measured the genes controlling their expression were functioning as early as 40 days' gestation. Detection of hereditary disorders related to these enzymes could theoretically be accomplished as early as five weeks.

Human placental barrier to glucagon-I-125 early in gestation. P. ADAM, K. KING, R. SCHWARTZ, and K. TERAMO. *Case Western Reserve Univ. Sch. Med. at Cleveland Metro. Gen. Hosp., and Univ. Helsinki, Central Hospital, Helsinki, Finland.*

Studies of hormonal transfer have shown that the human placenta is impervious to 2 labeled polypeptide hormones-insulin and human growth hormone; but the transfer of glucagon, a polypeptide hormone of lower molecular weight, has not been evaluated previously in pregnant human subjects. Although its placental transfer has been demonstrated in other species, the results are confusing because of the non-specific methodology used. In order to determine whether the human placenta permits maternofetal transfer of glucagon, nine pregnant women at 15 to 17 wks of gestation were evaluated during legal therapeutic abortions by abdominal hysterotomy. The plasma concentration of glucagon-I-125 was maintained until delivery of the fetus by continuous intravenous infusion of the labeled hormone at the following rates: 20 μc/hr for 3–4 hrs in 4 women; and 60 μc/hr for 1 to 1.5 hrs in the other 5. The plasma concentration of the labeled glucagon was measured by a *specific* immunoprecipitation. Even with maternal plasma concentrations of radioactive glucagon between 599 and 1289 cpm/ml, no glucagon-I-125 was detected either in the umbilical venous or arterial plasma, or in the amniotic fluid. Early in gestation, therefore, the human placenta is an effective barrier to the rapid maternofetal transfer of glucagon-I-125. Based on this concept, regulation of the fetal plasma glucagon levels would depend on its secretion by the fetal rather than the maternal pancreas.

The placental calcium pump. INGEBORG C. RADDE, YEHEZKEL SHAMI, and DAVID K. PARKINSON. *Univ. of Toronto and Hosp. for Sick Children, Toronto, Ont., Canada* (Intr. by Andrew Sass-Kortsak).

During fetal life an uphill gradient for calcium ions exists between maternal and fetal circulations (maternal Ca^{2+} 1.95 mEq/l, fetal Ca^{2+} 2.45 mEq/l). We postulate that this gradient is maintained by an active transport system for calcium ions, similar to the calcium pump in renal tubular and intestinal mucosal plasma membranes. To characterize the enzyme, placental plasma membranes from guinea pigs were prepared according to the method of Post and Sen (Methods Enzymol. 10: 762, 1967). Samples were incubated for 30 min at 37 C in solutions containing 70 mM Na^+, 20 mM Tris (pH 7.6), Ca^{2+} or Mg^{2+} or both, in concentrations varying from 0.025 mM to 20 mM, and 5 mM Na_2ATP. P_i and protein were determined and results expressed as umole P_i released per mg protein in 30 min. Ca^{2+} in the absence of Mg^{2+} stimulated P_i production. Mg^{2+} in the absence of Ca^{2+} also stimulated the enzyme but to a lesser degree. 5 mM Ca^{2+} produced maximal stimulation (15–25 μmole P_i/mg protein in 30 min). Mn^{2+}, but not Sr^{2+}, stimulated P_i production, as with other Ca^{2+} ATPases (renal, intestinal mucosal). The pH optimum was 8.2; at 7.2 and 9.5 the enzyme activity was 50% of the maximum. Ouabain (1 mM) was not inhibitory, but addition of increasing amounts of EDTA led to progressive loss of activity; total inhibition occurring at 5 mM EDTA. Further fractionation of samples with sucrose-gradient centrifugation doubled the specific activity of the enzyme in the plasma membrane fraction. We believe that this enzyme of the placental plasma membranes activates a calcium pump which maintains the gradient of calcium ions between maternal and fetal circulations and ensures normal calcification in the fetus.

Fetal malnutrition. T. YOSHIDA, A. BERNAL, J. METCOFF, A. ROSADO, P. YOSHIDA, J. URRUSTI, L. VELASCO, and S. FRENK. *Univ. Oklahoma Med. Ctr., Okla. City, Okla., and Centro Medico Nacional, IMSS, Mexico City, D. F., Mexico*

Many instances of intrauterine growth retardation may represent fetal intrauterine malnutrition (IUM) rather than "placental insufficiency". Clinical, physiologic, and biochemical features simulate those of protein-calorie malnutrition (PCM) of infants. Cell size (protein/DNA) often is increased in IUM, but its relation to energy functions of the cell is uncertain. The present studies explored whether patterns of cell energy metabolism in IUM resembled those found in PCM, and if these patterns were similar in fetal and placental cells. Energy charge (EC) = (ATP + ADP/AMP + ADP + ATP), pyruvic (PK) and adenylic kinase (AK) and energy capacity (EC_a) = AK (ATP + ½ ADP) of leukocytes isolated from cord blood and of placentas were related to cell size (protein/DNA). For 13 IUM infants, leukocyte cell size was increased. PK and AK activities were reduced, compared to 28 low weight but appropriately nourished premies (P) or 33 full term (FT) infants. Most, but not all, of the differences were statistically significant. EC of the IUM leukocytes was not decreased; ATP and EC_a were. For placentas, while total DNA and RNA were reduced in 20 IUM's, cell size and ribosomal mass (RNA/DNA) were increased compared to 17 FT and 10 P. AMP was the only nucleotide significantly decreased in IUM's. Placental AK and PK were increased and correlated with cell size and birth weight in IUM babies. While EC was slightly decreased in IUM placentas, EC_a was increased. Thus, energy metabolism of IUM leukocytes is like infants with PCM, and metabolic changes in placental cells differ from those found in the infant's leukocytes.

Oxygen (O_2) consumption as measure of cell number in intrauterine growth failure (IUGF). INGEBORG KRIEGER and P. V. WOOLLEY, JR. *Wayne State Univ. Sch. of Med., Detroit, Mich.*

O_2-cons. in the basal state is a measure of the metabolically active tissue mass and, as such, may reflect cell number rather than cell mass. This was tested by comparing conditions which have a different ratio between cell number and cell mass (CNo). 24 patients with IUGF and persistent linear growth failure after birth (PIUGF) were assumed to have a low CNo. 31 patients with growth failure of postnatal onset due to undernutrition, group A, were assumed to have a higher CNo than PIUGF because undernutrition in not rapidly multiplying tissues decreases cell size. 24 patients with growth failure of postnatal onset and congenital anomalies were placed in group B, with unknown relative CNo. Ages were 4/12–10 years and height ages <3 years. 37 normal controls were <3 years old.

O_2-cons. for age and height age was significantly lower in PIUGF than all other groups. O_2-cons. of groups A and B was not different, i.e. significantly lower than the normal for age and similar to the normal for height age. O_2-cons. per body weight was normal in PIUGF and elevated in groups A and B. O_2-cons. per weight predicted from height was negatively related to height in per cent of the normal height for age. The regression for PIUGF was significantly lower than in groups A and B. A good correlation was evident only in group A ($r = .723$). This suggests an increase in metabolically active tissue with increasing

degrees of linear growth failure due to undernutrition, which is compatible with the known effect of chronic undernutrition on cell size. In concluding therefore that changes in CNo were responsible for this observation, it is suggested that the lower regression for PIUGF was also due to a lower CNo. By testing patients with persistent growth failure after birth we apparently singled out cases with "hypoplastic" IUGF.

Experimental alteration of intrauterine growth pattern in rhesus monkeys. DONALD E. HILL, ALAN B. HOLT, and DONALD B. CHEEK. *Johns Hopkins Univ. Sch. of Med., Baltimore, Md.*

The purpose of these studies is to indicate the biochemical alterations in intrauterine growth produced by interference with placental circulation or the chemical ablation of the fetal pancreas. In a group of pregnant rhesus monkeys the injection of Streptozotocin (approximately 75 mg/Kg) directly into the fetus produced cytotoxic effects on the pancreatic B-cells. Five of nine surviving animals had significantly large adrenals and livers. Body weight was normal. Two animals were significantly small for gestational age and had normal adrenals and livers. The remaining endocrine organs were of normal size in all animals. The protein:DNA ratio in muscle was increased in four animals. The fetal insulin levels at term were normal as were the blood glucose values. Regions of pancreatic regeneration and B cell activity could be identified on histological section. In a second group of pregnant monkeys the interplacental fetal vessels were ligated at 100 days gestation producing a reduction in placental mass and a placental insufficiency. Nine of 16 surviving fetuses had significantly low birth weights (2 S.D. below the mean) for gestational age. The brain was least affected while the liver was most affected and the small animals had a high brain:liver ratio. Total DNA, RNA, and protein were significantly low in muscle and liver. The protein:DNA ratio and the number of nuclei (cell number) were significantly low in muscle. The percentage fat was reduced in the carcass as well as the total fat, protein and collagen.

Secretory function of isolated parotid acinar cells. JOHN A. MANGOS and N. R. MCSHERRY. *Univ. of Wisconsin Med. Sch., Madison, Wisc.*

Functionally and anatomically intact acinar cells were obtained from the parotid gland of the rat by enzymatic dispersion. After exsanguination of the animal, the duct system of the gland was filled with a solution of hyaluronidase and collagenase (0.1%) in Ca±Mg free Hank's salt medium. The parotid was removed, minced and the tissue was incubated in the same enzyme solution for 60 min at 37°C. Then, the suspension was filtered through nylon mesh, the clumps of cells were separated by centrifugation, were suspended in a solution of trypsin (0.1%) and reincubated for 20 min. Finally, the cells were dispersed by pipetting the suspension 10 times, washed and then suspended in balanced Hank's solution containing bovine serum albumin (2 gm/100 ml). Yield of cells was $\sim$60% of the original gland tissue. The cells were anatomically intact and did not stain with 0.2% trypan blue for up to 8 hours. They showed normal respiration (O_2 uptake: 21.4 ± 2.7 μl/hour·mg protein) and responded to stimulants of secretory activity (epinephrine, isoproterenol, dibutyryl cyclic AMP, theophylline and NaF) by secreting amylase into the medium at rates approximately equal to those observed in the intact gland in situ.

This method permits the investigation of aspects of the secretory processes of exocrine glands which cannot be studied in the intact glands of experimental animals in situ or in gland slices. Furthermore, as preliminary experiments have shown, living acinar cells for similar secretory studies can be obtained from human salivary glands and pancreas immediately after death.

Regulation of cellular growth: Control of pyrimidine biosynthesis. RODNEY L. LEVINE, NICHOLAS J. HOOGENRAAD, and NORMAN KRETCHMER. *Stanford Univ. Med. Sch., Stanford, Calif.*

Dividing cells require adequate amounts of purine and pyrimidine nucleotides for nucleic acid synthesis. The *de novo* pathway of pyrimidine biosynthesis is regulated to meet these growth requirements. In mammalian tissues the first enzyme in the pathway, carbamoyl phosphate synthesis (CPS), is inhibited by the pyrimidine nucleotide UTP. This inhibition may effectively limit *de novo* pyrimidine synthesis since CPS activity is the limiting enzyme for the pathway.

A detailed study of the kinetics of UTP inhibition provides support for the hypothesis that regulation of CPS is the physiological mechanism for control of the *de novo* pathway. CPS gives a sigmoidal velocity curve when the substrate ATP is varied in the absence of UTP. The curve fits a 2/1 function which suggests that ATP stimulates activity by acting both as a homotropic substrate and as an allosteric effector. UTP inhibits by competition with ATP and increases the sigmoidality of the curve. The K_i for UTP is 35 μM. The kintetic constants (K_m for ATP = 3 mM) are such that CPS should be extremely sensitive to deviations from the normal cellular concentrations of ATP and UTP.

The purine nucleotide ATP stimulates CPS while the pyrimidine nucleotide UTP inhibits so that precursors of nucleic acid interact on the enzyme. Thus CPS may play a pivotal role in the regulation of cellular proliferation. These findings might explain the observation that uridine greatly reduces urinary excretion of orotic acid in children with hereditary orotic aciduria. This action further supports the concept that regulation of CPS is a physiologically important control mechanism.

The relation between DNA polymerase activity and DNA synthesis in specific regions of proliferating rat brain. Jo ANNE BRASEL (Intr. by Myron Winick). *Cornell Univ. Med. Coll., N. Y., N. Y.*

In a previous report demonstrating that activity of DNA polymerase parallels the rate of DNA synthesis in rat brain, we theorized that activity of this enzyme might serve as an *ad hoc* index of proliferative cell growth in normal tissues. Since individual brain regions have different rates of cell division and different times when maximum rates are attained, regional patterns of DNA polymerase activity have been examined and the characteristics of the enzyme further delineated. The data demonstrate that the enzyme is replicative and not reparative, is stimulated by glycerol, is linear with concentration and time and has an almost absolute requirement for DNA primer. Activity is always higher in cerebellum where the rate of DNA synthesis is rapid than it is in forebrain where cell division is slower. Activity in forebrain peaks between 10 and 12 days precisely when the rate of DNA synthesis is maximal in this region. By contrast in cerebellum there are two peaks of DNA synthesis at 7 and 13 days. DNA polymerase activity is also biphasic with peaks just preceeding each of the synthesis peaks. These data reinforce the concept that activity of this enzyme parallels the rate of cell division during proliferative cell growth. As such it should provide a