771 SKIN FIBROBLAST AND CARTILAGE PROTEOGLYCANS (PG) IN OSTEOGENESIS IMPERFECTA (OI) TYPES I AND II. J.C. Ward and L.S. Levin. (Spon. by R.L. Summitt). Depts. Ped., U. Tenn., Memphis and Otolaryn., Johns Hopkins Hosp., Baltimore

PG, with collagen and elastin, are major constituents of connective tissue. No consistant genetic biochemical abnormality has been found in OI. Skin fibroblasts from patients with OI-I, both with (N=3), and without (N=3), detinogenesis imperfecta (DI), were labeled with ^{35}S-SO_4 for 48 hours. Medium (M) and cell layer (CL) macromolecular material (^{35}S-PG) was fractionated from ^{35}S-SO_4, and expressed as counts per minute (CPM)/mgm CL protein (P) or CPM/10_3 cells. No quantitative differences were seen in the two categories of OI-I fibroblasts. Autoradiography of ^{35}S-PG (M and CL) from dried large-pore polyacrylamide-agarose (LPPA) electrophoretic gels showed no major qualitative differences between the pattern in cells from OI-I, with or without DI. Qualitative and quantitative differences seen between the two OI groups and controls may be related to age. Cartilage (CA) from patients with OI-II (N=3) and controls (N=2) was maintained in organ culture. Approximately 100 mgm wet weight CA was labeled for 48 hrs with ^{35}S-SO_4. ^{35}S-PG in M and in CA extracts (CE, extraction in 4M GnCl of the labeled CA) revealed no major quantitative differences in CPM/mgm P or CPM/10_3 cells, although individual values were variable in the M. However, autoradiography in LPPA gels revealed an extra cathodal ^{35}S-PG band from CE occurring in the OI-II cartilage, not present in the control samples. Identification by enzymatic degredation is being pursued.

772 PERMANENT NEONATAL DIABETES MELLITUS (DM) IN AN INFANT OF AN INSULIN-DEPENDENT MOTHER. John A. Widness, Richard M. Cowett, W. Patrick Zeller, John B. Susa, Robert Schwartz and Collaborators; Dept. of Pediatrics, Brown University, Providence, R.I.

Familial, insulin-dependent diabetes (IDDM) is unusual in a newborn. A 27 year old Caucasian Class F diabetic whose onset was at 10 wks of age was delivered of a 2100 g male at 36 wks gestation. By 5 days plasma glucose was elevated (242 mg/dl) and thereafter ranged from 350-550 without ketonuria. Islet cell surface antibodies were negative (Chicago). At six weeks an I.V. tolbutamide tolerance test (20 mg/kg) produced a fall in plasma glucose and rise in C-peptide (max. 1.75 pmol/ml at 40 min.). Pl. cortisol and GH were normal, while glucagon was low (<50 pg/ml). Glucose production was elevated to 10 mg/kg/min (nl: 3.8± 0.1, M±SE) by the D-[U-^{13}C] glucose primed infusion technique. Although growth was adequate, insulin therapy was initiated at 3 mos. when ketonuria was associated with a URI. A repeat tolbutamide test at 4 mos. had a minimal C-peptide response (0.35 pmol/ml). Insulin binding (NIH) on Rbc's, cultured B-lymphocytes and fibroblasts from mother and baby was normal. Rat adipocytes metabolized labelled glucose normally in the presence of infant serum. HLA lymphocyte typing (Boston) of the family revealed no antigens commonly associated with IDDM (Dr_3 and Dr_4 were absent). With insulin therapy, growth and development have continued normal to 18 mos. The etiology of this early onset familial diabetes does not appear to be HLA linked, nor due to an abnormality in insulin receptors or in insulin sensitivity.

773 BOVINE α-GLUCOSIDASE DEFICIENCY: A MODEL FOR ENZYME REPLACEMENT THERAPY. Julian C. Williams, James P. Chambers, Peter N. DiMarco, Peter R. Dorling and John McC. Howell (Spon. by Frank H. Morriss, Jr.). The University of Texas Health Science Center at Houston, Department of Pediatrics, Houston; Murdoch University, School of Veterinary Studies, Murdoch, Western Australia.

A bovine species has recently been described, whose pathology and genetics are those seen in Pompe's disease (Neuropathol. Appl. Neurobiol. 3:45, 1977.). Further studies have proven a deficiency of lysosomal α-glucosidase and accumulation of glycogen (Aust. J. Exp. Biol. Med. Sci. 55:141, 1977.). Bovine hepatic α-glucosidase was purified to homogeneity with a sp. act.= 7 umoles/min/mg, a single band of M.W.= 107,000 by PAGE and a single subunit of M.W.= 59,000 by SDS-PAGE. The ^{125}I-labeled enzyme was injected intravenously into a homozygous α-glucosidase deficient calf. The $t_{½}$ of disappearance from the plasma compartment was 2 min. with accumulation of activity in the liver. Disappearance from liver ($t_{½}$= 20 min.) was paralleled by the reappearance of activity (non-TCA precipitable) in the plasma. Less than 1% of the administered dose accumulated in muscle.

The establishment of a breeding herd of heterozygotes with α-glucosidase deficiency has allowed the production of affected animals for enzyme replacement studies. Administration of purified bovine α-glucosidase indicates rapid clearance from the plasma with accumulation in liver but not muscle. This model will allow the further study of enzyme targeting via enzyme lipoprotein conjugates.

774 EARLY EVENTS IN HUMAN LYMPHOCYTE ACTIVATION MEASURED BY HYBRIDIZATION TO PURIFIED GENES. G. Wilson, B. Szura, L. Szura, S. Schwartz, and R. Schmickel. University of Michigan, C. S. Mott Children's Hospital, Department of Pediatrics, Ann Arbor, MI 48109.

A specific hybridization assay for ribosomal RNA (rRNA) sequences reveals a dramatic increase in rRNA synthesis 2-6 hours after stimulation of human peripheral lymphocytes with phytohemagglutinin (PHA). Preliminary experiments demonstrated a serum requirement for long term survival and ^3H-uridine incorporation by peripheral lymphocytes incubated in RPMI media at 37 . Purified proteins such as bovine serum albumin or lactalbumin could not substitute for this requirement and human serum gave lower background RNA synthesis than fetal calf serum. Lymphocyte rRNA synthesis, as measured by hybridization to cloned rDNA segments, closely parallels total RNA synthesis as measured by the incorporation of ^3H-uridine. An early linear phase (2-14 hours) of PHA-stimulated rRNA synthesis was followed by a biphasic increase in synthesis at later times (14-32 hours). A more delayed and less complex response was observed in purified T lymphocytes, indicating heterogeneity among lymphocyte populations for PHA stimulation of rRNA synthesis. Synthesis of rRNA or total RNA by T cells was enhanced 25 fold 30 hours after PHA stimulation. This response affords a model system for gene activation which defines factors regulating rRNA synthesis in particular and cellular differentiation in general.

775 A COMPREHENSIVE SEARCH FOR POLYMORPHISM IN A SPECIFIC REGION OF HUMAN RIBOSOMAL DNA. G. Wilson, L. Szura, and A. Dranginis. University of Michigan C.S. Mott Children's Hospital, Department of Pediatrics, Ann Arbor, MI 48109.

Sequence and length polymorphism near the putative transcription initiation region of human ribosomal DNA (rDNA) has been defined using the complementary techniques of restriction analysis and recombination cloning. Seventeen bacteriophage clones of the human rDNA EcoRI B fragment which contains non-transcribed spacer, external transcribed spacer, and 18S gene sequences were compared by partial restriction analysis of end-labelled DNA segments. Restriction patterns of the external transcribed spacer and 18S gene regions were strikingly similar among the clones, but variable restriction sites for the enzymes Sma I and Bgl I were located in the non-transcribed spacer region. A probe containing this variable region demonstrated the presence of certain restriction sites in genomic rDNA which do not vary among different individuals or tumor cell lines. In contrast, restriction with the enzyme Sal I reveals several variable fragments, one of which has been found only in a retinoblastoma cell line. This report emphasizes the utility of a detailed restriction map for locating variable regions among cloned DNA segments so that informative probes for polymorphisms in genomic DNA can be constructed.

776 GLYCOSYLATION PROCESSES IN CYSTIC FIBROSIS (CF). Yoav Ben-Yoseph, C.L. DeFranco and Henry L. Nadler. Northwestern U. Medical School, Children's Memorial Hospital, Dept. of Pediatrics, Chicago, Illinois.

Aberrant glycosylation of glycoconjugates has been suggested as a possible defect in CF. Previously we have shown that although α_2-macroglobulin (α_2M) from CF patients is a poor substrate for sialylation no defect could be demonstrated in the metabolism of sialic acid. In an attempt to evaluate glycosylation in CF, carbohydrate analyses were performed by gas-liquid chromatography on purified plasma glycoproteins from patients with CF and controls. Increased fucose and/or decreased galactose, N-acetylglucosamine and sialic acid were found in CF-α_2M preparations when compared to those from age-matched controls. In contrast, the carbohydrate composition of purified CF transferrin preparations did not differ from that of control preparations. Mannosylation of endogenous and exogenous glycoproteins was also examined in CF and control fibroblast lysates using alternatively GDP-[^{14}C] mannose or dolichol-P-[^3H] mannose as the mannosyl donor. No significant differences were observed between the two groups in the incorporation of radio-labelled mannose into fetuin, normal α_2M, CF-α_2M and fibroblast glycoprotein fraction (phosphotungstic acid-precipitable material). The rate of synthesis of dolichol-P-mannose from dolichol-P and GDP-mannose, in CF fibroblasts, was within the range found for age-matched controls. These findings suggest that the mannosylation processes in CF appear normal. Additional studies are required to determine if a faulty glycosylation is indeed involved in CF.