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 ~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 preceding 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

unique background upon which to study the effects of stimuli which alter DNA synthesis.

The normal oxygen consumption and respiratory quotient of the mammalian fetus. ELIZABETH J. JAMES, JOHN R. RAYE, EDWIN L. GRESHAM, EDGAR L. MAKOWSKI, GIACOMO MESCHIA, and FREDERICK C. BATTAGLIA. *Univ. of Colorado Med. Ctr., Denver, Colo.*

There are no data on the normal oxygen consumption and respiratory quotient of the mammalian fetus. Such data are badly needed, both for a proper understanding of fetal respiration and as metabolic reference standard. Previous studies were limited to acute experiments and showed a wide range of variability in oxygen consumption (range 3.7–8.6). In attempting to obtain baseline data, oxygen consumption, CO_2 production and glucose utilization were studied in the unstressed fetal lamb. Umbilical blood flow was determined by constant fetal infusion of antipyrine and umbilical arterial-venous differences of glucose, O_2 and CO_2 were measured during the infusion. The experimental design was such that during each study period at least 5 observations of O_2 consumption could be made and expressed with reference to fetal weight. The following table summarizes the data:

	Flow ml/min·kg	O_2 Consumption ml/min·kg	CO_2 Production ml/min·kg	Glucose Utilization mg/min·kg
Mean ± SEM	191 ± 12	5.71 ± 0.11	5.41 ± 0.18	2.86 ± 0.31

Contrary to previous reports, little variability in O_2 consumption over a wide range of umbilical blood flows was found. Fetal glucose uptake could account for approximately half of the O_2 consumed; the mean fetal RQ was 0.94 (95% confidence limits 0.90–0.99). As with our earlier studies, these data suggest that metabolites other than glucose are important substrates in fetal aerobic metabolism.

Measurement of fatty acid and glucose oxidation by fetal heart cells in monolayer tissue culture. JOSEPH B. WARSHAW and MIRIAM D. ROSENTHAL. *Harvard Med Sch., Mass. Gen. Hosp., Shriner's Burns Inst., Boston, Mass.*

We have utilized a method to investigate substrate oxidations of embryonic chick heart cells growing in monolayer culture. The cells are grown in small capped Falcon flasks in F-12 media supplemented with fetal calf serum and chick embryo extract. For the assay the cells are washed with buffer to remove the media and are then incubated with ^{14}C-palmitate for 1 hour at 37°. After perchloric acid is injected through a serum cap to terminate the reaction, Hyamine-OH is injected into a polypropylene well fixed to the serum cap to trap the evolved $^{14}CO_2$ which can then be counted.

The oxidation of palmitic acid is very active and further stimulated if carnitine is included in the media. Palmitic acid oxidation is decreased by over 75% if the cells are scraped from the flasks prior to the assay perhaps due to alterations of the surface properties of the cells. The specific activity of palmitic acid oxidation is constant regardless of days in culture or initial plating density. In contrast, the specific activity of glucose oxidation is high during cell proliferation and markedly decreases as the cells plateau. Scraping the cells from the flask had little effect on oxidation. The decrease in glucose oxidation is associated with a striking fall in the ratio of glucose-1-^{14}C to glucose-6-^{14}C oxidation indicating a shift away from the hexose monophosphate shunt. Insulin stimulates glucose oxidation by 13 day embryonic heart cells when the cells are proliferating and show high specific activity for glucose oxidation. This method provides a rapid and convenient system for investigation of substrate oxidations in tissue culture.

Binding of drugs to cord plasma. ALBERT W. PRUITT and PETER G. DAYTON (Intr. by Richard W. Blumberg). *Emory Univ. Sch. Med., Clin. Pharm. Prog., Atlanta, Ga.*

The purpose of the present work is to compare the binding of several drugs—diphenylhydantoin (DPH), cephalothin (CPT), cephaloglycin (CPG), imipramine (IMI), and diazoxide (DX) to plasma from adults and from newborns. Adult plasma was collected from 3 healthy subjects and cord plasma from 9 term newborns. The drugs, except for DX, were ^{14}C labeled. After equilibrium dialysis with the plasma sample, the % binding of drug to plasma was determined. In this series, the average albumin in the adult plasma is 4.1 and in cord plasma 3.4 gm%. For each of the drugs studied, the binding to adult plasma is greater than to cord plasma. The average value for binding of DPH to cord plasma is 74% compared to 83% bound in adult samples. Using CPT, 72% of the drug is bound in cord plasma and 80% in adult plasma. CPG is not highly bound and the difference is not striking (60% to cord plasma and 63% to adult plasma). The binding of IMI to adult plasma is 89% and to cord plasma is 74%, but this drug is bound to plasma proteins other than albumin since binding to 3% human albumin is only 61%. DX is a very highly bound drug in both cord plasma (88%) and adult plasma (92%). The differences in binding between adult and newborn plasma can be correlated most clearly with reduced albumin concentration in the newborn. If adult plasma is diluted to the albumin concentration of cord plasma, the binding data are similar to that for cord plasma. The infant, with normally lower plasma albumin levels, will therefore have a greater fraction of free drug in plasma which is available for tissue distribution and for glomerular filtration.

Some membrane effects of bilirubin. MARILYN L. COWGER and MOHAMMAD G. MUSTAFA. *Albany Med. Coll., and State Univ. of New York, Albany, N. Y.*

The respiratory lesions induced by bilrirubin do not seem to adequately explain the great toxicity of this bile pigment. Bilirubin is highly lipophilic; thus, it was postulated that bilirubin has a general effect on membrane systems. Experimental evidence to support this hypothesis comes from studies using modified L-929 tissue culture cells, rat liver, bovine heart, and brain of several species. At a bilirubin concentration of 25 μM the plasma membrane of L-929 cells following a lag phase of 60–90 minutes becomes permeable to large dye molecules concurrent with the extrusion of protein molecules. Earlier changes in the plasma membrane can be shown by alterations in Na^+ and K^+ transport occurring within the first hour of exposure to bilirubin.

Mitochondrial membranes are exquisitely sensitive to bilirubin. Low (<20 μM) concentrations increase and high (>50 μM) concentrations decrease the respiration of liver and heart mitochondria, but brain respiration is always inhibited without this biphasic effect. Bilirubin in micromolar concentrations abolishes respiratory control, uncouples oxidative phosphorylation, and induces high amplitude irreversible swelling (K_m is 2.5 μM for swelling in liver and heart. (Brain is slightly more sensitive (K_m