

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<sub>2</sub> 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<sub>2</sub> and CO<sub>2</sub> were measured during the infusion. The experimental design was such that during each study period at least 5 observations of O<sub>2</sub> consumption could be made and expressed with reference to fetal weight. The following table summarizes the data:

	Flow ml/min·kg	O <sub>2</sub> Consumption ml/min·kg	CO <sub>2</sub> Production ml/min·kg	Glucose Utiliza- tion 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<sub>2</sub> consumption over a wide range of umbilical blood flows was found. Fetal glucose uptake could account for approximately half of the O<sub>2</sub> 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., Shriners 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 <sup>14</sup>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 <sup>14</sup>CO<sub>2</sub> 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 as-

sociated with a striking fall in the ratio of glucose-1-<sup>14</sup>C to glucose-6-<sup>14</sup>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. FRUITT 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 <sup>14</sup>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 bilirubin 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<sup>+</sup> and K<sup>+</sup> 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<sub>m</sub> is 2.5 μM for swelling in liver and heart. (Brain is slightly more sensitive (K<sub>m</sub>