● 1099 ABNORMAL NEUROTRANSMITTER STIMULATED PHOSPHATIDYL-INOSITOL METABOLISM IN EXPERIMENTAL GALACTOSE TOXICITY

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Irreversible mental retardation occurs in transferase deficiency galactosemia. Because of the documented alteration of CNS levels of free and lipid-bound inositol in the disorder, we have examined the metabolism of phosphatidylinositol (PhI) in isolated brain synaptosomes of galactose toxic rats. Emphasis has been on the effects of acetylcholine (Ach) since enhanced PhI turnover is seen in response to neurotransmitters. Experimental galactose toxicity was produced by feeding a 40% galactose diet to weanling rats. Cortical synaptosomes were prepared from control and galactose toxic rats from 37-83 days of age and incubated for 40 min with $^{33}PO_4$ and [^{3}H]-inositol in the presence or absence of Ach. Phospholipids were extracted from synaptosomal membranes; GC analysis was employed to determine chemical levels (nmole/mg protein) and specific radioactivities (dpm/peak area). Ach stimulation of synaptosomes from control and galactose toxic rats produced similar changes in PhI levels and $^{33}PO_4$ incorporation into PhI, however, synaptosomes from galactose toxic rats showed a 50% decrease in [^{3}H]-inositol labeling of PhI compared to control synaptosomes. This abnormality in PhI metabolism could be important in the CNS disturbances associated with galactose toxic states.

1100 THE ARRHENIUS PLOT OF HEPATIC MICROSOMAL GLUCOSE-6-PHOSPHATASE ACTIVITY OF THE CONGENITALLY JAUNDICED (GUNN) RAT.

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The failure of the Gunn rat to conjugate bilirubin results from either defective (or absent) bilirubin UDP-glucuronyl transferase or from a defect in the membrane environment of that enzyme. The latter possibility was investigated by constructing Arrhenius plots from 38-8°C of microsomal glucose-6-phosphatase activity of Gunn (jj,n=6), Wistar (JJ,n=9) and heterozygote (Jj,n=8) rats. This enzyme was studied because the Gunn rat is not defective in glucose-6-phosphatase, and this enzyme is tightly bound to the microsome. The plots of jj, Jj and JJ were identical in the following ways: a) the specific activity at 37°C was 2.51 µmol hydrolyzed/mg protein/10 min indicating no jj defect in enzyme activity; b) there was a change in slope at 35°C probably indicating a change in protein structure; c) the energy of activation was 12800 cal/mol. However, a clear difference was observed in the following way: JJ demonstrated a discontinuous plot indicating a lipid phase change at 11°C, but the plot of jj was a continuous straight line to 8°C. Jj exhibited an intermediate discontinuity at 8°C. This shift in the phase change to colder temperatures indicates a difference in membrane fluidity among groups. The proposed explanation for the deficient activity of bilirubin UDP-glucuronyl transferase in the Gunn rat is a difference in membrane fluidity which results in an anomalous environment for the enzyme.

1101 HYPERLIPIDEMIA AND GLUCOSE INTOLERANCE WITH HYPOCUPRINEMIA IN MENKES' DISEASE.

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Two infants presented with fine brittle hair with characteristic kinking. Their clinical course was characterized by neurological degeneration and a tendency to gain weight. In Case #1 (6 m. male) the S. copper was 5.09 ug/dl (80-120) and ceruloplasmin 2.44 ug/dl (20-50). I.V. GTT demonstrated glucose intolerance with K values of 0.81 and 0.825 but with an insulin peak of 35 uU/ml. Triglyceride TG=66 mg/dl and cholesterol TC=178 mg/dl (normal TG=73±37, TC=150±25). Apoliproteins (Apo's) were Apo AI, 97.4 mg/dl and Apo B, 110.9 mg/dl. In Case #2 (13 m. male) the S. copper was 12.7 ug/dl and ceruloplasmin 1.58 mg/dl. The I.V. GTT K rate was 1.98; TG, 149 mg/dl; TC, 160 mg/dl; Apo's AI, 100.2 mg/dl; AII, 51.7 mg/dl; B, 110.6 mg/dl; CIII, 10.5 mg/dl; E, 7.0 mg/dl; post-heparin lipoprotein lipase activity was 14.94±3.62 and triglyceride lipase activity 10.94±4.63 units per ml per hour using heparin sepharose affinity chromatography for enzyme separation and ^{14}C labeled triolein as substrate (young adult normal LPL=14.9±3.6, TGL=10.9±4.6). Lipoprotein separation revealed a predominant increase in TG, TC and Apo B in VLDL in Case 2. Double diffusion analysis and crossed electrophoresis against anti-Apo B on agarose gels revealed normal immunoprecipitin lines. We conclude that in Menkes' disease, copper deficiency may cause secondary hyperlipidemia as suggested by Allen, K.G.D. and Klevay L.M. (Life Sciences 22, 1691, 1978).

● 1102 HEPATIC GLUCOSE METABOLISM AND GLYCOGENOLYTIC RESPONSE TO HYPOXIA IN FETAL LAMBS.

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To assess the role of the liver in glucose (G) metabolism, we studied G flux across the liver in 7 fetal lambs (118-123 days) 2-5 days after catheterization of descending aorta (AO), inferior vena cava (IVC), umbilical vein (UV), portal vein (PV), and right (RHV), left (LHV), or both hepatic veins. Blood flow to each liver lobe from hepatic artery, UV and PV were measured by injections of microspheres into IVC, PV, and UV. G concentrations were measured in all sites and G consumption ($\dot{V}G$) was measured by the Fick method. Studies were done before and during maternal hypoxemia. G concentrations (mg/dl) were:

	Control					Hypoxia				
	AO	UV	PV	HV	IVC	AO	UV	PV	HV	IVC
Right lobe	17.9 ±5.9	19.7 ±5.1	17.1 ±5.4	19.4 ±5.5	16.8 ±5.9	20.8 ±4.6	22.5 ±4.3	19.0 ±3.4	23.5 ±4.0	16.9 ±3.7
Left lobe	19.9 ±4.3	21.8 ±3.1	19.9 ±4.5	21.4 ±3.4	18.3 ±3.2	21.7 ±5.8	23.1 ±6.4	20.5 ±4.4	25.7 ±5.5	17.7 ±3.7

In C, RHV and LHV glucose are similar to UV G, and the liver is in zero G balance; with hypoxia, HV G rises rapidly above UV G, indicating release of glucose, presumably by glycogenolysis, from L and R liver lobes. $\dot{V}G$ uptake in placenta and liver were:

	Placenta	R lobe		L lobe	
	mg/min/kg	mg/min	mg/min/100g	mg/min	mg/min/100g
C	3.8±2.2	-0.5±0.9	-1.2±2.4	0.4±0.4	1.2±1.0
H	3.1±2.8	-2.2±0.8	-5.4±1.8	-2.4±0.2	-8.4±2.0

Glucose production by the liver is important in meeting increased fetal needs for anaerobic energy substrate during hypoxia.

● 1103 SCLERODERMA-LIKE SYNDROME AND THE NON-ENZYMATIC GLUCOSYLATION OF COLLAGEN IN CHILDREN WITH POORLY CONTROLLED INSULIN DEPENDENT DIABETES (IDDM).

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104 children with IDDM were examined for induration and thickening of the skin and for joint contractures. Five patients had both multiple joint involvement and skin changes; 3 were studied in detail. Their diabetes ranged from 7-16 years in duration and was characterized by short stature, hepatomegaly, 4 to 6 hospitalizations each year, and glucosylated Hgb levels of 12-16%. All 3 had restrictive pulmonary lung disease. Histopathology of skin biopsies (bxs) demonstrated increased accumulation of collagen in the lower dermis. Three skin bxs were homogenized and compared to 6 bxs from age matched controls. The extractability of collagen in 0.5 N acetic acid was decreased to about ½ normal in 2 of the patients, suggesting increased cross-linkage of collagen. The amount of hexose bound to protein in a ketoamine linkage was assayed as the 5-hydroxymethylfurfural derivative. In patients, the mean non-enzymatic glucosylation was 4.0 (range 0.9-8.5) ng hexose/ug hydroxyproline which was 13 times that of controls (mean=0.3, range 0.2-0.4, ng hexose/ug hydroxyproline). The results suggest that non-enzymatic glucosylation may alter the packing, cross-linking and turnover of collagen, thus contributing to the development of a scleroderma-like cutaneous syndrome in IDDM.

1104 DECREASED INSULIN BINDING AND PRODUCTION: PROBABLE MECHANISM FOR HYPERGLYCEMIA DUE TO THERAPY WITH PREDNISONE (PRED) AND L-ASPARAGINASE (ASP).

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Asp and pred (40 mg/M^2/day) were found to act synergistically in producing hyperglycemia during leukemia remission induction. To explain the mechanism, glucose and insulin (µU/ml) levels during oral glucose tolerance test (OGTT) and erythrocyte insulin binding studies were performed serially during the course of therapy in an obese 8-year-old girl with Down's syndrome.

Days of Pred	Fasting Insulin	% Insulin binding	Receptor No. (per cell)	Average Affinity Ke (10^8M^{-1})
0	11	10.0	278	0.71
5	60	4.5	133	0.62
6*	53	3.8	119	0.60

*One day after Asp (10,000 units/M^2)

Five days of pred therapy alone resulted in hyperinsulinemia (594 µU/ml) and decreased insulin binding without OGTT deterioration. Following Asp, fasting and postprandial glucose increased to 241 and 514 mg/dl, respectively whereas insulin response was inadequate (peak < 130 µU/ml). These data suggest that the mechanism of Asp and pred induced-hyperglycemia is a combined effect of reduced insulin binding and impaired insulin production or secretion (or both).