63 Bulk Fractionation of Donor Human Milk

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Milk donated by mothers of term infants may not be of optimal nutrient concentration if used to feed low birthweight infants (LBW), but since many milk constituents have functions other than classically 'nutritional' ones it is, in theory, qualitatively superior to artificial formulae. We describe a system for bulk preparation of a human milk protein concentrate which can be used in powder form to adjust the nutrient content of human milk to any specified protein/calorie content. Donor human milk is separated into skimmed milk (0.01g protein/g, 0.34kCal/g) and fat (0.01g protein/g, 2kCal/g). Skimmed milk is concentrated fourfold from 16-20L starting volume by a commercial ultrafiltration apparatus which contains 2.5 sq M of polysulfone membrane having a nominal molecular weight cut-off of 10,000. The resulting liquid concentrate (protein 0.04-0.05g/g) is lyophilised to a dry powder (protein 0.4g/g, energy 4kCal/g). Skimmed milk, protein-rich powder and fat are stored separately at -20°C. A microcomputer program has been developed which sepcified the weight of each fraction required to produce a human milk of any protein/calorie content desired. The human milk fractions can therefore be utilised to provide a feed of any composition required. Studies of the absorption and utilisation of milk constituents processed in this way are in progress.

64 Genetic complementation analysis of 3-Hydroxy-3-methylglutaryl-CoA lyase deficiency in cultured fibroblasts

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3-Hydroxy-3-methylglutaryl-CoA(HMG-CoA) lyase deficiency is an inborn error of leucine catabolism, characterized by metabolic acidosis and hypoketotic hypoglycemia, leading to vomiting, lethargy and coma. The clinical phenotype is variable, with severe illness neonatally in some patients, and a milder course in others. We studied cultured fibroblasts from 7 patients in an attempt to clarify the biochemical and genetic basis of this heterogeneity. The residual activity of HMG-CoA lyase was 1.1 ± 0.3%(mean±SD) of normal, with no significant differences between the patients. Genetic complementation was studied in heterokaryons obtained by fusion with polyethylene glycol. When cells with HMG-CoA lyase deficiency were combined with cells from patients with isovaleric acidemia or methylcrotonyl-CoA carboxylase deficiency, the incorporation of 1-14-C-isovaleric acid into protein increased from less than 5% to 30% of normal (positive controls). However, none of the fusions between the 7 HMG-CoA lyase deficient cell lines resulted in increased incorporation. Thus, no evidence was obtained for biochemical or genetic heterogeneity in this disease.

65 Temperature Variability of very low birthweight infants.

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Cold stress leads to increased mortality and morbidity in very low birthweight (VLBW) infants. We have studied temperature stability of VLBW infants in incubators controlled by air (air mode) and baby temperatures (servo mode) using a computer linked monitoring system, which continuously recorded 4 temperatures and humidity from the infant and environment. Thirteen infants (median bw 980 g, gest. 27 weeks) were studied using servo mode and five infants (median bw 1000g gest. 28wks) using air mode. No changes were made in unit policy and the infants were studied while undergoing routine intensive care in the first 4 days of life. Twenty four hour computer graphs showed clearly that the servo mode infants were exposed to wide swings in ambient temperature and to significantly more cold stress than those in air mode. These graphs also highlighted several other problems with the servo control system.

	n	Day 1	2	3	4
Servo	12	1.49 ± 2.55	.45 ± .68	.18 ± .09	.16 ± .10
Air	5	.22 ± .17	.15 ± .07	.26 ± .16	.13 ± .09

The table gives the mean (±SD) daily variance for core-toe temperature difference (ΔT) for the two groups. Infants in such servo mode showed a significantly greater variance of ΔT in the first 2 days, suggesting an increase in cold stress. Infant temperature became more stable with increasing gestational and postnatal ages. VLBW infants undergoing intensive care suffer less cold stress in air mode incubators.

66 INTERACTION OF ACTIVATED HUMAN PLATELETS WITH POLYMORPHONUCLEAR CELLS.

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The supernatants from human blood platelets activated by thrombin are chemotactic for polymorphonuclear cells(PMNs). We examined other functional and metabolic responses of PMNs to thrombin-activated vs unstimulated platelet supernatants. We showed that the incubation of PMNs with supernatant from activated platelets caused a significant increase in the rate of aggregation elicited by opsonized-zymosan(opZ), as compared to that from unstimulated platelets On the contrary, supernatants from activated platelets caused a 50% decrease in both the killing of staphylococcus aureus and in the luminol-enhaneed chemiluminescence by PMNs. No influence on O_2^- consumption and O_2^- production by opZ-activated PMNs was observed. Supernatants from activated platelets were able to reduce the release of myeloperoxidase from opZ-stimulated PMNs by 30 to 50%. The leakage of LDH from activated platelets was less than 3%, and no catalase and SOD activity was found in the supernatants. These data show that activated human platelets excrete some heat-stable factor(s), which modulate the PMN response to the stimuli.

67 I. Multicentre clinical trial on the effects of diet on low birthweight infants.

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Optimal dietary management of preterm infants is uncertain partly because it has been based largely on short term studies rather than on clinical outcome data. We introduce as a possible model for further trials, the structure of a large five-centre study designed to investigate dietary influences on morbidity and long term neurodevelopment, growth and clinical outcome. Currently, >600 unselected preterm infants (<1850g), both ill and well, have been randomised into 4 parallel trials involving paired comparisons of banked milk (BBM), adapted formula, or preterm formula (PTF) fed as sole diets or in conjunction with maternal milk. Overall postrandomization incidence of NEC (3.6%), death (5.8%) and serious metabolic complications are used to illustrate the very large calculated sample sizes required to provide adequate reassurance on clinical safety of available diets. In the short term BBM fed infants, compared with those fed PTF, have reduced steady gains in weight ($p<0.001$), length and OFC ($p<0.02$), different body proportions (reduced ratio; length or OFC: weight^2, ($p<0.01$), and marginally lower body water (by deuterium isotope dilution). Whereas at 2 kg, a PTF fed infant born at 1kg/28 weeks has maintained its birth centile, weight falls <3rd centile (Lubcenko) on breastmilk. These continuing trials will test the hypotheses that early growth influences neurodevelopment and programmes future growth.

68 Riboflavin deficiency and the preterm infant

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Riboflavin (B_2) plays a central role in intermediary metabolism and energy release; little is known about riboflavin requirements or pathophysiological consequences of deficiency states in preterm (PT) infants. 68 infants were allocated randomly to diets including human milk or a preterm formula containing 1.8 mg riboflavin/1. 18 of 23 human milk fed infants, who had not received supplementary riboflavin before day 7, developed biochemical riboflavin deficiency (erythrocyte glutathione reductase activation coefficient >1.3) compared with a low incidence in the formula fed group ($p<0.01$). Dietary effects of riboflavin status outweighed those of phototherapy. Further studies demonstrated significant photodegredation of riboflavin in breastmilk during home collection, handling and enteral infusion: controlled exposure to daylight of human milk in plastic storage bottles or infusion apparatus, resulted in 40-50% destruction over 6 h (also 70% vitamin A lost): these losses may compound deficiency. Phototherapy lighting was similarly destructive. Our preliminary work on neonatal and older rats demonstrate that experimental riboflavin deficiency decreases oxygen consumption ($p<0.05$) and decreases ($p<0.05$) iron utilization and fatty acid oxidation (the latter suggested also by early studies on PT neonates). We speculate that riboflavin deficiency in PT infants might impair energy metabolism and substrate utilization though early supplementation requires caution since light degraded riboflavin may release damaging free radicals.