DEVELOPMENTAL BIOLOGY

Monolayer Cultures of disaggregated human adipocytes, Festus O. Adebajo, Children's Hosp., Phila., Pa. (Intro. by Alfred Bongiovanni).

Two theories of adipose cell origin have been proposed. One suggests that they develop from specialized anlage whose cells spread throughout the body and accumulate fat. The other holds that they form by accumulation of fat in unspecialized cells of connective tissue. Each theory has some experimental support.

Animal adipose cells have been grown readily in tissue culture but human adipocytes have been more difficult to grow. This study was undertaken in an effort to understand the origin of adipocytes and their relationship to obesity. Disaggregated adipose cells taken from human infants of different ages were grown in tissue culture. Under conditions of culture used the cells changed from their usual spherical to a fibroblast appearance, gradually lost intracellular lipids, then divided. Adipocytes from older children also changed shape, lost intracellular lipids more slowly but were not observed to divide.

These findings, while shedding no light on the origin of adipocytes, suggest that their ability to divide may depend, at least in tissue culture, on the donor age.

POSTMORTEM VITREOUS HUMOR CONCENTRATIONS OF NA, K, CL, CA & MG IN CHILDREN. Thomas A. Blumenfeld, William A. Blanc, John Devlin. Columbia Univ. Col. of Phys. & Surg., N.Y., Dept. Ped. & Path., and The Office of Medical Examiner, N.Y.C.

This study was performed to determine postmortem vitreous concentration (PVC) of Na, K, Cl, Ca and Mg from newborns (1h-8d), group I, and older children (2mo-13yr), group II, with no known antemortem abnormalities of these substances. It is known that the PVC can reflect abnormal antemortem serum concentration but the relationship of PVC to patient age and to hours between death and aspiration of specimens [postmortem interval (PMI)] has not been studied in the pediatric age group.

	GP.	PMI	PVC	I vs II	PVC vs PMI (r)
Mg(mg/dl)	I(15)	3-29	5.8±0.7	signif.	.09 not signif.
	II(18)	4-24	3.3±0.5	p<.01	.12 " "
Ca(mg/dl)	I(8)	3-29	8.5±0.7	"	.45 " "
	II(17)	4-24	6.9±0.9	"	.26 " "
Cl(meq/L)	I(10)	3-29	113±4	"	.00 " "
	II(10)	4-24	122±8	"	.22 " "
Na(meq/L)	I(10)	3-29	139±7	not signif.	.35 " "
	II(10)	4-24	141±7	"	.50 signif. p<.01
K(meq/L)	I(15)	3-17	12.2±2.3	"	.50 signif. p<.01
	II(19)	4-24	11.1±2.8	"	.50 signif. p<.01

In this study the PVC of Ca, Mg, Cl, but not Na, K, is significantly different in groups I and II. The correlation of PVC and PMI is significant only for K and thus the PVC of K can give an indication of PMI ± 5 h.

RECOGNITION AND ADHESION OF EMBRYONIC CELLS. Jean-Jacques Cassiman and Merton R. Bernfield, Ped. Dept., Stanford Univ. Sch. of Med., Stanford, Calif.

To test the hypothesis that deviations in cell recognition and adhesion may be involved in abnormal development, chick embryonic cells were used to develop a quantitative assay for adhesive recognition and to investigate its molecular basis. The assay avoids dissociated cells containing altered surfaces, and is based upon the differential ability of pre-formed cell aggregates to bind to cultured cell layers. The proportion of aggregates bound was independent of the number added, was second-order with respect to time of incubation and this second order rate varied with the cell type. Adhesion between like types was greater than between unlike types, indicating that the assay measures cell recognition. Modification of binding by varying incubation temperature, treatment with metabolic inhibitors and with varying concentrations of protein cross-linkers showed that adhesive recognition involves mobile elements embedded within the plasma membrane and is not dependent upon metabolic activity; that the elements involved in recognition differ from those involved in adhesion, and that the effect of these modifiers varied with the cell type. These data suggest that intercellular adhesive recognition is due to complementary surface components which differ in concentration on various cell types. Mouse fibroblasts showed similar adhesive recognition properties, possibly allowing analysis of these properties in fibroblasts from individuals with birth defects.

ALANINE-GLUCAGON INTERRELATIONS IN HUMAN NEWBORNS. Paul V. Delamater, Mark A. Sperling, Robert H. Fiser, Jr., Dale L. Phelps, William Oh, Delbert A. Fisher. UCLA - Harbor General Hospital, Dept. of Pediatrics, Torrance, California.

The significance of key substrate-hormone interrelations in neonatal glucose (G) homeostasis, was ascertained by measurements of plasma G, alanine (A), glucagon (GLU) and insulin (I) in the immediate hrs and days after birth. The effect of an A infusion was also studied. In cross-sectional studies over the initial 12 hrs of life, G fell (77 ± 6 mg% @ 1/2 hr to 57 ± 5 mg% @ 1-1/2 hrs) while GLU increased (224 ± 27 pg/ml to 420 ± 81 pg/ml) significantly, but A and I remained unchanged. When infants were sampled sequentially the fall in G (cord 133 ± 21 to 49 ± 14 mg%) and rise in GLU (83 ± 27 to 139 ± 41 pg/ml) during the first 2 hrs post birth was confirmed. G and GLU remained stable during the next 10 hrs but rose after 24 hrs; highest values were observed on day 3 (61 ± 6 mg% and 243 ± 30 pg/ml). Plasma A rose progressively; the peak at 12 hrs coinciding with the nadir of G. An increase in A also occurred from days 1 to 3. I remained unchanged throughout the period of observation. An I.V. infusion of 1mM/Kg A into 6 fasting infants at 12 hrs produced an elevation of plasma GLU at 15 min (208 ± 27 to 293 ± 62 pg/ml) and an increase in G at 30 min (47.8 ± 3.1 to 54.7 ± 1 mg%). Thus, in the neonate GLU secretion occurs in response to the stimulus of G need or gluconeogenic amino acid infusion. No consistent relation of endogenous A to G or GLU levels was observed but the response to A infusion suggests a potential role for this substrate in neonatal G homeostasis.

EFFECT OF EXCHANGE TRANSFUSION AND CARBON MONOXIDE(CO) ON BRAIN AND HEART MITOCHONDRIA OF THE NEWBORN ANIMAL. Maria Delivoria-Papadopoulos, Lena Mela, and R.E. Forster. Univ. of Pennsylvania Sch. of Medicine, Philadelphia, Penna.

Adult mitochondria show a high degree of adaptability to large changes of systemic PO₂. The present studies illustrate response of mitochondrial respiratory activity in newborn piglets and lambs after exchange transfusions or rebreathing of 15% CO. Measurements of P₅₀, P₅₀O₂, and S_{O₂} were done in all animals. Brain and heart mitochondria were isolated, and State 4(substrate, no ADP) State 3(substrate ADP) respiratory rates were calculated as nmoles O₂/min/mg protein, as well as respiratory control ratios (State 3 to State 4) and ADP/O ratios. All preparations were well coupled having respiratory control ratios about 10 and close to theoretical ADP/O ratios. Control animals from birth to 4 days old showed a linear decrease of State 3 respiratory rates from a mean of 120 to 55 nmoles O₂/min/mg(46% of control) followed by a linear rise to the control level at 10 days of age. In the exchanged animals (low HbO₂ affinity) State 3 respiratory rates increased by 38-90%. In the animals with COHb (high HbO₂ affinity) changes of State 3 respiratory activity were more prominent in the brain than in the heart mitochondria, and were similar to changes observed during acute or chronic tissue hypoxia in adults. These data suggest that the respiratory activity of newborn mitochondria is sensitive to relatively small, acute changes of HbO₂ affinity.

LECITHIN SYNTHESIS IN THE FETAL AND NEONATAL PRIMATE LUNG AS MEASURED *in vitro*. Philip M. Farrell and Michael F. Epstein, (Intr. by Paul A. di Sant'Agnes), NIH, Bethesda, Md.

Two pathways are present in fetal primate lung for *de novo* synthesis of lecithin: the choline incorporation route (I) and phosphatidyl ethanolamine (PE) methylation (II). Based on fatty acid analyses it has been proposed (Pediat. Res. 6:81, 1972) that the latter pathway is predominant in the human fetus until late gestation. Accordingly, lung slices from Rhesus monkeys were incubated with isotopic precursors including either ¹⁴C-choline (I), ¹⁴C-methionine (II), or ¹⁴C-ethanolamine (II) and incorporation of isotope into lecithin was determined. Measurement of precursor uptake was also performed, and the phospholipid synthesis rates were adjusted for isotope transport.

LECITHIN SYNTHESIS (cpm/mg lung/hr)

Gestation	No.	Pathway I	Pathway II
102 days	1	246	11
130 "	2	517	25
144 "	6	470	8
150 "	5	927	13
Neonates	5	375	16

(24-72 hr old)

The major pathway in all animals examined is the choline incorporation route. Whereas II shows a relatively constant, low rate throughout gestation (term 164 days), I increases at 150 days gestation (p<.05) and then decreases in the neonate.

It is concluded that pathway I is predominant and accounts for increased lecithin synthesis in the mature fetus as well as for the surge in amniotic fluid lecithin/sphingomyelin ratio.