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

is 2 μM). All of these mitchondrial reactions are membrane linked. Electron microscopy confirms the biochemical data of swelling.

Lysosomes of rat liver and L-929 cells demonstrate increased permeability within one hour of exposure to bilirubin using acid phosphatase activity as a monitor of membrane fragility. This effect is seen at relatively high bilirubin levels (500 μM) but preliminary evidence suggests concentration of bilirubin by lysosomes.

Influence of early malnutrition on drug metabolism and effect. C. S. CATZ, J. A. BRASEL, M. WINICK, and S. J. YAFFE. *Sch. of Med., State Univ. of New York at Buffalo and Cornell University, Ithaca, N. Y.*

Male Sprague-Dawley rats were raised in litters of 10 (controls, C) and 18 (malnourished, M) until 21 days of age (weaning). Total body and liver weights were decreased by 30% in the M group. As a consequence liver weight remained at the same percentage of body weight (4.89 $\pm$ 0.1) as in the controls (4.75 $\pm$ 0.2). The microsomal components of the electron transport chain for oxidative pathways were similar in both groups. Metabolism for several different oxidative substrates (aminopyrine, aniline, and benzpyrene) was increased significantly in liver homogenates in the M group. Since drug metabolism is the most important determinant of drug effect, hexobarbital was used in correlative *in vivo* studies. The duration of sleep (the major action of hexobarbital) was surprisingly longer in the M animals (160 $\pm$ 9 minutes) than in the controls (112 $\pm$ 6 minutes) at a dose of 100 mg/kg. Since hexobarbital metabolism was not decreased in the M group these findings strongly suggest that brain sensitivity is altered. This may have important consequences for drug usage in malnourished children.

Pharmacologic modification of bilirubin toxicity in tissue culture. RICHARD P. WENNBERG and L. FRASER RASMUSSEN (Intro. by David Baum). *Univ. of Wash. Sch. of Med., Seattle, Wash.*

While several areas of bilirubin toxicity have been identified, the mechanism of bilirubin entry into cells and the mode of cell death are not understood. Several substances which have been associated with membrane functions were examined to determine if they would influence bilirubin toxicity. Strain 929 L-cells were washed four times in protein free media and incubated one hour with the test drug before adding bilirubin. Ten μM bilirubin killed >90% cells in four hours as determined by cell penetration of erythrocin B. Hydrocortisone totally protected the cells from bilirubin toxicity; prednisolone was slightly less effective. The rate of cell death was retarded by insulin, but only in very high concentrations (0.2 units/ml). Theophylline and caffeine, which inhibit the breakdown of cyclic AMP by the enzyme phosphodiesterase, offered partial protection. Paradoxically, epinephrine and glucagon, which stimulate adenyl cyclase, and cyclic AMP and dibutyrl cyclic AMP either failed to protect or even accelerated cellular death with bilirubin. These effects could be blocked with theophylline and caffeine, suggsting that AMP or phosphodiesterase itself may be involved in bilirubin toxicity.

These studies reveal additional parameters of bilirubin toxicity and suggest the possibility of altering susceptibility for kernicterus with pharmacologic agents.

A six year prospective controlled study of neonatal hypoglycemia. ROSITA S. PILDES, IRVINA S. WARREN, SALVATORE DIMENZA, EDWARD PAGE-EL, SANDRA NEUWELT and MARVIN CORNBLATH. *Univ. of Illinois, Abraham Lincoln Sch. of Med., Chicago, Ill., and Univ. of Maryland, Sch. of Med., Baltimore, Md.*

Between 1961–1964, thirty-nine newborns with transient neonatal hypoglycemia (Group I) were matched with 41 controls (Group II) on the basis of 9 weighted clinical criteria. On-going medical and social service care was provided and yearly EEG's, neurological and psychological examinations were done. Computer analysis indicated the infants to be well matched according to medical criteria as well as socio-economic background. The incidence of R.D.S., sepsis, hyperbilirubinemia, polycythemia and C.N.S. problems was similar in both groups. Nevertheless, the clinical course of Group I was more severe due to the manifestations of hypoglycemia. Recurrent hypoglycemia was seen in 4 children; there were no deaths in either group. The follow-up data on physical development indicate that Group I showed a significant lag in height and weight until 3 years of age, after which both groups were in the 25th percentile. Head size, significantly smaller at birth in Group I, remained below the 3rd percentile at age 6. An analysis of 214 EEG's failed to reveal any significant differences in abnormalities between the groups. Stanford-Binet scores at age 5 showed a mean IQ of 87 $\pm$ 4 in Group I (22) vs 94 $\pm$ 4 in Group II (20) children. At age 6, the mean IQ was 88 $\pm$ 4 in Group I (14) and 96 $\pm$ 3 in Group II (18) children. These differences are not significant. W.I.S.C. scores at age 5 and 6 were similar in both groups. To date, the prompt and vigorous treatment of symptomatic neonatal hypoglycemia would appear to obviate marked differences in development.

GENETICS

Normal, Duarte Variant, and galactosemic alleles code for immunologically identical gal-1-P uridyl transferase enzyme protein. THOMAS A. TEDESCO and WILLIAM J. MELLMAN. *Univ. of Pennsylvania Sch. of Med., Philadelphia, Pa.*

Human galactose-1-phosphate uridyl transferase was purified from post-mortem liver to a preparation having a single band in polyacryamide gel electrophoresis. This preparation was used successfully to produce a rabbit antibody that precipitates transferase activity from solution and that forms a precipitin band in double immunodiffusion. Hemoglobulin-free erythrocyte preparations from homozygous normal (Gt^+/Gt^+), Duarte Variant (Gt^D/Gt^D), and galactosemic (Gt^G/Gt^G) individuals show immunoprecipitin bands in double immunodiffusion against this antibody that are identical with that of the purified transferase preparation. The results indicate that the three alleles code for immunologically similar enzyme proteins suggesting that the functionally less active Duarte Variant and inactive galactosemic enzyme proteins have resulted from "point" mutations.

Fabry's disease: Evidence for structural mutation of α-galactosidase. GIOVANNI ROMEO and BARBARA R. MIGEON, *Johns Hopkins Hosp., Baltimore, Md.*

Fibroblasts from a patient with Fabry's disease have an α-galactosidase activity corresponding to 10–20% of control values, and the same difference has been found between the 2 clonal populations derived from the patient's mother and sister (Science 170: 180, 1970). The α-galactosidase present in fibroblasts of 2 unrelated patients and in "negative" clones of 2 heterozygotes shows a slower rate of heat inactivation than the enzyme of