753A LIPOSOME ENTRAPMENT OF DNA AND TRANSFER TO MAMMALIAN CELLS. W.B. Rizzo, J.D. Schulman, and A.B. Mukherjee, NICHD, NIH, Bethesda, MD 20205.

We investigated techniques for entrapment of DNA in phospholipid vesicles (liposomes) and transfer to mammalian cells. Large unilamellar vesicles prepared by reverse-phase evaporation were superior to other methods of liposome preparation and entrapped 25-35% of radioactively labelled lambda DNA into neutral phosphatidylcholine:cholesterol liposomes (7:2 molar ratio). The proportion of DNA entrapped was dependent on the lipid concentration and independent of the DNA concentration between 0.2 - 20 ug/ml. Entrapped lambda DNA remained intact and resistant to deoxyribonuclease digestion as judged by agarose gel electrophoresis. The reverse-phase method was also used to incorporate DNA into negatively-charged phosphatidylserine:phosphatidylcholine: cholesterol liposomes (2:7:2). To test for transfer of functional DNA into mammalian cells, we incubated liposome-entrapped SV40 DNA with monkey kidney cells in vitro. Infectious plaque formation was not observed using the neutral liposomes but did occur under similar conditions when the DNA was encapsulated into phosphatidylserine-containing vesicles. Preliminary experiments with the negatively-charged liposomes suggested that post-treatment of the cells with 10% dimethylsulfoxide increased frequency of plaques ~ 2-fold while 30% glycerol stimulated plaque formation at least 10-fold, confirming the recent report of Fraley et al. (J. Biol. Chem. 255:10431, 1980). This method of packaging exogenous DNA for delivery to mammalian cells may prove useful in gene transfer experiments.

● **754** TYPE I COLLAGEN SYNTHESIS IN OSTEOGENESIS IMPERFECTA, David W. Rowe, Michael J. Poirier, Univ. of CT Health Center, Department of Pediatrics, Farmington, CT and Jay R. Shapiro, NIH, Bethesda, MD. (Spon. by Arnold J. Altman)

We have examined the collagenous protein synthesized by dermal fibroblasts from 35 patients with osteogenesis imperfecta (OI) and 12 controls. Fibroblasts were incubated with ^{3}H-proline; the total collagenous proteins were determined by collagenase digestion and the ratio of type I to type III collagen estimated by interrupted acrylamide gel electrophoresis. At present, four patterns of type I synthesis have been observed roughly correlating with the clinical phenotypes: 1) Low type I synthesis without an abnormality in migration or ratio of the α_1(I) and α_2 chains. 2) Low or normal type I synthesis with an abnormality in the α_1/α_2 ratio or a structural abnormality of the α_2 chains. Patients in either group have dominantly or sporadically inherited OI which is mildly to moderately deforming. 3) Normal to elevated type I synthesis with a normal type III to type I ratio. Both the α_1(I) and α_2 chains show delayed migration. These patients belong to the progressively deforming group as described by Sillence since they have severe growth retardation and are uniformly wheelchair bound. 4) Low to normal type I synthesis with structural abnormalities in the α_1(I) chain. The α_1(I) chain findings have only been found in patients with the neonatal lethal form of OI. The results of this biochemical survey indicate that a wide range of identifiable abnormalities of type I collagen are present in most patients with OI reflecting the genetic heterogeneity of this disease.

755 GLYCOPROTEINS IN CULTURE MEDIUM: A COMPARISON FROM CYSTIC FIBROSIS (CF) AND CONTROL SKIN FIBROBLASTS. Thomas F. Scanlin, Judith A. Voynow, Edwin L. Thomas and Mary Catherine Glick. (Spon. by Stanton Segal). University of Pennsylvania School of Medicine, Children's Hospital of Philadelphia, Department of Pediatrics, Philadelphia.

Glycoproteins of high molecular weight are found associated with the cell surface or in growth media of fibroblasts and other cell types in culture. Several abnormalities of glycoproteins in CF have been reported. Therefore the glycoproteins from the media of CF and matched control skin fibroblasts were isolated and partially characterized. Cells were labeled with L-[^{3}H]fucose and grown in medium containing 10% serum. For some experiments the cells were subsequently grown in serum free medium for 48 hours prior to harvest. Media were examined from cells in both log and confluent phases of the growth cycle. The four methods used to isolate the glycoproteins were cryoprecipitation, ammonium sulfate precipitation, heparin precipitation and a heparin-sepharose column. The precipitated proteins were characterized by polyacrylamide gel electrophoresis, isoelectric focusing and protein and carbohydrate content. Different classes of glycoproteins were obtained with each of the four methods. Only heparin precipitation yielded a single radioactive glycoprotein (M_r>200,000) demonstrated by gel electrophoresis. Several qualitative and quantitative differences were noted. In all cases less protein per cell was precipitated by heparin from the CF medium when compared to control. USPHS grant AM16859 and CF Foundation Student Traineeships.

756 TRIMETHYLAMINURIA: RARE OR UNRECOGNIZED CAUSE OF BODY ODOR? Heinrich K. Schedewie, Marge A. Brewster, Lawson Glover, University of Arkansas for Medical Sciences, Arkansas Children's Hospital, Departments of Pediatrics, Pathology and Medicine, Little Rock.

Trimethylaminuria (TMAU) is a rare disorder of choline metabolism reported in 6 patients since its first description 10 years ago. We have found 3 more cases in unrelated caucasians, ages 9-14yrs, with body odor of putrid fish. Urinary TMA concentrations were determined by gas chromatography of alkalinized urine volatiles. Basal TMA concentrations (ug/ml) ranged from 25-100 in patients. As controls random urines were obtained from 96 patients with a variety of disorders without regard to choline ingestion, including 51 children 0-15 yrs. 84/96 values were $<$2.0, 91/96$<$ 7.5. Of the 5 outliers, 3 had vaginal disorders (14-27.8), 2 are unexplained (26.5, 400.8). The 2 TMA-patient mothers assayed showed intermediate concentrations of 10 and 21 ug/ml. For 2 patients, oral choline loading (50mg/kg) produced a several-fold increase in urinary TMA while dietary choline restriction markedly relieved odors. The third patient has not shown this strong choline correlation and is undergoing further metabolic study. We speculate that the incidence of TMAU among individuals with body odors may be much higher than suggested by the few case reports. Physician awareness and wider availability of the assay may help to diagnose and dietarily manage a greater number of patients with this repugnant odor, avoiding the sequelae of psychosocial disturbances and severe emotional disorders.

● **757** CHROMOSOME MAPPING OF HUMAN GENES BY GENE PURIFICATION SOMATIC CELL HYBRIDIZATION AND RESTRICTION ENZYME ANLAYSIS. Roy D. Schmickel, Susan L. Naylor, Mechthilde Knoller, Alan Y. Sakaguchi, and Thomas B. Shows. The University of Michigan, University of Michigan Medical Center, Department of Pediatrics, Ann Arbor, Michigan and The Roswell Park Memorial Institute, Buffalo, New York.

A combination of techniques permits mapping of genes which can by chromosomally located by no other means. Purified genes were labelled with ^{32}P by nick translation. Chromosomes were segregated separately in mouse/human hybrids. The purified genes were mapped on segregated chromosomes by the restriction of the DNA of the somatic hybrid cells and molecular hybridization with the radioactivity labelled genes. In the human, 300 ribosomal genes are located on five homologous chromosome pairs (13, 14, 15, 21 & 22). These genes are polymorphic and contain several populations of different genes. These experiments were designed to study whether this polymorphism is due to mutational events on a single chromosome or if they are a result of interactions between chromosomes. In these experiments a single human chromosome was analyzed by utilizing a 15/X translocation. This permitted the chromosome to be selected in a mouse HPRT^{-}/human HPRT^{+} somatic cell hybrid. This 15/X chromosome could also be removed by selection against that chromosome using 8-azaguanidine. This technique permitted us to localize a specific variant to and only to the #15 chromosome. Evidence of the restriction analysis indicates that the polymorphism of this family of genes is a result of interchromosomal exchange.

758 CYSTIC FIBROSIS (CF) IN A LARGE KINDRED. Robert H. Schwartz, Robert J. Holzhauer, Barbara Kehoe, Ronald G. Perciaccante and Paul R. Patterson. University of Rochester, School of Medicine and Dentistry, Rochester, New York

Consanguineous matings increase the chance for the appearance of homozygous autosomal recessive disease. When the frequency of a recessive gene in the population is high, as with CF, the finding of consanguinity among the parents need not be common. There have been few reports of consanguinity in CF families.

In four families, 6 of 21 children with documented CF can trace their roots back 6 and 7 generations to a common ancestor who was born in 1789 in Lorraine, France. Since 1833 when this patriarch came to the U.S. there have been over 5,000 descendants. Most have lived in upstate New York.

The parents of the CF proband are distant cousins (4 and 5 generations removed) via two lines of descent.

Two other CF children in a second family and one in a third family are cousins. They are also second cousins to the proband. Each parent pair contains a sibling descended from the patriarch via a second line of descent. The opposite spouses, not of the same lineage, are also siblings.

Two other CF children in a fourth family are related to the other CF individuals via a third line of descent.

The inherited nature of CF is evidenced by;(1)four CF families with common ancestry, and (2)brothers and sisters having children with CF. Autosomal recessive inheritance is evidenced by; (1) consanguinity in parents of the proband, (2) first cousins with CF, and (3)a 29% CF incidence when the expected incidence is 25%.