55 ENAMEL DEFECTS IN LOW BIRTHWEIGHT CHILDREN WITH NEONATAL PROBLEMS: A HISTOLOGICAL STUDY.

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A clinical study has shown that low birth weight (LBW) children (≤2kg) had significantly more enamel defects of their deciduous dentition than normal birthweight controls. There was an increased prevalence in children who had severe neonatal problems, or a 1 min Apgar of <4 irrespective of whether or not they had subsequent illness ($p<.001$). In a follow up histological study, 100 µm sections of deciduous incisors were examined with light and polarization microscopy. LBW children often had a more marked neonatal incremental line in enamel than the controls. Defects were seen corresponding to enamel formation at the time of stress to the infant, but later enamel formation was normal as the child recovered and the insult to the developing tooth was removed. By studying dental tissues from these LBW children with well documented perinatal histories it is hoped to determine the value of deciduous teeth as a potential tool for studying disturbances of general growth and development during this period.

56 INFLUENCE OF FOOD RESTRICTION AND OF LOW-PROTEIN DIET ON URINARY EXCRETION OF MODIFIED RNA CATABOLITES AND OF 3-METHYLHISTIDINE (m^3HIS) IN RELATION TO N-BALANCE IN GROWING RATS

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For assessing the nutritional and metabolic status in man, we have shown in man and animals that by measuring quantitatively excreted urinary modified RNA-catabolites the whole-body turnover of tRNA (from t^6A, N^6-threoninocarbonyl-adenosine), rRNA (from Ψ, pseudouridine) and mRNA (from m^7Gua, 7-methyl-guanine) can be estimated similarly as that of actin + myosin is determined from urinary m^3His. Growing rats (41-54d) were fed either a control diet(C, 20 energy% as protein) or 1/2 C or 1/4 C, or they were given a low-protein (0.4 energy%) diet (LP) ad libitum. N-retention(NR): in control animals NR was 1-1.5g/kg/d. With C/2, NR briefly fell to 0, then returned to ~1g/kg/d. With C/4 NR was close to 0 for 3 days, then fell to -0.75g/kg/d during days 4-5. With LP, NR dipped to -0.5g/kg/d and gradually returned to 0. Food restriction: in the C/4 group Ψ and m^7Gua increased within a day by 22-25%, then fell to normal (Ψ) or subnormal (m^7Gua, -18%) levels until day 5. m^3His was elevated by 50% from day 2 onwards. We conclude that there was a rapid transient breakdown of ribosomes and mRNA followed by a longer lasting breakdown of actin and myosin. Protein restriction: with LP, m^7Gua fell by ~20% within a day and stayed near −25% (→day 14) while Ψ started to decline on day 2, reached −30% on day 5 and stayed there (→day 14). m^3His was markedly increased only on days 2-3. These changes suggest the occurrence of an ordered sequence of efficient RNA and protein sparing mechanisms as an adaptation to protein deficiency.

57 IN SITU FLUORESCENCE ANISOTROPY MEASUREMENTS IN CULTURED CELLS BY A COVERSLIP TECHNIQUE USING 1-(4-TRIMETHYL-AMMONIUMPHENYL) -6-PHENYL -1, 3, 5- HEXATRIENE (TMA-DPH)

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Fluorescence anisotropy (rG) was determined in monolayers of cultured cells grown on glass coverslips under various conditions with 5µM TMA-DPH in Hank's buffered salt solution at 37°C. rG was inversely related to membrane fluidity and increased with density of cells and age in culture. rG (±SD) was different in confluent human fibroblasts (0.348±0.016), rat astrocytes (0.315 ±0.011) and rat Roc-1 hybridoma cells (oligodendrocytes x C6) (0.304 ±0.018). Environmental factors like temperature, Ca^{++} and osmolarity as well as X-ray irradiation modified anisotropy in human fibroblasts. rG decreased in absence of extracellular Ca^{++} (0.301±0.027) and under hypotonic conditions (30 mosmol mannitol reduced rG to 0.298 ±0.015). On the other hand rG was shown to increase at deeper temperatures and under hypertonic conditions (550 mosmol mannitol led to rG of 0.388±0.013).

Conclusions: TMA-DPH is a marker of superficial layers of the membrane. Fluorescence anisotropy measurements in situ allow to study cellular and environmental factors in cultured cells and provide a sensitive method for detecting of possible genetic or acquired alterations of the membrane.

58 TRACE ELEMENT OVERLOAD IN PKU - DIET ?

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The knowledge of the trace element requirements of infants fed a phenylalanine restricted diet due to PKU (Phenylketonuria) is very limited. We studied 3 infants with PKU under their diet (Milupa PKU 1) longitudinally in balance studies (≥ 72 hours) under home conditions at the age of 2,5,8,12,16 weeks. Mn,Cu,Fe concentrations in diet, feces and urine were determined by Atomic Absorption Spectroscopy. Median concentrations found in the final diet were 1.7 mg/l Cu, 6 mg/l Fe, 0.5 mg/l Mn. The comparison of the retention rates with the data of 10 healthy breast-fed (BF) infants studied before (s. table) showed a higher range of Mn and Fe retention.

Mn, Cu and Fe retention (median and range)

Diet	Mn (µg/kg x day)	Cu (mg/kg x day)	Fe (mg/kg x day)
PKU	6.5 (- 33-39.7)	0.15 (0.06-0.25)	0.23 (-18-0.48)
BF	0.49 (-1.75-1.63)	0.098 (-0.05-0.23)	0.03 (-0.03-0.15)

No. of balances: PKU: Fe=10,Cu=10,Mn=10 --- BF: Fe=39,Cu=43,Mn=44

The median absolute retention of copper from PKU-diet exceeded that from human milk. Iron supplementation leading to median retentions sixfold the retentions from human milk seems to be unnecessary. Manganese supplementation should be avoided as neither symptoms of deficiency in formula-fed infants have been described nor possible side effects have been excluded.

59 FACTOR ANALYSIS OF CORD BLOOD LIPIDS.

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Analysis of cord blood lipids has been proposed for the early detection of familial hypercholesterolaemia and the assessment of the nutritional status of newborns. Lipid metabolism at birth, however, is affected by several variables wich must be taken into account for a correct evaluation of plasma lipid values. We studied the relation between gestational age and birth weight and plasma lipids at birth, making a factor analysis of the normalized values (i.e. mean = 0, DS = 1) for total cholesterol (TC), triglycerides (TG), HDL cholesterol (HDL), birth weight and gestational age of 70 newborn. The range of birth weight was 2160-4730 g; the range of gestational age was 32-42 weeks. This analysis led to the extraction of three factors that explained 84,7 % of the observed variance. The results are shown in a sequence of decreasing "eigenvalue"; only the coefficients above 0.20 are shown. The first factor links, as expected, gestational age and birth weight. The second is positively related to HDL and TC. The third factor, that summarizes an important degree of variation, shows that birth weight is negatively correlated with TC and positively with HDL.
These data indicate that cord blood lipids are related to birth weight. This variable must therefore be considered when screening for familial hypercholesterolaemia and assessing the nutritional status of newborns.

VARIABLE	FACTOR 1	FACTOR 2	FACTOR 3
Gestational age	0.925		
Birth weight	0.788		-0.420
TC		0.884	
HDL		0.835	-0.218
TG			0.969
Eigenvalue	1.831	1.308	1.094
% of Variance	36.6	26.2	21.9

60 RESPONSE OF PROSTAGLANDIN (PG) SYNTHESIS AND MEMBRANE FLUIDITY TO CHANGES OF OSMOLALITY IN CULTURED HUMAN SKIN FIBROBLASTS (HSF)

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The effect of changing osmolality on basal and bradykinin (BK) stimulated 6-oxo-PGF1α, PGE2, TxB2 and arachidonic acid (AA) synthesis of human skin fibroblasts was studied. In parallel, its influence on membrane fluidity was determined using 1-(4-trimethylammoniumphenyl)-6-phenyl-1, 3, 5 -hexatriene (TMA-DPH) as a marker of superficial cell membrane layers. Hypoosmolar mannitol (35 mosm/kg) onhanced basal 6-oxoPGF1α (152%), PGE2 (292%), TxB2 (181%), AA (549%) and BK stimulated 6-oxoPGF1α (194%), PGE2 (342%), TxB2 (152%) and AA (402%) production, when compared to isotonic control (100%). rG (fluorescence anisotropy, inversely related to membrane fluidity) was significantly decreased from 0.362±0.009 to 0.281±0.024 ($p < 0.001$). In hyperosmolar mannitol (610 mosm/kg) the basal and BK simulated PG synthesis was either unaltered or inhibited (in BK stimulated release PGE2 was 77% and AA 70%) . rG was slightly increased from 0.362±0.009 to 0.368±0.026 ($p > 0.05$), when compared to isotonic mannitol.

Conclusions: Osmolality modifies prostaglandin production and membrane fluidity in cultured human skin fibroblasts. Since changes in osmolality result in parallel changes of PG production and membrane fluidity it might be assumed that PG production is at least in part due to changes of membrane fluidity.