methionine (Met) was replaced by homocysteine (HC) despite the presence of the Met synthesizing activity, 5-methyl-$H_4$-folate: homocysteine cobalamin methyltransferase (Ashe et al. BBRC 57: 417, 1974). In contrast, normal human, rat and hamster cells proliferate well under these conditions. In order to determine the basis for the Met auxotrophy in transformed cells, we have compared in vivo Met synthesis in the Walker 256 carcinoma line and 2 SV40-transformed human fibroblast lines, all of which are unable to proliferate on HC-containing, Met-deficient media, with that in 2 normal human skin fibroblast strains, which proliferate well under these conditions. The total methyltransferase activities in extracts of the transformed and normal cells were comparable. 5-($^{14}$C)methyl-$H_4$-folate was taken up by normal and transformed cells, and this uptake was greatly stimulated by HC. On amino acid analysis of the total cell acid hydrolysate, most of this label was recovered in Met. Surprisingly the rates of Met synthesis were at least as high in transformed as in normal cells and, in one transformed line, 2-3 fold higher. Further, the proportion of newly synthesized Met incorporated into high molecular weight substances was substantially greater in the transformed cell lines. The requirement for exogenous Met for growth despite high levels of endogenous synthesis is presently unexplained but appears to distinguish at least certain oncogenically transformed but otherwise widely varying cell lines from normals. (Supported in part by NIH Grants HD06356, CA16838, and CA04670.)

●CORRELATION BETWEEN CLINICAL PHENOTYPE AND HYPOXANTHINE PHOSPHORIBOSYLTRANSFERASE (HPRT) ACTIVITY IN INTACT MUTANT FIBROBLASTS. M. J. C. Holland, J. Dancis, M. E. Balis, and R. P. Cox. New York U. Medical Center, New York, and Memorial-Sloan Kettering Cancer Center, New York.

Discordance between clinical phenotype and the level of mutant enzyme activity may reflect differences between enzyme function in vivo and that measured by the customary enzyme assays. Clinical determination of HPRT activity is usually performed on cell extracts in the presence of excesses of the cosubstrates, hypoxanthine and phosphoribosyl pyrophosphate, at optimum pH and $Mg^{+2}$ concentration. Although there is general correlation between clinical symptomatology and the level of residual HPRT activity detected by this method, there are notable exceptions. In order to investigate HPRT activity under more physiological conditions, an intact cell assay was developed. Late log phase skin fibroblast cultures growing in monolayer were incubated in medium containing 4 X $10^{-4}$ M [8-$^{14}$C] hypoxanthine (15 mCi/mmole) and, following disruption of the cells, the reaction products were isolated. In fibroblasts with normal HPRT the rate of conversion of hypoxanthine to inosinate (mean $\pm$ SE: 5.10 nmoles $\pm$ 0.13/mg protein/h) is linear for at least 2 h. HPRT activity was measured in intact fibroblasts from seven HPRT deficient patients, ranging in phenotype from asymptomatic hyperuricemia to the Lesch-Nyhan syndrome. The residual HPRT activity in intact mutant fibroblasts was consistent with the clinical picture for each patient and correlated closely with clinical symptomatology. Studies on intact skin fibroblasts derived from patients with heritable diseases of metabolism may provide a more accurate assessment of the in vivo function of a mutant enzyme than assays using cell homogenates or purified enzymes. The approach used in this study may be applicable to other inborn errors of metabolism.

ZINC QUINACRINE MUSTARD. D. H. Hollander, L. E. Litton, and Y. W. Liang. Johns Hopkins U., Baltimore, Md.

The cation population of the staining solution markedly influences the intensity of fluorescence after quinacrine mustard (QM). Variability in QM staining may be related to absence of suitable ions. Monovalent ions give poor stains, while, in general, divalent ions produce good stains. Rapid consistent stains of excellent quality are obtained with added zinc ions.

50 µg/ml QM in 0.5% $ZnSO_4$ (w/v) solution is generally useful for staining. 5 minutes followed by 5 minutes differentiation in 0.5% $ZnSO_4$ is sufficient for metaphase chromosomes. Longer staining accentuates brilliant polymorphic bands but obscures dark bands. 30 minutes staining followed by 10 minutes differentiation in 5.0% $ZnSO_4$ solution stains Y chromatin brilliantly in buccal smears. Formalin fixed tissue requires 30 minutes staining followed by 30 minutes differentiation in 5.0% $ZnSO_4$ solution.

Thanatophoric dwarfism is a severe, short-limbed chondrodystrophy resulting in death in the neonatal period; characteristic radiographic and pathologic alterations of the skeleton have been documented. At present, the homogeneity and mode of inheritance of this syndrome are in doubt.

We have studied cartilage specimens from 2 pathologically-proven cases of thanatophoric dwarfism, and one additional clinically-diagnosed case. We find distinctive identical abnormalities of cartilage collagen by polyacrylamide gel electrophoresis in the first two cases, but not in the third. Whole cartilage collagen alpha chains, prepared by pepsin digestion, exhibit an apparent decrease in molecular weight, as do the smaller peptides derived from alpha chains by cyanogen bromide (CNBr) digestion when compared to normal human cartilage collagen. Direct CNBr digestion of thanatophoric cartilage confirms the previously noted abnormalities of CNBr peptides observed with isolated alpha chains. These findings suggest (1) thanatophoric dwarfism may be biochemically as well as clinically heterogeneous, (2) some forms of this disorder exhibit an altered cartilage collagen, and (3) the observed apparent lower molecular weights of whole collagen chains and CNBr peptides may result from lack of the normally-occurring sugar residues and/or hydroxylation of thanatophoric cartilage collagen. Elucidation of the molecular defect(s) in thanatophoric dwarfism may serve to clarify the heterogeneity, pathogenesis and inheritance of these disorders.

●CHARACTERIZATION OF A PURINE AUXOTROPH UNABLE TO CATALYZE THE FIRST REACTION OF PURINE BIOSYNTHESIS DE NOVO. E. W. Holmes, A. Leyva, G. King (Introd. by J. B. Sidbury) Duke U., Durham, NC Synthesis of phosphoribosylamine (PRA) is the initial rate limiting step of purine biosynthesis de novo. Three enzymatic reactions have been reported to catalyze the synthesis of PRA in mammalian cells: PP-ribose-P amidotransferase (glutamine utilizing), PP-ribose-P aminotransferase ($NH_3$ utilizing) and Ribose-5-phosphate aminotransferase ($NH_3$ and ATP utilizing). The recent isolation of a purine auxotroph from a Chinese hamster cell line by Chu et. al. has permitted the following analyses of these reactions. (1) The mutant cell line (P-1-2) does not synthesize DNA in a purine free media, while wild type cells (743) synthesize DNA in this media. (2) Extract from 743 can synthesize 104 and 238 nMoles of PRA/hr/mg of protein with glutamine (PP-ribose-P amidotransferase) and $NH_3$ (PP-ribose-P aminotransferase) as substrates, respectively. Extract from P-1-2 has no detectable ($<$1 nMoles/hr/mg) PP-ribose-P amidotransferase or PP-ribose-P aminotransferase activity. (3) Extract from P-1-2 can synthesize phosphoribosylglycinamide (PRG) from ribose-5-phosphate, $NH_3$, ATP and glycine. (4) Both cell lines have comparable activities of PP-ribose-P synthetase, IMP dehydrogenase, S-AMP synthetase, hypoxanthine-guanine phosphoribosyltransferase and adenine phosphoribosyltransferase. We conclude the following from these studies: (1) The inability to detect either PP-ribose-P amidotransferase or PP-ribose-P aminotransferase activity in mutant extracts suggests that these two enzymes may be structurally or genetically related. (2) The synthesis of PRG by mutant extract, which is dependent upon the prior formation of PRA, suggests that the in vitro activity of Ribose-5-phosphate Aminotransferase may not be functional intracellularly in this purine auxotroph under the conditions studied.

SPONTANEOUS MUTATIONS FOR AUTOSOMAL DOMINANT DISORDERS IN 18,000 NEWBORNS. L. B. Holmes and J. Alper. Massachusetts General Hospital, Boston.

The average mutation rate for autosomal dominants has been estimated as $2.6 \times 10^{-5}$, one of the highest rates being neurofibromatosis at $13 \times 10^{-5}$. We have evaluated all spontaneous mutations for dominants among 18,000 newborns, including a study of polydactyly and infants with multiple café-au-lait spots at risk for neurofibromatosis. There were 3 known dominants, Pfeiffer's syndrome, spondyloepiphyseal dysplasia congenita and Moebius syndrome, plus 2 more suspected disorders.

49 infants had postaxial polydactyly; 46, type B; 3, type A. Only 13 of 57 parents examined had polydactyly. The low rate of occurrence in parents could reflect: lack of penetrance in one parent, spontaneous mutation in the child or etiology other than dominant gene.

4,641 infants were examined for 3 or more café-au-lait spots. 13 were identified, only 1 with an affected parent. None of the infants had other signs of neurofibromatosis. As all 13 were black, a normal racial variation is a more likely etiology.

An additional 80 infants had major malformations of unknown etiology, none recognized as dominant disorders at this time.

CYTOGENETIC STUDIES IN PATIENTS WITH THE BASAL CELL NEVUS SYNDROME AND THEIR RELATIVES. A. A. Horland, S. R. Wolman, E. Reich, and R. P. Cox. New York U. Medical Center, New York.

The basal cell nevus syndrome is an autosomal dominant condition consisting of multiple basal cell carcinomas, jaw cysts, "pits" of the palms and soles, calcification of the falx cerebri, and other skeletal and endocrine abnormalities. Recently, two families with this syndrome were referred for counseling and evaluation. Our interest in chromosomal instability in association with human malignancy, and the appearance of a few reports of chromosome aberrations in this syndrome prompted a cytogenetic study of the families. The first proband was a 62 year old woman in whom significant increases of chromatid breaks (6%) chromosome breaks (2%) and rearrangements (10%) were found in cultured lymphocytes. One of her sons also showed some chromosome abnormalities, although he is asymptomatic at age 36. The proband in the second family is a 78 year old woman with multiple basal cell nevi and jaw cysts. Studies of her lymphocytes and fibroblasts are in progress. Analysis of the family reveals one surviving daughter aged 48 who is asymptomatic, but her peripheral lymphocyte cultures exhibit a high percentage of chromosome aberrations (30%). This syndrome may constitute another example of association of the familial occurrence of neoplasia with chromosome aberrations.

$\alpha_1$ ANTITRYPSIN PHENOTYPE AND PULMONARY FUNCTION IN SASKATCHEWAN GRAIN HANDLERS. S.L. Horne, C. Deutscher, R.P. Singh. University of Saskatchewan, Saskatoon, Canada.

**While persons with an $\alpha_1$ antitrypsin phenotype of ZZ are known to have an increased risk of developing chronic obstructive pulmonary disease (COPD), the effects of other phenotypes i.e. MZ, SS, MS and SZ on the development of COPD are not clear. Conflicting reports may be due in part to methodology but could also be due to systematic selection of individuals exposed to different sets of environmental factors.**

**Men employed in Saskatchewan grain elevators have a high incidence of lung problems presumably because of the enormous amounts of grain dust in the elevators. We have done pulmonary function screening tests and Pi typing on 1000 of these men and have found a significantly higher frequency of abnormal pulmonary function (as measured by $FEF_{25-75}$ values) in MZ heterozygotes, possibly also in MS heterozygotes. Examination of smoking histories and ethnic background suggests that these also may make a contribution to the risk of developing abnormal pulmonary function.**

●RESPONSE TO A CARRIER DETECTION PROGRAM IN DUCHENNE MUSCULAR DYSTROPHY. E.M. Hutton and M.W. Thompson. Hospital for Sick Children and University of Toronto, Toronto, Canada.

A genetic counseling program, initiated in 1964, has been assessed to determine what factors influence the family planning decisions of women at risk of producing sons with DMD. The women were classified as having a high, moderate or low risk on the basis of serum creatine kinase activities and pedigree data, analyzed by Bayesian techniques.

Response to a follow-up questionnaire was obtained from 256 of 336 females in the childbearing age range. In a group of 122 females who indicated that at the time of counseling their families were incomplete, limitation of reproduction correlated directly with the magnitude of genetic risk. The percentage deterred from further childbearing was greater in families with at least one child, and much greater in families with an affected son. The reduced reproductive rate of women at risk is reflected by the finding that, among newly ascertained cases, the proportion in which the mother was aware of a positive family history declined from 30% in 1965-69 to 15% in 1970-74. In 1973 and 1974 more than 90% of the patients ascertained were isolated cases; thus there is an increasing need for counseling on the basis of Bayesian analysis in order to identify and reassure female relatives who may be at low risk.

Women with one or more children are less likely to reproduce than are women without children. To eliminate this source of bias, we have also examined the reproductive attitudes and performance of 110 women in the study who were childless at the time of counseling. The responses of these women and the factors affecting their responses will be described.
(Supported by The Muscular Dystrophy Association of Canada.)

COLCEMID INDUCED ACCELERATED DNA SYNTHESIS IN CULTURED HUMAN LYMPHOCYTES AS SHOWN WITH MICROSPECTROPHOTOMETRY. C. Hux, L. Sciorra. Rutgers U., New Brunswick, N.J.

Previous work by Hux and Tegenkamp has shown increases in tetraploidy and endoreduplication in human amniotic fluid and lymphocytes with elevated levels of colcemid (0.1-3.0mcg/ml media). Because the exposure time of colcemid was two hours it was not possible to explain the presence of tetraploidy and endoreduplication on the basis of a normal 24 hr. cell cycle. Published work indicates that high levels of colcemid can accelerate DNA synthesis in cultured mammalian cells. Microspectrophotometry was used in a series of measurements of Feulgen stained diploid, tetraploid and endoreduplicated mitoses to quantitatively determine DNA content. Tetraploid and endoreduplicated cells contained twice the amount of DNA as diploid cells. Several known aromatic hydrocarbons have been shown to produce cancer in mammalian cells. The two general characteristic changes exhibited by tissues treated with these hydrocarbons are cell membrane disruption and an increased DNA synthesis resulting in accelerated cellular and nuclear division and polyploidy all of which are characteristics of colcemid induced tetraploidy in our investigations. We hope this phenomenon can be used as a model system to obtain a better understanding of the relationship between an aromatic hydrocarbon and the regulation of DNA synthesis.

Cell fusion experiments are in progress, however, it seems highly unlikely that this phenomenon would produce the endoreduplicated cell.

A STUDY OF SUICIDE IN HAWAII. I. Ibrahim, C. Carter, D. McLaughlin, and M. N. Rashad
U. of Hawaii, Honolulu.

A study of death due to suicide among the Japanese, the Caucasians and the Filipinos in Hawaii was carried out for the years 1910-1965. Rates per 100,000 were computed for each racial group. A study of the effect of age, sex, race and year was undertaken using the least square method on computor.

Suicide rates increased with age and were lower in females than males for all three races. The rates were highest in Japanese and lowest in Filipinos. Caucasians were intermediate. A study of the time trends showed significant decrease with time in the Japanese. The Filipinos showed a significant increase with time, approaching the rates in Caucasians, which were intermediate.

●MANNOSIDOSIS: CHARACTERIZATION OF THE α-D-MANNOSIDASE ISOZYMES. J.U. Ikonne and R.J. Desnick.
University of Minnesota, Minneapolis.

Three components of α-D-mannosidase activity were isolated and purified from normal human tissues by ion-exchange and affinity chromatography. These components, designated A, B, and C, were resolved by DEAE-cellulose chromatography at pH 6.0; the A form was eluted with the initial 10mM sodium phosphate buffer, and the B and C forms were eluted at 0.08M and 0.12M KCl, respectively. Affinity chromatography on Concanavalin A-Sepharose at pH 6.0, retained the acidic A and B components which were subsequently released by elution with D-glucose, α-methyl-D-glucoside or α-methyl-D-mannoside at pH 7.0; the C form did not bind to the column and was recovered from the initial buffer wash. These isozymes were separated electrophoretically on cellulose acetate gels at pH 6.5.

The α-D-mannosidase A and B components had optimum activities at pH 4.5 and were thermostable at 60°C for up to 4 hr; the Km values for the purified A and B components were 0.38 and 0.44 x $10^{-3}$M, respectively. In contrast, the C component had an optimum activity at pH 6.5 and was rapidly inactivated at 60°C. Furthermore, the C activity was stable at 4°C but was partially inactivated following storage at -20°C.

In plasma, leukocytes, and tissues from a homozygote with mannosidosis, the A and B isozymes were deficient but the C form was present at normal levels. Immunological characterization of the molecular and genetic interrelationships among the α-D-mannosidase components in health and disease have been undertaken in order to elucidate the biochemical defect in mannosidosis at the molecular level. Supported by The National Foundation (1-273) and NIH (AM 17154).

●IMPROVED COMPUTER IDENTIFICATION FOR HUMAN CHROMOSOMES USING R BANDED CELLS. P.S. Ing, R. S. Ledley, R.S. Verma and H. A. Lubs. U. of Colorado Medical Center, Denver, and Georgetown U., Washington, D.C.

The goal of the present work is to develop an automated system of chromosome identification which correctly identifies more than 90% of chromosomes, i.e. 2 pairs might be misidentified, and permits quantitation of chromosomal variation. In Q and (ASG) G banded cells, only 2/3 of cells are correctly identified by the present system when Fourier coefficients derived from other cells are employed. The clearly demarcated bands and ends of arms in acridine-orange R (AO-R) banded cells makes these cells potentially more amenable to computerized identification. Several means of analyzing variation in the densitometric curves have been developed. For example, by plotting curves derived from the mean and standard deviations of the Fourier coefficients, the areas of greatest variability in the karyotype can be determined. Analysis of these curves can indicate whether this is due to genetic or technical variation. Using the FIDAC/MACDAC automated chromosome identification system developed by Ledley, Lubs and Ruddle 79 cells from 3 female and 2 male subjects were used to generate 5 sets of Fourier coefficients for R banded cells. After eliminating an average of 3.2 chromosomes per karyotype because of overlapping chromosomes and other technical problems, 96% (range 91-99%) of the remaining chromosomes were correctly identified when chromosomes and coefficients of the same subject were used. When coefficients of one subject were used to identify the chromosomes of another subject, 89% of the chromosomes were correctly identified (range 84-98%).

PROVIDING GENETIC COUNSELING FOR MORE COMMON GENETIC MALFORMATIONS: EXPERIENCE WITH A CLEFT LIP AND PALATE CLINIC. G.B. Ingall, A. Easton, B.A. Bernhardt, R. M. Bannerman. State University of New York at Buffalo.

In a recent follow-up study of 200 discharged patients from a cleft lip and palate evaluation clinic where genetic counseling was not previously a regular service, 76% expressed a desire for more genetic information. 33% of the group thought that fate was responsible for their cleft, 9% thought environmental factors were involved, 17% indicated heredity was responsible and 41% did not know. When asked their views on recurrence risks, 9% stated there was no risk of recurrence, 39% had no idea, and 16% thought the risk was greater than 50%.

There is a need for ready access to genetic counseling for individuals and families with the more common genetic diseases and birth defects. Referrals should be made directly from the specialty clinic or hospital to the counseling center. Genetic counseling has now been instituted for the Cleft Lip and Palate Clinic and is becoming a part of other specialty clinics as well. For example, the parents of newborns, the affected individual and his or her sibs when they reach high school age all benefit from understanding recurrence risks which may actually be much lower than thought.

The use of health professionals, informed parents' groups and high school workshops will be discussed.

●DECREASED PROTEIN SYNTHESIS IN MUSCLE CULTURES FROM PATIENTS WITH DUCHENNE MUSCULAR DYSTROPHY (MD) (PRELIMINARY REPORT). V.Ionasescu, R.Ionasescu, H.Zellweger. U.Hospitals, Iowa City, IA

Muscle cells were grown from muscle biopsies of 3 patients with early stage of Duchenne MD, 3 patients with myotonic dystrophy and 8 controls matched for age and sex. Primary cultures from trypsin-dissociated myoblasts were grown in Eagle's Minimum Essential Medium (MEM) with 20% calf serum. After 4 weeks, cells were trypsinized, counted and plated in 100 mm dishes at densities of $3 \times 10^5$ cells per dish. The subcultures were grown for 5 days in MEM with 5% horse serum and finally incubated for 4 hours with 10 µCi of L(4,5$^3$H) - leucine (Schwarz-Mann, 50Ci/mmol). Labeled protein from monolayer culture was collected as 5% trichloroacetic acid - insoluble material on glass filters (Whatman, GF/C) and counted in toluene PPO-POPOP. Duplicates of muscle subcultures were supplemented with A23187 ionophore, calcium chloride, or both. Leucine uptake (cpm/dish) showed a significant decrease (½ of control values) in muscle cultures from patients with Duchenne MD and normal values from patients with myotonic dystrophy. Leucine incorporation reflects noncollagen protein synthesis, particularly myosin heavy chain synthesis. Addition of both A23187 and calcium chloride normalized protein synthesis in muscle cultures from patients with Duchenne muscular dystrophy. A 2X increase in protein synthesis was noted in muscle cultures from one case with congenital myotonic dystrophy while no significant changes were noted from the 2 cases with late onset myotonic dystrophy as well as from our normal controls. Our findings suggest that the alteration in protein synthesis in muscle cultures of Duchenne muscular dystrophy might be secondary to a defect in permeability of muscle membrane.

●DEREPRESSION OF ARGININOSUCCINATE SYNTHETASE IN CULTURED LYMPHOBLASTS. J. D. Irr, L. B. Jacoby and R. W. Erbe. Genetics Unit, Massachusetts General Hospital, Boston.

Argininosuccinate synthetase (ASS) and argininosuccinate lyase (AL) are present in normal human lymphoblasts in long-term culture. Deficiencies of these urea cycle enzymes have been documented in patients with citrullinemia and argininosuccinic aciduria, respectively. Normally these two enzymes mediate the conversion of L-citrulline to L-arginine and thus enable lymphoblasts to grow in arginine-deficient Eagle's minimal essential medium supplemented with citrulline. The ASS specific activity in cells grown in citrulline-supplemented medium was 10 times greater than in cells grown in 0.08-0.60 mM arginine, while AL specific activities remained relatively constant under these culture conditions. No comparable difference in ASS activity was found in diploid human fibroblasts grown in the same media. Lymphoblasts growing exponentially in arginine-containing medium lagged for 48 hours after being shifted to arginine-deficient medium. Subsequently the cells grew exponentially and the ASS activity increased 10 fold in about 1.2 generations. The lower ASS activity in the arginine-grown cells was not due to feedback inhibition nor to the presence of inhibitors, nor was it affected by the citrulline concentration. The simplest interpretation of these results is that exogenous arginine represses the synthesis of ASS. Thus, once the regulation of these enzymes has been characterized, lymphoblasts cultured from patients will likely provide a useful means for studying urea cycle disorders. (Supported by NIH grant CA14534 and the National Foundation.)

●REDUCED DISULPHIDE BONDING IN KERATIN FROM TWO SIBS WITH AN AUTOSOMAL RECESSIVE FORM OF ECTODERMAL DYSPLASIA (R.E.D.). S. Isenberg, C.E. Jackson & R.J.M. Gold, MRC Genetics Group, McGill U., Montreal, & Ford Research Institute, Detroit.

Cephalic hair from two sibs with R.E.D. and from two normal subjects was subjected to acid hydrolysis in triplicate. R.E.D. hair yielded 20% less cystine than normal hair. However, on pretreatment with performic acid, which converts both cystine and cysteine to cysteic acid, R.E.D. and normal hair yielded similar amounts of cysteic acid. Combustion analysis showed that R.E.D. and normal hair have similar sulphur contents in agreement with their cysteic acid yields (see Table).

| | CYS (MOLES%) | CYSTEIC (MOLES%) | S BY COMBUSTION % |
|---|---|---|---|
| Normal Hair | 15.3 ± .69 | 16.78±.85 | 4.65±.20 |
| R.E.D. | 12.27±1.26* | 16.9 ±.68 | 4.62±.35 |

* $p < .01$

The amino acid composition of R.E.D. hair was otherwise normal. These results are consistent with a deficit of disulphide bonds in R.E.D. hair. There is, apparently, no change in protein composition as is the case with dominantly inherited E.D. This is the first inherited dysplasia, where loss of disulphide bonding has been found in the absence of other changes of protein composition.

SEX CHROMOSOME MOSAICISM IN TWO SIBLINGS WITH FEMALE PHENOTYPE 45XO/46X, i (Yq) AND 45XO/46XX. H. T. Jackson, I. M. Irwin, P. G. Sullivan, H. M. Pashayan, G. W. Mitchell, Jr. New England Medical Center Hospital, Boston, Massachusetts.

An unmarried 50-year-old phenotypically normal female with a history of primary amenorrhea presented with vaginal bleeding of three days duration. Adenocarcinoma in an endometrial polyp was demonstrated by endometrial curettage. Abdominal surgery revealed a small septate uterus, long Fallopian tubes, and bilateral streak gonads. Histopathology showed hyperplasia of the endometrium with moderate atypicality, and no evidence of malignancy. Streak gonads exhibited ovarian type stroma including Wolffian and Walthard rests. Chromosome studies established sex chromosome mosaicism with one line 45XO, and the other line 46X with an abnormal metacentric chromosome slightly larger than Number 16. Q-banding showed brightly fluorescing areas on the distal ends of this metacentric chromosome with each arm corresponding to the normal pattern of the long arm of the human Y chromosome. G-banding also showed the Y banding pattern on each arm. Buccal smears stained for "F" bodies showed two brightly fluorescing bodies in close proximity, one slightly larger than the other in 95 percent of the cells. Chromosome studies on a 60-year-old married sister, who menstruated but never conceived revealed sex chromosome mosaicism with 45XO/46XX. Chromosome studies on a remaining childless sister, four brothers and the father are in progress.

GILLES DE LA TOURETTE SYNDROME-IS IT INHERITED? A.V. Jayam, J.W. Cann, Howard U., Washington, D. C.

Gilles de la tourette's syndrome is a relatively rare condition characterized by the occurrence of multiple motor tics and explosive involuntary utterances, including both inarticulate noises and articulated obscenities with a mean age of onset at 7 years and a notably consistent course with frequent waxing and waning of symptoms.

A 11-1/2 year old black child with onset of symptoms around the age of 7 years is presented. Though ethnic background, race and religion have been thought to play no part in the incidence or prevalence of the syndrome, in a recent abstract on clinical and genetic observations of 21 families with the syndrome, both parents in 17 families were of similar European ethnic origin (Ashkenazic Jews in 13 families). Moreover, in 20 families, 61 members had one or more symptoms of the syndrome. Only 2 black children with the affliction have been described and one of them had an English mother and a West Indian father. The father of the propositus has had motor tics since early childhood and a paternal uncle is also reported to have a similar problem. At least 2 reports in the literature cite a familial occurrence of the full-blown syndrome; the first, in a father and son and the second, in three cases occurring in the same family involving 2 sisters and the son of one of them. Chromosomal studies done in the latter family were normal. This case is being presented to draw attention to the possibility that this syndrome may have a genetic basis.

HUMAN HGPRT: ASSAY AND PROPERTIES. G.G. Johnson, S. Nash and J.W. Littlefield. Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore.

A simple quantitative assay of human HGPRT (E.C.2.4.2.8.) in unfractionated lysates of human lymphoblasts and fibroblasts can be carried out at pH 10.5 with dithiothreitol. Under these conditions IMP nucleotidase is not active, so that neither inhibitors of nucleotidase nor electrophoresis of the reaction products is required. The activity at pH 10.5 is 2-fold greater than the activity at pH 7.4 and is exclusively due to HGPRT as judged by substrate requirements, undetectable activity in lysates of fibroblasts from a Lesch-Nyhan patient, and heat stability, which follows first-order kinetics during loss of 85% of the initial activity.

Further, at least one property of the HGPRT activity of human red blood cell lysates appears to be different from that of the HGPRT activity of cultured fibroblast or lymphoblast lysates. The ratio of the activity at pH 10.5 to that at pH 7.4 in red cell lysates is regularly about 1.4 while the ratio in fibroblastic or lymphoblastic cell lysates is near 2.1. This difference in relative activity is maintained when red cells are mixed with lymphoblasts prior to lysis or when unfractionated lysates are mixed. Purification of the red cell HGPRT activity approximately 80-fold by DEAE cellulose fractionation to two elution peaks does not alter this activity ratio. Further studies to understand the molecular nature and/or significance of this differential activity are in progress.

THE QUANTITATIVE USE OF TESTS OF SKILL TO DEMONSTRATE THE SPECTRUM OF LATERALITY. R. C. Juberg and C. C. Faust. L.S.U. School of Medicine in Shreveport, Louisiana, and L.S.U. at Eunice, Louisiana.

Our objectives were to assess the utility of selected tests of skill to differentiate lateral orientation and to show that there is a continuous spectrum of laterality from one extreme of right handedness or footedness to the other of the left.

We timed or graded the ability of 217 right handed and 15 left handed white subjects on 5 tests with each hand (grip strength, finger tapping, stereognosis, grooved pegboard, and dowel driving) and 1 test with each foot (tapping) and to write with each hand. There were 128 females and 104 males from 8 to 66 years in 54 families. We expressed the result of each test as proportionate superiority (difference in right and left values/inferior value) with right as plus and left as minus and averaged 4 of the 5 hand tests.

Values for the averaged hand tests ranged from -0.19, indicative of 19% left handed superiority, to +0.36 with mode between +0.161 → +0.200 when the data were grouped by 0.04 intervals. They appeared to be continuously distributed in a normal curve. Handwriting values appeared normally distributed for the right handed. Values for foot tapping seemed normally distributed with mode between +0.001 → +0.050 thus showing considerable left footed superiority but with more scatter than in other tests.

We interpreted these results as showing that laterality is a continuously distributed trait and not an all right or all left phenomenon. In this respect it resembles certain polygenically determined structural, physiological, and mental characteristics.

MONOSOMY FOR SHORT ARM OF CHROMOSOME 20, 46, XX, del (20) (p 11). D. K. Kalousek and S. Therien. Montreal Children's Hospital, Montreal, Canada. In a newborn with multiple congenital anomalies chromosomal analysis employing G - and R - banding techniques revealed deletion of short arm of chromosome 20. Karyotypes of both parents were normal.

Clinically at birth this girl showed dysmorphic facial features, congenital heart disease and multiple skeletal defects. Heart catheterization revealed mitral atresia, single ventricle and pulmonary stenosis. At the age of 10 months height, weight and head circumference were all below the third percentile. She is a fourth child of 30 years old mother and 31 year old father. Both parents and her sibs are in good health.

To our knowledge this is the first reported case of monosomy for short arms of chromosome 20.

PLASMA CHOLESTERYL ESTER FATTY ACIDS IN HUMAN TWINS AND THEIR BROTHERS. K.W. Kang, J.C. Christian, B. Hedges, F.P. Harmath, L.A. Corey and M.M. Evans. Indiana U. School of Med., Indianapolis.

Cholesteryl ester fatty acids were measured in plasma from 64 male twin pairs (28 MZ and 36 DZ) ranging in age from 45-55, and from brothers of these twins, ranging in age from 38-71. The study was designed to partition plasma cholesteryl ester fatty acid variation into genetic and environmental components, and to compare environmental variance in these twin types and brothers.

Gas chromatographic analysis showed that the most common fatty acids of cholesteryl esters were palmitic, palmitoleic, stearic, oleic, linoleic and arachidonic.

There were no significant differences among the means of MZ and DZ twins and their brothers for any of the fatty acids. There was, however, a significant difference in the total variance of MZ and DZ twins and their brothers for four of the six fatty acids. The total variance of the brothers was generally largest and the MZ twins the smallest for most of the fatty acid components, even after adjustment for age differences. There was little evidence for significant genetic variance after adjustment for total variance.

The heterogeneity found in the total variances of MZ and DZ twins has been postulated to be due to environmental effects which exert different influences on the two types of twins. We have found evidence of differences in the environmental influences on twins as well as their brothers, but, in this sample, little evidence of significant genetic variance for cholesteryl ester fatty acids.

SICKLE CELL TRAIT: A CRITICAL EVALUATION. S.H. Katz and M.K. McCormack. W.M. Krogman Center, Children's Hosp. of Philadelphia, Philadelphia, Dept. of Biological Sciences, Rutgers U., New Brunswick, N.J.

Our studies for the past 3 years indicate that significant, subtle differences do exist in children with sickle cell trait which are measurable by carefully executed, well-controlled studies. We have focused on an in-depth evaluation of various hematologic and anthropometric measures of 3 independently ascertained populations of children with sickle cell trait in Philadelphia. The standard red cell hematologic data have been evaluated and our findings indicate significant decreases in hematocrit among females with sickle cell trait. Anthropometric measures taken on sickle cell trait children have included measures of skeletal maturation, physical growth parameters and body composition. The findings from these studies indicate significant delays in measures of biological maturation and decreased measures of body fat.

Thus, although sickle cell trait does not represent a disease entity, heterozygotes for the sickle hemoglobin gene do show detectable differences from normal hemoglobin children.

●INCONSISTENT SPECIFIC 5α-DIHYDROTESTOSTERONE (DHT) BINDING (B) IN HUMAN FIBROBLASTS CULTURED FROM NONGENITAL SKIN. M. Kaufman and L. Pinsky. Cell Genet. Lab., Lady Davis Inst., Depts. of Ped. and Biol., McGill U., Montreal.

We have used Sephadex G-25 chromatography and dextran-coated charcoal adsorption to separate free from specifically bound DHT in the cytosol of confluent fibroblast monolayers exposed to varying concentrations of $H^3$-DHT. High affinity, low capacity ($K_d \simeq 10^{-9}$ Molar$^{-1}$; $B_{max}$ = 15-50 fmole/mg protein) binding has been identified reproducibly in preputial skin fibroblasts derived from 8 normal males of various ages. The binding is stable for 10 min at 37°C but is unstable at 55°C.

Recently Keenan et al. (J. Clin. Endocr. 38, 1143, 1974) have suggested that the absence of B in cultured skin fibroblasts is a predictor of androgen insensitivity and therefore a laboratory aid in the diagnosis of complete testicular feminization (T.F.). Such binding activity was indeed absent in all but one of nine strains derived from both genital and nongenital skin of patients with the clinical diagnosis of complete testicular feminization. However, 11 of 18 strains (9 tested more than once) developed from various nongenital skin sites of normal males and females of various ages also lacked B on at least one occasion. The patient and control strains had attained comparable mean population doubling levels. Whatever is responsible for the inconsistent results in control nongenital strains does not appear to affect preputial strains. In view of our results, the usefulness of this assay as a diagnostic test for complete T.F. in nongenital skin fibroblast strains from male pseudohermaphrodites with unambiguous female genitalia remains to be determined.

●"TURNING-UP" ADULT HEMOGLOBIN SYNTHESIS IN FETAL DEVELOPMENT: ASSOCIATION WITH DECREASING $\gamma/\alpha$ mRNA RATIO. H.H. Kazazian, Jr., A. Silverstein, P.G. Snyder and R.J. Van-Beneden, Johns Hopkins U., Baltimore, Md.

In man the mechanism of switching from $\gamma$ chain to $\beta$ chain production is unknown. By gel electrophoresis and cell-free assay, we have observed a two-fold excess of $\gamma$ mRNA in the 13-20 week fetus. In contrast, at 32 weeks when adult hemoglobin (Hb) synthesis is "turned-up," the $\gamma/\alpha$ mRNA ratio $\sim 1$. To further study the relationship of the changing $\gamma/\alpha$ mRNA ratio with increased $\beta$ synthesis, we obtained blood from fetal sheep at various times of gestation and determined in reticulocytes 1) adult to fetal Hb synthesis ratio, 2) $\gamma/\alpha$ synthesis ratio, and 3) $\gamma/\alpha$ mRNA ratio on polysomes ($\gamma$ and $\alpha$ mRNAs of sheep can be separated by formamide electrophoresis). We find 1) a decrease in $\gamma/\alpha$ mRNA ratio from 2 at 80 days to 1 at 98 days of gestation, 2) the "turning-up" of adult Hb synthesis begins at 98 days, and 3) $\gamma/\alpha$ synthesis ratio approximates the $\gamma/\alpha$ mRNA ratio at all times, i.e., $\gamma$ synthesis exceeds $\alpha$ synthesis at 80 days. Two sheep were phlebotomized (1/3 of blood volume removed) at 81, 90, and 97 days, and sacrificed at 105 days. Premature "turning-up" of adult Hb synthesis was definitely observed in one fetus following phlebotomy. However, a premature decrease in the $\gamma/\alpha$ mRNA ratio was not demonstrable in these fetuses. We conclude that "turning-up" of $\beta$ synthesis 1) is associated temporally in man and sheep with a decrease in the $\gamma/\alpha$ mRNA ratio (perhaps by decreased $\gamma$ mRNA transcription) and 2) can be manipulated in the fetal sheep.

●THE ROLE OF THE INTAKE INTERVIEW IN GENETIC COUNSELING. P. T. Kelly. U. of California, San Francisco and Berkeley.

Comprehension and credibility are essential factors in the genetic counseling process. Without comprehension, families cannot make informed decisions. Without credibility, families can believe information is being withheld from them, is incorrect, or is being presented in a distorted manner. In a recent study, open-ended interviews were held with 19 couples and 5 individuals selected at random from the University of California, San Francisco Birth Defects Center (genetics clinic). These interviews have elucidated some of the factors which can decrease comprehension and credibility during a genetic counseling session: (1) lack of discussion about issues often considered outside the realm of traditional genetic counseling (e.g. prognosis, care and cure), (2) the family's emotional response to the problem and to genetic counseling (e.g. guilt, blaming, embarrassment, poor self-image, and mistrust due to previous unpleasant experiences with the medical community), and (3) lack of background knowledge about genetics, medicine and biology (e.g. about meiosis and probability). We have found that an intake interview during which people are encouraged to raise their non-genetic concerns, helped to express their feelings about the genetic disease, and given a general framework into which they can fit information given during a counseling session enables families to progress more smoothly to the intellectual and informational aspects of genetic counseling. Intake interviews have been successfully conducted by Ph. D. geneticists, Genetic Advising students, and a public health nurse.

EXPRESSION OF A GENETIC DEFECT BY FIBROBLASTS INITIATED FROM SKIN FROZEN FOR FIVE YEARS. H. Kihara, A. L. Fluharty, R. T. Miller, S. D. de la Flor and R. L. Stevens. Neuropsychiatric Institute-Pacific State Hospital Research Group, Pomona, California.

A deficiency in L-α-iduronidase was observed in cultured fibroblasts initiated from tissue stored in liquid nitrogen for five years. Skin obtained at autopsy of a patient with mucopolysaccharidosis I was sectioned into explant-size fragments. A portion was used directly to initiate a fibroblast culture. The remainder was suspended in growth medium containing 10% dimethylsulfoxide, frozen, and stored in liquid nitrogen. After five years the frozen tissue was retrieved and a second fibroblast culture was initiated. The "direct" and "delayed" cultures were compared morphologically, cytogenetically and biochemically. They were indistinguishable by these criteria. Both strains grew like typical fibroblasts but the cells were somewhat larger than normal cells; both were diploid; and both were deficient in L-α-iduronidase and normal in other lysosomal hydrolases. These observations demonstrate that initiation of cultures for biochemical investigations can be deferred by freezing tissue. Initiating cultures as tissue becomes available from all patients with genetic disorders, characterized or suspected, is not always feasible. The practice of freezing such tissue will preserve the option for tissue culture studies in the future. (Supported in part by NIH Grants HD-4331, NS-11665 and HD-4612.)

●CYTOGENETIC MONITORING OF CHEMICAL INDUSTRY POPULATIONS. D. J. Kilian and D. J. Picciano. Texas Division, Dow Chemical U.S.A., Freeport, Texas.

Cytogenetic monitoring of suitable industrial populations is both feasible and desirable for the protection of humans exposed to possible genetic hazards. The Texas Division of Dow Chemical U.S.A. started human cytogenetic evaluation studies more than ten years ago and has accumulated findings on more than 69,000 shorthand and conventional karyotypes from over 3000 individuals. Persons being considered for employment are given a pre-placement examination that includes chromosomal analysis. Medical and cytogenetic evaluations are repeated at intervals determined by the worker's physical condition and job requirements. This procedure allows us to obtain a reference point for future clinical investigation of the individual and permits the accumulation of a large pool from which to draw control subjects. For individual studies, the person serves as his own control. As one would expect, cytogenetic analysis of a large population will occasionally turn up individuals with heritable chromosomal abnormalities that have reproductive implications. Automated equipment and electronic data processing techniques are being developed for use in the metaphase location and data record phases of the monitoring process. Computer assistance is expected to reduce time-cost expenditure; more sophisticated and intensive utilization of computer technology is planned. Although there are limitations to present day cytogenetic techniques, the human-orientation of this approach makes its findings more valuable than those of animal or bacterial systems. The greatest need in the field of cytogenetic studies is for comprehensive, large scale surveys of general population groups.

A CASE REPORT OF CEREBRO-TENDINO-XANTHOMATOSIS. D. S. Kim and F. J. Detterbeck. Hillcrest Center, Howell, Michigan.

This is to present a case of cerebro-tendino-xanthomatosis. The patient is a 50-year old man, diagnosed clinically, with one sibling (sister) who has had the same diagnosis suggested on review of medical record.
This disease is inherited as autosomal recessive pattern and only a dozen of cases have been reported in the literature. The main clinical findings are: juvenile cataract, cerebellar ataxia in adolescence, mental deficiency which is slowly progressive, and xanthomas on the major tendons, with normal serum cholesterol level.
We would like to present clinical features of this patient and life long medical history.

PRENATAL DETECTION OF PERICENTRIC INVERSION OF CHROMOSOME 11. H.J. Kim, L.Y.F. Hsu, and K. Hirschhorn. Department of Pediatrics, Mount Sinai School of Medicine of CUNY and Beth Israel Medical Center, New York.

The principal indication for prenatal cytogenetic diagnosis has been to detect numerical chromosome aberrations. Thus far the majority of cases have fallen into two groups: advanced maternal age and women who have had a child with Down's syndrome. Since 1975 we have applied Q and/or G banding to every case of prenatal cytogenetic diagnosis, and have identified two cases of pericentric inversion of No. 11. The indications for both cases were advanced maternal age. The first case was a de novo pericentric inversion of No. 11 and thus gave us difficulty in predicting its outcome. The baby was born and was found to be phenotypically normal. The inversion in the second case was familial. It was only identifiable by banding karyotypes since the chromosome segments involved in this pericentric inversion were almost identical in size, resulting in an unchanged arm ratio. The same inversion was found in the phenotypically normal father and two siblings. It appears that this inversion is compatible with a normal phenotype. This couple decided to continue the pregnancy; the baby is not yet born. We believe that banding studies are necessary in order to detect all types of structural aberrations. While these two cases fortunately showed balanced types of chromosomal aberrations, an unbalanced chromosomal aberration may have been missed without banding studies. The latter abnormality could have arisen from parents with balanced chromosomal aberrations and is associated with either congenital abnormalities or fetal wastage.

●HAIRBULB TYROSINASE ACTIVITY IN OCULOCUTANEOUS ALBINISM. R. A. King, and C. J. Witkop, Jr. U. of Minnesota, Minneapolis.

A method has been developed that allows tyrosinase activity in single plucked anagen hairbulbs to be quantitated, based on the production of $^{3}HOH$ from the oxidation of L-tyrosine-3,5-$^{3}H$ to L-dopa-5-$^{3}H$, and expressed as pmoles of tyrosine oxidized in 2 hours. A mean hairbulb tyrosinase activity can be determined for an individual from a sample of hairbulbs. Two tyrosinase-negative oculocutaneous albinos had no tyrosinase activity in their hairbulbs, a finding consistent with the suggestion that tyrosinase-negative albinism is analogous to c/c albino animals that have been shown to lack tyrosinase. Five tyrosinase-positive oculocutaneous albinos could be divided into two groups, those with high enzyme activity (mean = 4.742 pmoles tyrosine oxidized at 2 hours) and those with moderate enzyme activity (mean = 1.837 pmoles tyrosine oxidized at 2 hours). The mean hairbulb value for each of the three with high activity was greater than that found for any normal control with brown, black, blond or red hair. The mean hairbulb value for the two with moderate activity was similar to levels found in brown hair.

●ANTENATAL DIAGNOSIS: EFFECTS OF PARTIAL REPRODUCTIVE COMPENSATION, OVER-COMPENSATION, AND PRECOCITY, ON FUTURE GENE FREQUENCIES. H.N. Kirkman. U. of North Carolina, Chapel Hill.

Concern has been expressed about the prevalence of harmful genes in future generations if reproductive compensation or precocity accompany the increasing availability of antenatal diagnosis and selective abortion. Earlier workers have estimated the effects of complete replacement, through reproductive compensation, when abortion of the affected fetus occurs for autosomal and X-linked recessive disorders, or when abortion of all males occurs for the latter problem. The present work led to derivation of general equations allowing prediction of future gene frequencies for any degree of reproductive compensation, C. These allow the effect of partial compensation, or over-compensation, to be examined. Precocity (decreased maternal age) may be unlikely to result from the availability of antenatal diagnosis; moreover, its effect is negligible when the human population size is stable or expanding at only a moderate rate. A very small and transient rise in gene frequency occurs, in a manner analogous to the Doppler effect, while the maternal age is falling.

A LARGE FAMILY WITH MYOCLONIC EPILEPSY IN A SWISS ISOLATE. D. Klein and T. Rabinowicz. Institute of medical Genetics, University of Geneva, and Neuropathologic Division, University of Lausanne.

The authors have investigated a large family with myoclonic epilepsy from upper Valais, where 13 patients within five sibships, four of which issuing from the same ancestor, who lived at the beginning of the 18th century, are affected. Familial occurrence and parental consanguinity clearly indicate a recessive transmission of the disease.

The post-mortem examination of the first patient did not reveal classical Lafora bodies. However, small corpuscles were found in the neuropil, showing a rosette structure by PAS-reaction. Furthermore, PAS-positive deposits (mucopolysaccharides) were found in several visceral organs. (D. Klein et al., Humangenetik 6, 237-252, 1968).

Since this first description, pathological examination of two other patients revealed the same distribution of mucopolysaccharides and absence of Lafora bodies. The proposed name "acorpuscular form" for this type of myoclonic epilepsy thus seems justified.

●THE EFFECT OF MAJOR GENES ON THE HUMAN DENTITION. D. Kolakowski and H. Bailit. U. of Connecticut, Storrs.

Nearly all the studies so far which purport to demonstrate some genetic involvement in human dental traits are to some extent controversial. The argument for a genetic component usually rests solely on the finding of a non-zero heritability index, which results in little understanding of the causes of individual and population differences even if the underlying assumptions can be assumed to be valid. Mendelian models, on the other hand, can tell us how genetic variation causes phenotypic variation; i.e. what the mode of inheritance is and what average metrical deviation is associated with the allelic genes at the relevant loci.

The present research effort has sought to implement Mendelian models by means of pedigree analysis and the detection of linkage relationships between genetic marker loci and the cites of qualitative variance in the dentition. The data consist in dental casts, serology and demographic information for approximately 4,000 Solomon Islanders among whom genetic microdifferentiation has already been documented. The indications of major-gene influence within and among subpopulations are discussed and the presence of linkage to the available marker loci is investigated. Finally, the usefulness and explanatory value of quasi-Mendelian models for dental data is discussed and compared to similar analyses of human behavioral and physiological measures.

●IDENTIFICATION OF MALE SPECIFIC REITERATED DNA IN MAN. L. M. Kunkel, K. D. Smith and S. H. Boyer. Johns Hopkins U., Baltimore, Maryland.

Reassociation of radiolabelled human-XY DNA in the presence of vast excess of unlabelled human-XX DNA has led to the identification of reiterated DNA specific for the Y chromosome.

Labelled DNA was prepared from continuously labelled lymphocyte cultures or by nick translation of isolated DNA utilizing DNA polymerase. Labelled reiterated sequences were prepared by self reassociation to Cot 46 and then collection on hydroxylapatite. These sequences were reassociated with a 1000 to 10,000 fold excess of XX DNA. The sequences which failed to reassociate were again reassociated with an excess of XX DNA.

The DNA unreassociated after two exposures to vast excesses of XX DNA reassociates specifically with human-XY DNA. It does not reassociate with human-XX or gorilla-XY DNA and is thus specific for the human Y chromosome. Y specific DNA reassociates with excess XY DNA between cots 0.46 and 100 indicating a complex group of sequences whose of reiteration frequencies ranges between 3,000 and 300 copies per genome.

The Y specific DNA isolated in this manner represents 5 to 10 percent of the Y chromosome. The amount of Y specific DNA isolatable from lymphocytes of individuals with increasing numbers of Y chromosomes (XY, XYY, XYYY) follows a linear relationship with Y dose.

Self reassociation of the Y specific sequences yields duplexes which are highly stable and resistant to single strand specific nuclease. The formation of extensively paired duplexes and the failure to reassociate with gorilla DNA suggests that these Y specific sequences are a recent addition to the human genome.

CHROMOSOME ABNORMALITIES AND SISTER CHROMATID EXCHANGES (S.C.E.) IN SENESCENCE. Y. Lacassie, W. Fu, and D. S. Borgaonkar. Johns Hopkins U., Baltimore, Maryland.

Numerical and structural chromosomal abnormalities, using standard staining techniques, have been reported in older people. With the differential sister chromatid staining techniques a high frequency of S.C.E. has been described in Bloom syndrome, a genetic disorder predisposing to chromosome instability and cancer.

To test the hypothesis whether senescence could be associated with a defective DNA repairing system leading to chromosomal instability, we studied the chromosomes of six "healthy" persons, ranging in age from 86 to 91 years.

Preliminary results show that the frequency of sister chromatid exchanges (S.C.E.) are not increased when compared to young controls. Characteristics of aneuploid cells will be presented and discussed.

●THE EXPRESSION OF HEXOSAMINIDASE A DEPENDS ON THE PRESENCE OF HEXOSAMANIDASE B. P. A. Lalley and T. B. Shows. Roswell Park Memorial Institute, Buffalo, New York

The genetic relationships of the two major molecular forms of hexosaminidase (Hex A and Hex B) have been questioned. This question is important for understanding hexosaminidase deficiencies associated with Tay-Sachs and Sandhoff diseases. A gene coding for Hex B has been assigned to chromosome 5, and genes coding for Hex A, mannosephosphate isomerase (MPI) and pyruvate kinase-3 (PK-3) have been assigned to chromosome 15. The genetic relationship of Hex A and Hex B was investigated by employing man-rodent cell hybrids, utilizing human cells which possessed an X/5 or an X/15 translocation. The HAT/8-azaguanine selection system was employed to select for or against the translocations. Improved procedures increased the resolution of Hex A and Hex B in hybrid cells. In 131 control hybrids from eleven hybrid sets utilizing 5 different human parental cells, 51 clones were Hex $A^+$/Hex $B^+$; 21 were Hex $A^-$/Hex $B^+$; and 59 were Hex $A^-$/Hex $B^-$. Hex $A^+$/Hex $B^-$ hybrids were not observed. Eleven clones of the Hex $A^-$/Hex $B^-$ phenotype were $MPI^+$/$PK\text{-}3^+$ indicating that the chromosome carrying the Hex A gene was present, but that Hex A was not expressed in the absence of Hex B. All primary clones from the X/5 translocation hybrids grown in HAT selection media expressed Hex B together with the translocated X-linked genes HPRT (required for growth) and G6PD confirming the assignment of the Hex B gene to chromosome 5. Clones which also expressed Hex A, MPI and PK-3 were counterselected in the presence of 8-azaguanine. Eight clones lost the X-linked markers, Hex B and Hex A, but continued to express MPI and PK-3. These results suggest that Hex A expression depends on the presence of Hex B. Reciprocal experiments utilizing the X/15 translocation further confirmed these results.

●**CALCULATION OF GENETIC VARIANCE COMPONENTS FROM PEDIGREE DATA.** **K. Lange, M. A. Spence, and J. Westlake. University of California, Los Angeles.**

**The classical variance components for simple polygenic traits--additive, dominance, and environmental variance--have traditionally been estimated from sample covariances between first-degree relatives. If data is gathered on pedigrees, this statistical procedure wastes information. Recently Elston and Stewart suggested an alternative likelihood procedure that uses all the information in a set of pedigrees. A refinement of their method, based on the scoring technique, gives rapidly converging maximum likelihood estimates of the variance components and of the male and female means. Tests of statistical hypotheses about the various parameters can then be made by the likelihood ratio method. Furthermore, using classical regression analysis, the estimated parameter values allow prediction of unknown trait values from known trait values within a pedigree. These methods should apply to traits like total finger ridge count and activity levels for certain enzymes. Since the model postulates independent environmental effects and no assortative mating, its utility in human behavior genetics seems limited.**

INTER- AND INTRA- HOMOGLOGUE VARIATION IN THE DNA REPLICATION PATTERNS OF HUMAN AUTOSOMES. J.T. Lanman Jr., and C.G. Palmer*. Indiana U. School of Medicine, Indianapolis.

The terminal S-phase DNA replication patterns in the p and q arms of all 22 pairs of autosomes were examined using a technique of sequential autoradiography and trypsin banding. Analysis of a polymorphic pair of chromosome 16's indicated that there were no significant differences between the two homologues either in time of cessation of DNA synthesis or in the total amount of $^{3}$H-TdR incorporated; however there was significant heterogeneity among cells in the proportion of $^{3}$H-TdR incorporated into each of the two homologues at the end of S-phase. This suggests that, while on the average the two homologues replicate at the same time, they do exhibit different degrees of asynchronous DNA replication which change significantly from cell to cell.

Control of terminal S-phase DNA replication at the chromosomal level was demonstrated by comparing the kinetics of DNA synthesis in the p and q arms of homologous chromosomes. The comparisons were made in two ways. (1) The within cell variances of the differences in grain count between the p and q arms of the same chromosome were significantly less than the within cell variances of the differences in grain count between the p arm of one homologue and the q arm of the other homologue. (2) The frequency of homologous chromosome pairs where the p and q arms of the same homologue were the latest replicating p and q arms was significantly greater than the frequency of homologous chromosome pairs where the latest replicating p and q arms were on separate homologues. These results indicate there is a measurable amount of variation in DNA synthesis at the chromosome level which contributes to differences in DNA synthetic activity observed between homologues. Supported by PHS GM 1056 and P01 GM 21054

PRENATAL GENETIC DIAGNOSIS (PGD) IN NORTH AMERICA - RESULTS OF A 1974 SURVEY. M.D. Levine, M.M.Kaback and C. Griffin. UCLA - Harbor General Hospital, Torrance, California

A 20 question survey was developed to assess the structure of 2nd trimester prenatal genetic diagnostic centers, the extent of their experience, and the ancillary use of and belief in the efficacy of pre-amniocentesis ultrasound through 1974. These data represent the experience of 55 centers who perform PGD in continental N. America. On average, centers have fewer than 3 years experience and process fewer than 50 fluids a year. 69.5% of fluids are obtained within the center. In most centers, amniocenteses are performed by 1 or 2 experienced physicians. Despite the continued growth in the total number of centers involved (over 55) and in the number of amniocenteses (over 2600) in 1974, the data indicate that the rate of this growth may be decreasing and may soon plateau. Ultrasound is not used by 19 centers usually because of equipment lack. In 35 centers, ultrasound is used for placental localization, determination of fetal number, site for amniocentesis, and fetal age, and detection of fetal neural tube abnormalities. The more experienced the center is with ultrasound, the more likely the center is to believe that ultrasound a) makes amniocentesis safer for mother and fetus and b) reduces the frequency of bloody taps. Centers were asked their frequency of "bloody taps" defined as "the unspun fluid is at least blood tinged". The reported frequency of bloody taps is not reduced by pre-amniocentesis ultrasound. Furthermore, there is a peculiar distribution of ultrasonically located anterior placentas: 23% of centers found < 20% anterior; 12% of centers found > 60% anterior. These findings suggest that placental localization may frequently be incorrect. Two conclusions are drawn. 1) Prospective studies need to be continued to assess the usefulness, limitations, and complications of ultrasound. 2) The potential availability of PGD may be far less than what future needs may require.

● THE MEASUREMENT OF CROSS-REACTIVE MATERIAL (CRM) IN CELL EXTRACTS FROM PATIENTS WITH HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE (HGPRT) DEFICIENCY. A. Leyva, K. S. Upchurch, and W. N. Kelley. Duke U., Durham, N. C.

Hemolysates and extracts of cultured cells from a number of patients with a genetic deficiency of HGPRT activity were examined immunologically for the presence of material which cross-reacts with the normal enzyme. The quantitative measurement of CRM in HGPRT deficient hemolysates was performed using an immunoprecipitation-inhibition assay. Only one out of 14 hemolysates examined was found to have a normal level of CRM, while the remainder had less than 3% of normal CRM. It has been previously reported that the HGPRT of the patient with the normal level of CRM has altered kinetic properties which suggests a structural gene mutation. This provides evidence indicating that at least in one patient there is a normal level of enzyme protein, however, structurally and catalytically defective. With some modification of the CRM assay it was possible to analyze extracts of cultured cells derived from patients with HGPRT deficiency. Three fibroblast cell strains and two lymphoblast cell strains, derived from 5 different patients, were found to have less than 5% of normal CRM. Extracts from the two lymphoblast cell strains contained sufficient enzyme activity to perform a direct immunoprecipitation assay for CRM. HGPRT activity of only one of the two cell strains could be precipitated by anti-HGPRT serum. The markedly reduced or undetectable levels of CRM found in most of the patients examined suggests that there may be 1) a reduction in the concentration of HGPRT protein or 2) the presence of structurally defective enzyme molecules with altered antigenic sites. This study provides evidence that either of these possibilities may exist.

●IDURONATE SULFATASE DEFICIENCY IN SERUM AND LYMPHOCYTES OF HUNTER PATIENTS. I. Liebaers and E. F. Neufeld, NIAMDD, National Institutes of Health, Bethesda, Maryland 20014.

The Hunter syndrome (the only mucopolysaccharidosis which is X-linked) results from a deficiency of iduronate sulfatase. The biochemical test presently used ($^{35}$S-mucopolysaccharide accumulation and correction in cultured fibroblasts) is an indirect measure of iduronate sulfatase activity. We have now developed a direct assay for the enzyme in serum and lymphocytes. The substrate is a disulfated disaccharide composed of a sulfated iduronic acid residue linked to sulfated anhydrommanitol-($^{3}$H) (Lim et al., Carbohyd. Res. 37, 103, 1974). The iduronate sulfatase produces a radioactive monosulfated disaccharide, from which radioactive sulfated anhydrommanitol is released by the iduronidase present in lymphocyte homogenates (but not in serum). Substrate and products are separated by high voltage paper electrophoresis and counted. The simplicity and speed of the serum assay for iduronate sulfatase make it the method of choice for the differential diagnosis of the Hunter syndrome.

Iduronate Sulfatase Activity (nmoles/mg protein/24 hr)

| | SERUM | | LYMPHOCYTES | |
|---|---|---|---|---|
| | n | Mean (range) | n | Mean (range) |
| Hunter | 7 | 0.1 (0-0.3) | 5 | 1 (0.7-1.2) |
| Normal | 16 | 9.4 (6.5-14) | 16 | 42 (18-112) |
| MPS I, III | 5 | 8.3 (3.9-17) | 3 | 57 (42-83) |
| Mucolipidosis II, III | 5 | 280 (100-470) | 1 | 27 |

DIFFERENTIAL FLUORESCENT STAINING OF HUMAN CHROMOSOMES WITH DAUNOMYCIN AND ADRIAMYCIN - THE D-BANDS. C.C. Lin and H. van de Sande. U. of Calgary, Calgary, Alberta, Canada.

Human chromosome preparations were treated with a group of anthracycline antibiotics. Well-defined, orange-red fluorescent bands were observed on chromosomes after the slide was stained with daunomycin and adriamycin but not with nogalamycin. The characteristic differential bands appeared to be similar to the banding patterns obtained by the quinacrine techniques. However, these bands (D-bands) appeared to be more stable than the Q-bands and may have some usefulness for routine clinical cytogenetic analysis.

CLINICAL AND CYTOGENETIC FINDINGS OF THREE PARTIAL TRISOMIES.
L.H. Lockhart and J. Meyne. U. of Texas Medical Branch, Galveston.

During a two year chromosome banding study of infants with multiple congenital anomalies, three new partial trisomies were identified. One child had a partial duplication of 8q and a partial deletion of 8p due to a "de novo" translocation, t(8;8)(p21;q13). A second "de novo" translocation, t(9;20)(q21;p11), resulted in partial trisomy for 9q and partial deletion of 20p. This child also had a paternally transmitted inversion of the centromeric region of one No. 9 chromosome. A third child was partially trisomic for 3q and had a partial deletion of 4q derived from segregation of a paternal translocation, t(3;4)(q25;q33). All three showed delayed mental and physical development and unusual facies. The specific clinical findings in each case aid in delineation of these new partial trisomy syndromes.

CHROMOSOME ABERRATIONS IN LIVEBIRTHS OF EAST TENNESSEE. C.B. Lozzio. U. Tennessee Memorial Hospital, Knoxville, Tn. 37920.

The number of liveborn births in 16 counties of East Tennessee during 5 years (1970-74) was compared to the number of cases with chromosome aberrations found among the infants referred to us for chromosome analyses. An incidence of 0.09% for all autosomal trisomies and 0.08% for trisomy 21 was estimated for this population. This represents 75% of the frequencies obtained in surveys of consecutive newborn (P.A. Jacobs, Human Genetics, 1974, p. 232). These results suggest that a high proportion of the cases were accurately diagnosed clinically and referred early to our clinic. Another likely possibility is that a high incidence of autosomal trisomies is present in this population since 78% of the cases with nondisjunction type of trisomy 21 had mothers younger than 35 years. A total of 46 cases with trisomy 21 were observed among 57,240 liveborn infants (1 in 1244) and 36 of them had a mother with a mean age of 23 years (1 in 1590). The distribution of these cases with young mothers was also remarkable. Seventeen cases were found in four counties with 12,432 livebirths. This represents a very high incidence of 1 in 731 for a group with low maternal age. Fifteen cases were observed in 3 counties with 24,226 livebirths (1 in 1615) and only 4 cases were referred from 9 other counties with 20,582 livebirths (1 in 5545). During the same period five cases of trisomy 18, two of trisomy 13, two cases of Turner's syndrome, two with triple X and 8 unbalanced structural rearrangements were diagnosed among children born in the 16 counties. The influence of genetics and/or environmental factors in the incidence of chromosomal aberrations is under investigation with detailed computerized prenatal and family history. Tentatively, it is suggested that regional differences in the frequency of autosomal trisomies might be related to the incidence in the population of genes increasing the rate of nondisjunction.

MEDITERRANEAN ORIGIN OF LONG Y CHROMOSOMES IN CAUCASIANS. H. Lubs and S. Patil. U. of Colorado Medical Center, Denver.

Since European surnames mimic Y-linked inheritance, the country of origin of each Y chromosome can be determined in a high proportion of newborns by classifying each surname as Italian, French, English, etc. In the sample of 2,183 consecutive New Haven male newborn infants the length of the Y chromosome was first determined by visual comparisons with F group chromosomes in the same cell. In all children with a presumptive long Y chromosome and several other subsamples 5-10 cells were measured with X,Y digitizer. A Y/F index $\geq$1.0 was defined as a long Y chromosome. Of the 401 children whose Y/F index was determined, 48 were $\geq$1.0 (2.2%). Since most Caucasian Protestants were of northern European origin and the largest group of Caucasian Catholics of southern European origin, determination of the frequency of long Y chromosomes in various religious groups gives an indication of geographic differences in respect to the Y chromosome. One percent of Protestant, 2.6% of Catholic and 3.6% of Jewish male infants had long Y chromosomes. The 959 Catholic infants were then subclassified by surname according to the probable country of origin. The frequencies of long Y chromosomes were as follows: Spanish 6% (Puerto Rican parents were excluded), Italian 4%, French 1.2%, German 1.2%, English, Scotish, Scandinavian 0.6%, Irish, Polish, Czech and unknown 0%. Although 45% of the Catholic population were Italian, 80% of the long Y's came from Italian families. Since the highest frequencies of long Y's were found in those of Jewish, Italian and Spanish origin and the lowest in those of northern European origin it seems likely that the long Y chromosome is of Mediterranean origin and that there is a gradual south to north decreasing cline.

CYTOGENETIC ANALYSIS OF PREIMPLANTATION EMBRYOS FROM XO MICE. F. M. Luthardt. U. of California, Los Angeles.

In mammals the absence of an X chromosome is lethal in males and results in increased prenatal death in females. No cytogenetic data is available on the survival capacity of OY and XO embryos or the cleavage stage at which the presence of at least one X chromosome is mandatory for survival. These questions were examined by analyzing metaphase chromosomes of embryos from XO females during first and second cleavage stages. The XO status of females was established by either presence or absence of the X-linked tabby (Ta) phenotype and confirmed by chromosome counts from corneal epithelial preparations.

Second meiotic metaphase chromosome counts obtained from cultured ovarian oocytes indicated that the X chromosome in XO females remained preferentially in the oocyte subsequent to the first meiotic division confirming previous results. The sex ratio observed during first cleavage metaphase in embryos from XO females was not significantly different from the control sex ratio. Individual cleavage metaphase chromosomes were identified on the basis of their trypsin-giemsa banding pattern and morphology. Several XO and OY embryos were observed at first cleavage metaphase suggesting that the absence of one X chromosome, at least in one-cell embryos, is not detrimental. Since some XO embryos survive to birth these results are not surprising. Analysis of two-cell embryos must be completed to determine the fate of OY embryos during second cleavage.

BREAST CANCER GENETICS: FAMILIAL TUMOR ASSOCIATIONS. H.T. Lynch, H.A. Guirgis, F.D. Brodkey, G. Mulcahy, P. Lynch, J. Lynch, K. Maloney, L. Rankin & R. Thomas. Creighton U., Omaha, Neb.

Until very recently, few studies in cancer genetics dealt with extensive pedigrees wherein cancer of all anatomic sites were meticulously documented. Tumor associated patterns in certain breast cancer families have now been identified (Lynch, et al. JAMA 22:1631, 1972), in addition to families showing predominant site specific occurrences of this disease. Tumor associations with breast cancer include: (1) carcinoma of the gastrointestinal tract; (2) carcinoma of the ovary and endometrium; (3) sarcoma, leukemia, and brain tumors; (4) multiple hamartoma syndrome (Cowden's disease) (Weary, et al. Arch. Derm. 106:682, 1972).

We have analyzed cancer of all anatomic sites in our clinical resource comprised of 52 breast cancer prone families. Approximately 45% of these families demonstrated associated malignancies. These families showed a statistically significant increase in the prevalence of those associated tumors when compared with the New York State Tumor Registry. The particular patterns in those families suggest that multiple genotypes are responsible for the diversity of cancer, observations consistent with genetic heterogeneity.

One example of familial breast cancer associated genotypes is the association of breast and ovary cancers. Our data show a statistically significant increase in the age specific incidence of cancer of the breast and ovary ($p<0.001$) when compared with New York State Statistics. Another example namely including dominantly inherited breast cancer in association with sarcoma, leukemia, and brain tumors has shown interesting pathology findings: prominance of multinucleated tumor cells with rather distinct cell borders; large amounts of eosinophilic cytoplasm in relation to nuclear size, and variably sized and generally sparse nuclear and/or cytoplasmic inclusion. Supported by Contract No. NO1-CB-3901.

PARTIAL TRISOMY 18 (47,XX,18q- or 18pi) ASSOCIATED WITH DEVELOPMENTAL RETARDATION AND MULTIPLE CONGENITAL ANOMALIES. C.C. Mabry, L. Smith, K. Schlich, and D.M. Goldenberg. Depts. Ped. and Path., U. Kentucky, Lexington.

Cases with a supernumerary small, non-satellited, metacentric chromosome have been described, and the phenotype in some has been considered a discrete genetic syndrome. We have studied a 2-year-old female with mental retardation (DQ 35), multiple congenital anomalies, and a supernumerary small metacentric, non-satellited, chromosome. The physical anomalies included microcephaly, asymmetrical head, low set, malformed ears, short palpebral fissures, strabismus, corneal stromal opacity, and slender fingers and toes. Chromosome preparations of blood lymphocytes and skin fibroblasts were made by the trypsin-Giemsa banding technique, and revealed that this extra chromosome, which is smaller than chromosomes no. 19 or 20, is either a no. 18 with a portion of the long arms deleted (18q-) or an isochromosome of the short arms of no. 18 (18pi). Chromosome studies of both parents did not reveal any abnormalities. All 3 siblings are normal, and there is no history of fetal wastage.

It appears that this case represents a partial trisomy 18, yet the clinical abnormalities seen are quite dissimilar to those of complete trisomy 18 patients, since the child has survived considerably longer than in such cases. This suggests that an entire chromosome 18, or at least the long arms of the supernumerary 18 chromosome, is required for the manifestations of the traditional trisomy 18 syndrome.

●ASSIGNMENT OF PYROPHOSPHATASE GENE LOCUS (PPT) TO CHROMOSOME 10 AND PEPTIDASE D GENE LOCUS (PEP D) TO CHROMOSOME 19 IN MAN. P.J. McAlpine, T. Mohandas, M. Ray, and J.L. Hamerton. Health Sciences Children's Centre and U. of Manitoba, Winnipeg.

The correlation of the presence of precisely identified human chromosomes, or parts of these chromosomes, with the expression of human gene loci in human-rodent somatic cell hybrids has been widely used in studies designed to map the human genome. Using such an approach, we have obtained data that permit the tentative assignments of the pyrophosphatase gene locus (PPT) to chromosome 10 and the Peptidase D locus (Pep D) to chromosome 19 in man.

●COMPLEX ALPHA THALASSEMIA: A CAUSE OF NEONATAL NORMOBLASTEMIA. M.K. McCormack,* G.R. Geller, S.J. Zak, D.P. Tukey and W. Krivit. Dept. of Peds., U. of Minn., Minneapolis, and *Dept. of Biological Sciences, Rutgers U., New Brunswick, N.J.

A new syndrome with hypochromic erythrocytes and marked normoblastemia during the newborn period has been characterized. One male (proband), 2 female siblings and a first cousin had a marked normoblastemia (300-900 normoblasts/100 WBC) at birth. Globin chain synthesis studies on peripheral blood of the father, the proband, and two (2) affected full-siblings indicated depressed α globin chain synthesis as evidenced by calculated globin chain ratios (α/β = .45-.54).

The globin chain synthesis studies done during the newborn period on the proband showed depressed γ chain synthesis as well as depressed α chain synthesis. Other supporting evidence for the γ-thalassemia trait are: (1) no hemoglobin Bart's ($\gamma_4$) present in the newborn period and (2) low percentage of fetal hemoglobin in the newborn period.

Pedigree analysis indicates this complex α thalassemia is inherited as a single autosomal dominant trait. The unique features of this condition are: (1) marked neonatal normoblastemia; (2) lower α/β synthetic ratios; (3) no hemoglobin H present and (4) depressed γ chain synthesis in the neonatal periods. This suggests this syndrome as a new complex thalassemia condition which is best characterized as α-γ thalassemia.

●APPARENT DUPLICATION OF THE β-CHAIN GENE IN MAN. P. R. McCurdy, J. Fox and W. Moo-Penn. Georgetown Medical Division, D.C. General Hospital, Washington Regional Blood Program, ARC, Dept. Pathology, Providence Hospital, Washington, D.C.; Hematology Division, Center for Disease Control, Atlanta, Georgia.

A 25-year-old black woman was discovered to have three distinct electrophoretic hemoglobin bands migrating in the positions of Hb A, J and N on cellulose acetate (pH 8.6), and two bands between Hb A and F and one at Hb A on agar gel (pH 6.2). Globin chain electrophoresis in a urea buffer system and Clegg column analyses show that there are two abnormal β-chains in addition to normal β and α-chains. Sequence analysis indicates that the abnormal J-like chain has an amino acid substitution at position 82 of Lys → Asn: a new substitution named Hb Providence I. Sequence analysis of the N-like chain is still in progress. The proposita who is clinically well, has a hematocrit value and a red cell mass that is slightly elevated. Red cell life span ($^{51}Cr$) is minimally reduced (T 1/2 = 23.5 days). Her two children are normal. An 18-year-old sister and her two children have the same electrophoretic pattern and clinical picture as the proposita. Hence, in this family there appears to be duplication of one of the β-chain genes.

AN ANALYSIS OF THE SIGNIFICANCE OF SICKLE CELL TRAIT IN THREE MICHIGAN POPULATIONS. A.K. Mack, U. of Miami, Miami, Florida.

A survey was undertaken to obtain a reliable estimate of the prevalence of sickle cell trait and to gather evidence for its effects on blacks in three Michigan populations.

Laboratory diagnoses of hemoglobinopathies were determined from blood specimens of more than 3,500 respondents. Prior to the diagnosis a medical and social history questionnaire was administered. Comparisons were made between those individuals found to have sickle cell trait and those with normal hemoglobin.

Prevalances of health problems and symptoms revealed that weakness in legs, pain in bones, sense of exhaustion and epistaxis were significantly associated with sickle cell trait in the Lansing random sample, while swollen joints, numbness in legs and epistaxis were more prevalent among trait persons in the non-random Grand Rapids survey. Analysis by a single test of association of health problems and sickle cell trait revealed no significant difference between trait and control subjects.

Age trend analysis among trait subjects in the random sample showed no decrease in the proportion of carriers in older age groups. Neither population revealed a differential in primary or secondary infertility among heterozygous females. Nor was there any statistical difference between trait and control females of reproductive age for miscarriages or stillbirths.

A comparison of means and standard deviations of seven hematological indices between trait and normal subjects sampled in the Michigan State University study revealed no significant differences. The findings suggest that the trait is not appreciably associated with chronic illness.

RELATIVE RATES OF ALCOHOLISM AMONGST RACIAL GROUPS IN HAWAII. D. McLaughlin, C. Carter, and M. N. Rashad. U. of Hawaii and Hawaii State Dept. of Health, Honolulu.

A study of 442 patients receiving a diagnosis of alcoholism and admitted to the public mental health service in the State of Hawaii in 1973-74 showed that there were highly significant differences in admission rates amongst racial groups. The Caucasian males had an age-adjusted ratio of actual to expected admissions of 1.81. All other racial groups had fewer admissions than expected. For males, the age-adjusted ratios of actual to expected admissions were 0.11 for Chinese, 0.21 for Japanese, 0.32 for Filipinos, and 0.76 for Hawaiians. The females showed a similar pattern. The peak age range for admissions was 35-54 years with condiderable spread for all racial groups and both sexes. There was a significant tendency for the proportion of females within a race to decrease as the rate of alcoholism decreased for the races. In all races the proportion of males was at least 3 times that of females.

AUTOSOMAL RECESSIVE INHERITANCE IN ONE FORM OF IDIOPATHIC ADOLESCENT SCOLIOSIS. R. Marcus, H. Rothschild, I. Redler, S. Stone, W. Johnson. Louisiana State U. Medical Center, New Orleans.

Idiopathic adolescent scoliosis (IAS) (rotary curvature of greater than $25^{o}$) has an increased incidence among family members. Various modes of inheritance have been postulated to explain the 7 to 10 times increased incidence of this disorder in females. We examined the vertebral column in 142 randomly selected, clinically unaffected individuals, and also the parents and siblings of 18 patients with IAS. Approximately 20% of the male and 35% of the female controls had radiologically detectable curvatures ($5^{o}$ - $20^{o}$) as calculated by Cobb's method. Although the parents of five of the six male probands were not clinically affected, they all had radiologically detectable curvatures ($5^{o}$ - $20^{o}$).The observed frequency in parents of male probands exceeds expected by an amount that is statistically significant ($p = 0.01$). However, the parents of less than half of the 12 female probands had radiologically detectable curvatures. The difference between observed and expected for parents of female probands is not statistically significant. Therefore, we suggest that the IAS is a genetic heterogeneous disease, one form of which is inherited as an autosomal recessive disorder; the heterozygote has a curvature which is detectable radiologically but not clinically. Most of the affected males, but only a minority of the affected females, have this form of IAS. Apparently there is another type which may or may not be inherited, is more common among females, and accounts for the preponderence of females with IAS. Serum levels of testosterone and estradiol were measured by radioimmunoassay and found not to correlate with either form of the disorder.

TWO CASES OF PERICENTRIC INVERSION OF THE HUMAN CHROMOSOME NUMBER 9. H.F.Mark, T. Mendoza, and L. Beauregard. Genetics Laboratory, Rhode Island Hospital, Providence, and Eastern Maine Medical Center, Bangor.

Two cases of pericentric inversions of human chromosome 9 were reported. The first proband was referred for genetic counseling because of a history of two molar pregnancies. The second proband was referred to this laboratory because of a history of infertility and two premature deliveries which succumbed in early infancy. Routine chromosome analysis of 30 peripheral blood leukocytes revealed in both cases a modal chromosome number of 46 per cell with a female karyotype. In both cases a pericentric inversion of the $C_9$ chromosome was noted in 100% of the cells using the G- and C-banding techniques. The breakpoints for the two inversions are similar, one at p11 and q13 and the other at p12 and q13. The various theoretical risks due to exchange within the limits of the inversion were discussed. The literature was reviewed and a summary of various possible clinical manifestations of inversion heterozygotes was presented. It was emphasized that even though the majority of inversion heterozygotes from the literature seemed to be entirely within normal limits, the possibility cannot be excluded that zygotes resulting from the union of gametes from a homozygous normal and an inversion heterozygote run a risk of being abnormal with some yet ill-defined clinical manifestations.

COMPUTER-AIDED ANALYSIS OF HUMAN CHROMOSOMES:TWO STRUCTURAL REARRANGEMENTS.A.O.Martin and C.W.Thomas,Northwestern U.,Chicago,Ill., and Case Western Reserve,Cleveland,Ohio.

An Optronics P=1700 scanner has been used to digitize human chromosomes stained by the trypsin-Giemsa method. After being digitized,the images are normalized,filtered,and quantified preparatory to statistical analysis.

Two of the structural rearrangements which we have investigated are the so-called tan(21;21) and inv(9qh). Various mechanisms of formation have been proposed for the translocation between two number 21's. We have been able to exclude the"pericentric inversion followed by translocation"model,at least in our two families. We have found some interesting evidence of topographical change at the fusion point. Comparisons have been made of the translocation among families in order to see if this rearrangement might represent an identical recurrence. Comparisons have also been made with parental chromosome 21's in order to determine the source of this aberration.

The internal shifts of the rearranged chromosome 9 have been analyzed within and between families, and within and between clinically normal and abnormal individuals. Our aim has been to determine the nature of this rearrangement,i.e.is it really an inversion; to see whether it is the same among families,which will help answer the question of why it appears in such high frequencies in the population; and to decide if there is a cause and effect relationship between the variant and clinical abnormalities. One of the hypotheses we have tested is whether the amount of genetic material differs in clinically normal and abnormal individuals who bear what appears to be the same rearrangement visually.

THE TEAM APPROACH TO GENETIC COUNSELING: EXPANDED ROLES OF THE NURSE GENETICIST AND GENETICS ASSOCIATE. A. L. Matthews, J. Blu and V. M. Riccardi. Genetics Unit, U. of Colorado Med. Ctr., Denver.

Modern genetic counseling depends on the team-approach for maximal effectiveness: professionals with varying levels of training (MD, PhD, RN, MA, etc.) can thus effectively complement each other's areas of responsibility. This is essential since the counseling effort usually involves many basic and clinical disciplines, the collection, collation and formulation of data from many sources, a long duration between initial contact and final disposition, and the need for prolonged follow-up and reassessment. Primary responsibility for many components of this complex type of health care has been assumed by the Nurse Geneticist (NG) and Genetics Associate (GA) members of the Colorado-Wyoming Regional Genetic Counseling PROGRAM (RGCP). They have thereby had the opportunity to expand the roles and expectations of NG and GA personnel in general. The net results have included more efficient use of the physician-geneticist's time, increased numbers of patients seen, more effective follow-up, improved liason with local health professionals at all levels and a more extensive training and teaching effort. Our experience has demonstrated that for optimal use of genetic counseling resources more extensive involvement of the NG and GA is requisite, especially for center-satellite programs as the RGCP. These points will be demonstrated by means of specific examples. In addition, we will present a model for the deployment of such professionals on a semi-autonomous basis as part of the RGCP. By this presentation we thus wish to document and clarify the current expanded role of the NG and GA and suggest a plan for their assumption of even more responsibilities in the near future.

ALLELISM AND GENETIC COMPOUNDS AT THE MPS I LOCUS. I.H. Maumenee, T.E. Kelly, and V.A. McKusick. Johns Hopkins U., Baltimore, Maryland.

In 1972, we first suggested the existence of a syndrome caused by compound action of the genes for the Hurler (MPS I H) and the Scheie (MPS I S) diseases, the so-called Hurler-Scheie compound heterozygote or MPS I H/S. In the following years we have come to delineate in six patients, among them two sib pairs, a clinical phenotype with which we now identify the Hurler-Scheie compound. All six had $\alpha$-L-iduronidase deficiency. On clinical grounds alone we now label as MPS I H/S patients who show reduced height and characteristic facial appearance with coarseness, mild prominence of the supraorbital ridges, and striking micrognathia; they have visceral involvement in form of mild hepatosplenomegaly; their intelligence is normal or only mildly impaired. Since the disease is intermediate in severity between MPS I H and MPS I S and since we did not observe consanguinity among any of the parents, in spite of the rarity of the syndrome, we postulate compound actions of the genes for the Hurler and the Scheie phenotype as its basic mechanism. The confirmation of this hypothesis has to await further biochemical elucidation. In several isolated cases with $\alpha$-L-iduronidase deficiency, further distinct phenotypes were observed which are clearly distinct from the three first mentioned by degree of mental involvement, size, facial appearance, and X-ray changes. Our clinical findings will be described. The X-ray changes will be compared and the relative severity in these different mucopolysaccharidoses at various ages will be outlined.

SOMATIC CELL GENETIC ANALYSIS OF CYSTIC FIBROSIS. B.J. Mayo, R.J. Klebe, B.J. Lankford, N.R. Morris, D.R. Barnett, and B.H. Bowman. U. of Texas Medical Branch, Galveston.

Studies concerning the linkage relationships of the cystic fibrosis (CF) ciliary inhibitor (CFCI) have been carried out by somatic cell genetic analysis. Human skin fibroblasts from CF patients, which elaborate the CFCI into culture media, were hybridized with the mouse cell lines LM(TK-) and RAG. The resulting CFxRAG and CFxLM(TK-) hybrids, designated CFR and LC respectively, were isolated by the HAT half-selection system. Analysis of the hybrid clones for production of the CFCI gene product via the oyster ciliary assay revealed that some clones retained the ability to synthesize the CFCI. These results confirm previous evidence that the ciliary inhibitor is produced in the hybrid configuration. The clones not expressing the CFCI also proved to contain the fewest number of human chromosomes, which implies that the gene coding for the ciliary inhibitor segregates in a Mendelian fashion. Concommitant isozyme studies on the CFR hybrids have shown that all clones, regardless of CFCI production and chromosome number, express the human G6PD isoenzyme which concurs with population genetics findings that the inheritance of cystic fibrosis is not sex-linked. Currently more extensive linkage analyses of the CFCI gene product are in progress.

Permanent cell lines of CF skin fibroblasts have been produced by simian virus - 40 transformation. Oyster ciliary assays reveal that the transformed cells maintain the ability to synthesize the CFCI. By eliminating the biochemical variations arising from senescence, these SVCF cells may aid in universalizing the biochemical characterization of cystic fibrosis cells. (Supported in part by NIH grant AM 17040, The National Foundation-March of Dimes and The National Cystic Fibrosis Research Foundation and aided by a Basil O'Connor Starter Research grant from the National Foundation-March of Dimes.

INHIBITION OF HUMAN β-GALACTOSIDASE AND β-GLUCOSIDASE BY N-BROMOACETYL-β-D-GALACTOSYLAMINE. M. H. Meisler, Department of Biochemistry, SUNY School of Medicine, Buffalo, N.Y.

N-bromoacetyl-β-D-galactosylamine is an irreversible inhibitor of the 'acid' and the 'neutral' β-galactosidases of human liver. The inactivation of 'acid' β-galactosidase appears to involve a group with a pKa = pH 4.5. The inhibition of 'neutral' β-galactosidase only occurs above pH 8.0. Both enzymes are protected against inhibition by the presence of substrates, indicating that the inhibitor reacts with the active site of the enzymes.

Other lysosomal hydrolases are not inhibited by N-bromoacetylgalactosylamine, with the exception of β glucosidase. The pH dependence of β-glucosidase inactivation is essentially identical to that of the neutral β-galactosidase. Inhibition of β-glucosidase by this galactose derivative supports previous evidence that human liver 'neutral' β-galactosidase also has β-glucosidase activity. We have compared the heat lability of neutral β-galactosidase and β-glucosidase activity: both are inactivated at 52° with a half-life of 7.5 min. The presence of a single enzyme with β-glucosidase and β-galactosidase activities is also indicated by mixed-substrate experiments. (Supported by The National Foundation Grant #5-53.)

A FAMILY STUDY OF PREGNANCY OUTCOME. M.P. Mi, T. Yamashita, and M.N. Rashad. U. of Hawaii, Honolulu.

A population-based file in which 400,002 pregnancies reported in Hawaii during a 25-year period (1942-1966) were linked to construct 216,236 families based on common identifying information was developed. Families with two consecutive pregnancies were selected to study the effects of the outcomes of previous pregnancies on subsequent ones. The outcomes included livebirths, and fetal, neonatal, and post-neonatal deaths. There were 21,624 first-second, 16,348 second-third, and 10,720 third-fourth pregnancy pairs with complete information on sex, type of birth, weight, duration of pregnancy, mother's age and race, father's age, race and occupation, pregnancy complication, labor, birth injury, and congenital malformation for each pregnancy. Multiple births were not included in the present study. Analyses were based on regression models with simultaneous consideration of race, sex, maternal age and obstetric factors.

A fetal or neonatal death appeared to be associated with an increased risk of subsequent fetal and neonatal death. Similar results were obtained in all three types of pregnancy pairs. Associations between various obstetric variables of two consecutive pregnancies were also observed.

DETECTION OF CARRIERS FOR HEMOPHILIA BY "MAIL ORDER". C.H. Miller, E.M. Barrow, R.C. Elston, H.M. Reisner, and J.B. Graham. U. of North Carolina at Chapel Hill.

In recent years it has become possible to reliably detect heterozygous carriers for hemophilia "A" by combined study with coagulant assays (VIII-C) and immunologic assays (VIII-Ag). As reported at the ASHG meeting two years ago, there is, however, a sizeable false negative diagnostic rate (ca. 20%) resulting from the lyonization mechanism. It has been suggested that the population of hemophilia carriers might be identified by a system in which local laboratories determine VIII-C while VIII-Ag is determined on samples shipped to reference laboratories. We have tested this possibility by examining 34 "obligate carriers" for severe hemophilia "A". Samples were obtained at home three times, 2-4 weeks apart, by traveling medical students and transported to the laboratory. Assays for VIII-C were done in Chapel Hill by standard assay procedures immediately and after storage at -70° C. The levels of VIII-Ag were assessed by a radioimmunoassay and by quantitative immunoelectrophoresis (Laurell) using rabbit anti-F.VIII. Controls consisted of a comparably managed group of 33 normal women and 24 women who are "obligate carriers" of hemophilia "B" (Christmas disease), the other X-linked form of hemophilia. Preliminary analysis of the data confirms that the false positive diagnostic rate is less than the rate of false negative diagnosis. It also points to several important sources of variability and suggests that much work needs to be done before reliable detection of hemophilia carriers will be possible by "mail order".

●THERAPEUTIC EFFECT OF VITAMIN C: A CO-TWIN CONTROL STUDY. J.Z. Miller, J.A. Norton, R.L. Wolen, R.S. Griffith, W.E. Nance. Indiana U. School of Medicine and Clinical Research Division, Eli Lilly and Co., Indianapolis.

To assess the effects of pharmacologic doses of Vitamin C on the incidence and morbidity of the common cold, 44 school-aged monozygotic twin pairs were enrolled in a double-blind co-twin control study. Zygosity was determined by dermatoglyphic analysis and extensive genotyping, and the sample included 18 male and 26 female pairs. The panel was divided into 3 dosage groups (500 mg, 750 mg, and 1000 mg) based on body weight, and daily observations of the presence and severity of 14 symptoms were recorded by the mothers over a 5-month period. Compliance was monitored by collection of monthly urine specimens for measurement of ascorbic acid excretion and multiple blood chemistries, and 24-hour urine collections were obtained prior to both the initiation and the conclusion of the study. All 44 twin pairs completed the 5-month study, but two male pairs were omitted from the analysis because of incomplete data. The average number of upper respiratory illnesses was 4.9 with an average duration of 7.2 days per incident, and an average severity index of 26.9. Analysis of the nested paired comparisons showed no significant overall treatment effect. However, there were significant dose x sex interactions for average severity ($P<0.01$) and total duration ($P<0.05$). Consequently, the data were subdivided and analyzed by dosage groups and sexes. The combined two lower dose group females showed significant ($P<0.05$) treatment effects on 5 variables of duration and severity while the lowest dose group had significant treatment effect ($P<0.05$) on total severity. It is possible that the age and sex effects detected in this study may in part account for the inconclusive results of previous investigations.

FIBRIN/FIBRINOGEN DEGRADATION PRODUCTS (FDP) ASSAY FOR PRENATAL DIAGNOSIS OF NEURAL TUBE DEFECTS. A. Milunsky, and A.C. Carvalho. E.K. Shriver Center, Massachusetts General Hospital, and Harvard Medical School, Boston.

The search has continued for protein markers in amniotic fluid (AF) as an aid to the prenatal diagnosis of neural tube defects (NTD). Both $\alpha$-fetoprotein (AFP) and $\beta$-trace protein analysis have been shown to be non-specific for the prenatal diagnosis of NTD. One report (Lancet 1:1013, 1975) suggested a good correlation between the fetus with NTD and elevated values of AF FDP. We have made preliminary studies of FDP in 28 non-blood contaminated AF samples from affected fetuses where elevated AFP levels have been found, four cases with abnormally elevated AFP where the fetus was free of a NTD, and 21 cases with normal pregnancy outcome. The assay used for measuring FDP was the staphlococcal clumping test. The +2 S.D. values for FDP in serum in our lab is 9.6 $\mu$G/ml.

| | Cases | AFP ( > 2 S.D.) | FDP ( > 9.6 $\mu$G/ml) |
|---|---|---|---|
| Neural Tube Defects | 28 | 28 | 24 |
| Other Fetal Abnormalities | 4 | 1 | 2 |
| Normal Pregnancy Outcome | 21 | 0 | 5 |

Results shown in the table suggest that assays for FDP in AF are less useful than AFP determination. While still in the process of assaying many more normal and variously abnormal samples and collecting samples prospectively with an antifibrinolytic agent, the preliminary conclusion is that these results and the frequency of bloody AF's mitigate against the use of FDP assays for the prenatal diagnosis of NTD.

TISSUE SPECIFIC TRISOMY 8. C. M. Moore, and C. I. Scott. U. of Texas Medical School, Houston.

A 3 year, 3 month old boy presented with many of the clinical features now recognized to be associated with trisomy 8: mild mental retardation with poor speech development, unusual facies, strabismus, contractures of fingers and toes, three deep furrows on the sole of each foot, and numerous skeletal abnormalities, including dysplastic, displaced patellae, vertebral anomalies, metatarsus adductus, and coxa vara. Chromosomal analysis was performed on PHA-stimulated lymphocytes. A 46,XY karyotype with normal banding patterns was found in the 100 cells examined. Because of the strikingly similar clinical appearance of this child to the individuals reported with trisomy 8, we obtained a skin biopsy for karyotyping. The fibroblasts had a 47,XY,+8 karyotype determined by Q-banding in each of the 100 cells examined. A bone marrow asperate revealed only cells with a 46,XY karyotype. The remarkable specificity of the trisomy to one tissue, the characteristics of the trisomic cells in culture, and the clinical significance of these findings in the diagnosis will be discussed.

●BLOOD GROUP GLYCOSYLTRANSFERASE AND GENETIC BACKGROUND OF BLOOD GROUP DETERMINATION. H. Muensch and A. Yoshida. City of Hope National Medical Center, Duarte, California.

The human blood groups (A,B, and O) are known to be related to the terminal sugar residues attached to common carbohydrate chains on red cell surface. Specific glycosyltransferases, i.e., UDP-N-acetylgalactosaminyltransferase (A-enzyme) in A type persons, and UDP-galactosyltransferase (B-enzyme) in B type persons, are responsible for transferring the sugars to the terminal fucosyl-galactose of the O substance, while both enzymes are absent in blood type O persons. It has not been clear whether the expression of these transferases is related to allelic genes or is under regulatory control.

A-and B-enzymes were purified from human plasma by several steps of column chromatography, and they were obtained in homogeneous form. Their molecular weight (about 100,000), sedimentation constant (S20,w=5.8s), and subunit molecular size (about 52,000) were determined by analytical ultracentrifugation, Sephadex gel filtration and by SDS disc electrophoresis. The purified B-enzyme cross-reacts with an antibody which was produced by immunizing rabbits with the purified A-enzyme. Furthermore, the anti-A-enzyme antibody cross-reacts with a component of blood group O plasma, indicating the presence of an enzymatically inactive but immunologically active protein in O plasma. Therefore, the genes for A-, B-enzymes and inactive O-enzyme are allelic, excluding the possibility of involvement of regulatory genes in blood group expression.

●HISTONE ACETYLATION IN MIXED LYMPHOCYTE CULTURES: IMPLICATION IN RENAL TRANSPLANTATION. A.B. Mukherjee & J.D. Fabricant. Children's Hosp. of Buffalo, New York; McGill U.,Montreal, Canada.

Blast transformation in mixed lymphocyte cultures is used to assess histocompatibility in human kidney transplantations. In phytohemagglutinin (PHA) stimulated lymphocytes, blast transformation (at 72hrs.) is preceded by histone acetylation (at 15min.). If detectable histone acetylation preceded blastogenesis in mixed lymphocyte cultures, a substantial reduction in the time required to evaluate histocompatibility could be achieved and many more cadaver kidneys could be utilized successfully. Histone acetylation was assayed in mixed lymphocyte cultures by labeling the histones with $C^{14}$ Na-acetate for 15 min. followed by extraction, purification and scintillation counting. Blast transformation was quantitated from slides stained with Giemsa-9. Histone acetylation was studied in the following combinations: 1) unrelated normal male x normal female, 2) unrelated normal male x normal male,3) unrelated normal female x normal female, 4) monozygotic twins, 5) dizygotic twins and 6) non-twin sibs of the same family. In all experiments several cultures of each individual were set up as controls. The results indicate that i) there is a direct correlation between histone acetylation and blast transformation in mixed lymphocyte cultures. ii)Acetylation begins within 30min. following the initiation of mixed lymphocyte cultures from unrelated individuals. iii) No detectable acetylation occured in mixed lymphocyte cultures of monozygotic twins. iv) 15% acetylation occured in dizygotic twins compared to 60% in completely unrelated individuals. There were varying degrees of acetylation (25-50%) in cultures of non-twin sibs. These results could be available within 3-4 hrs. after blood samples from the donor and recipient are available in the laboratory.

●ASSIGNMENT OF A GENETIC ELEMENT FOR HUMAN β-GLUCURONIDASE TO CHROMOSOME 9. M. J. Murnane R. S. Kucherlapati, S. Chen, and F. H. Ruddle. Dept. of Biology, Yale U., New Haven, Conn. 06520.

Human β-glucuronidase has only recently been studied in disease states and in cultured cells. Genetic relationships among multiple electrophoretic forms of the human enzyme have not yet been elucidated.

To obtain genetic linkage information on human β-glucuronidase, a series of Chinese hamster x human hybrids has been analyzed for enzyme and chromosome data. Cellogel electrophoresis was employed to separate the major human β-glucuronidase form having an acidic pH optimum from the corresponding Chinese hamster form of this enzyme. Twenty-three primary cell lines and 23 secondary cell lines were tested for β-glucuronidase expression using 4-methylumbelliferyl-β-D-glucuronide as substrate. In addition, each cell line was analyzed for the presence of 20 known enzyme markers. Chromosome information based on quinacrine A banded preparations was also obtained on 23 cell lines.

The resulting data indicate that an assignment can be made to human chromosome 9 for a genetic element responsible for the expression of the major acidic form of human β-glucuronidase. No other human chromosome was required for the expression of this enzyme.

PRENATAL DETECTION OF LEIGH'S DISEASE: CURRENT STATUS. J. V. Murphy, L. J. Craig and W. F. Diven. Children's Hospital of Pittsburgh and University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213.

Leigh's disease (LD) is a clinically nonspecific neurodegenerative disease with death generally occurring in the first several years of life. Patients with this condition have a deficiency of thiamine triphosphate (TTP) in brain and a factor in body fluids which inhibits the cerebral synthesis of TTP. We have also been able to demonstrate the inhibitor in patient fibroblasts, when these fibroblasts are grown in thiamine deficient medium (Pediat. Res. 8: 393, 1974).

Prior attempts by others to detect LD prenatally by direct examination of the amniotic fluid for inhibitor have been unsuccessful. Since 1974 we have obtained amniotic fluid cells from three fetuses at risk for LD. Utilizing the same techniques employed in the fibroblast studies we were able to predict that none of the fetuses were afflicted. Presently all three children are over one year of age and in excellent health; one is probably heterozygous for the LD gene. As the siblings with LD died between the ages of 4 and 6 months, our prenatal predictions are accurate in this small sample.

THE USE OF PEDIGREE LINKAGE FOR THE DIAGNOSIS OF HEREDITARY DEAFNESS. W.E. Nance, S. Rose, A. Proksch, and P.M. Conneally. Indiana U. School of Medicine, Indianapolis.

Profound prelingual deafness is unusual in that genetic cases and environmental phenocopies occur with approximately equal frequencies. In the absence of a syndromic diagnosis, consanguinity, or a positive family history, it may be difficult if not impossible to distinguish sporadic cases from chance isolated recessive cases. To explore the value of the establishment of remote genealogic relationships ("pedigree linkage") as an aid in diagnosis, systematic pedigree data were obtained from students at Gallaudet College during the 1973 school year and matched against a roster of some 30,000 individuals in the families of 4471 matings among the deaf that was established by E.A. Fay in 1898. Data were obtained from the parents of 555 probands. Segregation analysis revealed that 23% of the cases were sporadic and 77% genetic. The high proportion of genetic cases is not surprising in this high-achieving college group since many known environmental causes of deafness are often associated with other neurologic defects. Among the genetic cases, 76% were autosomal recessive and 23% autosomal dominant, and there was evidence for considerable variation in penetrance among the autosomal dominant cases. In 322 families, the parents were able to trace the family history back to one or more ancestors who were born in the U.S. before 1900, and among these, 8 possible and 10 definite pedigree linkages were established with the Fay data by a comparison of names, birthdates, birthplaces, and other identifying information, demonstrating that pedigree linkage is a useful adjunct to the diagnosis of hereditary deafness. A unique feature of pedigree linkage is that in order to utilize the roster, pedigree data must be submitted which will in turn augment the file. Thus, the more the system is used, the more efficient it will become.

ULTRASTRUCTURAL STUDIES OF LYMPHOCYTES IN BATTENS DISEASE. S.M. Noonan, L. Weiss, and J.M. Riddle. Department of Pathology, Wayne State U., School of Medicine and Department of Pediatrics, Henry Ford Hospital, Detroit, Michigan.

Although vacuolated lymphocytes are a well recognized feature in Battens Disease, their significance was unknown. Ultrastructural studies of circulating lymphocytes from a patient with Battens disease revealed two forms of abnormal intracytoplasmic inclusions with distinctive patterns of internal morphology. One form is a single membrane bounded vacuole which contains a collection of hollow cylinder-like structures. The second inclusion form is a single membrane bounded structure which contains five different arrangements of internal contents. These range from granules scattered in a low electron density matrix to the simulated "fingerprint" that has been described in the neurons from patients with Battens disease. The "fingerprint" inclusion is thought to represent the abnormal deposition of lipofuscin pigment. Some of the inclusion contents had an admixture of granules, vesicles, paracrystalline arrays and "fingerprint" formations within the same inclusion. The structure of these molecular entities suggests their progressive synthesis from a basic molecular unit. Acid phosphatase staining at the ultrastructural level was performed to further delineate the origin of these inclusions. These ultrastructural studies are the first which detail the morphology of the abnormal storage materials contained within cytoplasmic inclusions of lymphocytes from patients with Battens disease. It documents the direct involvement of lymphocytes, describes the fine structure of the abnormal inclusions and confirms the direct involvement of lysosomal enzymes.

●SPONDYLOEPIPHYSEAL DYSPLASIA, CORNEAL CLOUDING, NORMAL INTELLIGENCE AND ACID β-GALACTOSIDASE DEFICIENCY. J.S. O'Brien*, E. Gugler**, A. Giedion***, U. Wiessmann+, N. Hershkowitz+, C. Meier+, and J. LeRoy++. *U. of California at San Diego, La Jolla, **Children's Hosp. of Aarau, Aarau, Switzerland, ***Children's Hosp. of Zurich, Zurich, Switzerland, +U. of Bern, Bern, Switzerland, and ++U. of Antwerp, Antwerp, Belgium.

A 14-year-old girl with a unique type of progressive spondyloepiphyseal dysplasia, corneal clouding, and no evidence of neurological abnormality, was found to have a remarkable deficiency of acid β-galactosidase activity in cultured skin fibroblasts and in leucocyte preparations. In fibroblasts, ganglioside $GM_1$ β-galactosidase activity averaged 7% of the normal mean while asialofetuin β-galactosidase and 4-methylumbelliferyl-β-galactosidase averaged 1.4% and 3.5%, respectively. Activities for all three substrates in leucocytes from both her parents were close to 50% of the normal mean indicating that the patient is homozygous for a mutation or mutations affecting $GM_1$ β-galactosidase. We postulate that her phenotype is explained by the significantly higher residual activity of the mutant enzyme for ganglioside $GM_1$ than for galactose-containing proteoglycans which are important bony constituents.

●LINKAGE OF GENES FOR THYMIDINE KINASE AND GALACTOKINASE IN CHIMPANZEE AND AFRICAN GREEN MONKEY. K.G. Orkwiszewski, C.M. Croce and W.J. Mellman. Dept. Human Genetics, U. of Pa. Human Genetics Center and Wistar Institute of Anatomy and Biology, Philadelphia.

Thymidine kinase (TK) and galactokinase (GALK) are linked in man and are located on chromosome 17. Linkage of the structural genes for these enzymes was investigated in two other primates (chimpanzee and African green monkey [AGM]). Primate fibroblasts were fused with a TK-deficient mouse cell line (Cl 1D). Hybrid clones were selected for the persistence of primate TK by their ability to grow in HAT media.

Ten AGM x mouse hybrid clones selected in HAT medium contained a chromosome similar to the human E-group. This chromosome is lost when the clones were back-selected in BrdU-containing medium, and is presumed to contain the TK gene. The 10 HAT-selected clones contain monkey GALK (by starch gel electrophoresis), and GALK disappeared when the clones were back-selected in BrdU.

Twenty-two chimpanzee x mouse hybrid clones selected in HAT medium contained the chimpanzee chromosome 17, which is similar in size but different in arm ratio to the human 17. Chimpanzee GALK was present in these clones.

This investigation concludes that the linkage relationship between TK and GALK has been maintained in three divergent primate species. We have further found that two other genes involved in galactose metabolism, galactose-1-phosphate uridylyltransferase and UDP-glucose-4-epimerase, which are closely linked in bacteria and yeast, are unlinked in man, chimpanzee and AGM.