35 THE EVALUATION OF TREATMENT WITH ORAL ZINC FOR WILSON'S DISEASE.

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Copper toxity in liver, brain, kidney due to a congenital failure of the copper biliary excretion mechanism in the liver is the main problem in patients with M. Wilson. Oral zinc sulphate as sole treatment has been used succesfully for seven years as an alternative therapy for D-Penicillamin (D-P) which has sometimes serious side effects.
A comparison of both forms of treatment was done in 2 sets of children with M.Wilson. Quantitative balance studies were performed using a standardized hospital diet and identical food portions frozen for analysis. All fecal and urinary excretions were sampled each day for a seven day period.
Separately an oral 64-Cu test was done to measure copper uptake, and a single test dose of D-P given to determine copper excretion. Symptoms were absent and liver functions normal in all patients at time of the study.

Results:

I Patients	Treatment	Balance (% of intake)		Plasma levels (ug/dl)	
		copper	zinc	copper	zinc
1	zinc	-13,5	+0,5	11,7	209
2	zinc	-14,2	+1,9	3,4	215
3	D-Penicillamin	-20,3	-1,6	7,5	110
4	D-Penicillamin	-45,4	-4,7	5,1	81

II 64-Cu uptake from the gut was considerably decreased in zinc treated patients. Plasma peak levels were 0,6 and 0,45 % of administered dose.
III A significant rise in copper excretion after test dose of 500 mg D-P was noted in both groups indicating still incomplete decoppering.
IV Liver biopsy copper content showed still incomplete decoppering in both groups although histological improvement was seen in zinc treated patients.

Conclusion: oral zinc treatment for M.Wilson in children is effective by blocking the intestinal uptake of copper.

36 ENERGY-SOURCE RELATED DIFFERENCES IN WHOLE BODY PROTEIN METABOLISM MEASURED IN PARENTERALLY FED INFANTS, BY COMBINED STABLE ISOTOPIC LABELLING AND INDIRECT CALORIMETRY.

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The relative effect of glucose or lipids on whole body protein metabolism was investigated by a randomised crossover study in 5 infants (age 3-8 months) steadily growing on parenteral feeding, with constant protein (2,8 + 0,2 g/kg.d) and energy (103 + 3 kcal/kg.d) intakes. Energy was provided as glucose and as an isocaloric glucose lipid mixture, with 50 mg/kg.d L carnitine. Net glucose and fat oxidation rates were measured by indirect calorimetry (IC) for 6 hours. After 120 mn, a primed-constant ($L1^{13}C$) leucine infusion was started. Breath CO_2 and plasma samples were obtained at 0, 60, 120 and every 30 mn. Enrichments of plasma ketoisocaproate and leucine were measured by mass spectrometry (MS) and $^{13}CO_2$ production rate by using IC and MS data at steady state enrichment. Lipogenesis (2 + 0,8 g/kg.d) was present with glucose, whole body protein synthesis (PS) and breakdown (PB) rates amounting to 8,3 + 0,6 and 8,1 + 0,9 g/kg.d, respectively resulting in a 0,2 + 0,7 g/kg.d net synthesis (PNS) rate ; with the glucose-lipid mixture, fat oxidation rate was 3,8 + 0,6 g/kg.d ($p<0,001$), PS rate 7,4 + 0,5 but PB was only 5,5 + 0,8 g/kg.d ($p<0,01$), resulting in a 1,9 g/kg.d PNS. Thus, the glucose-lipid mixture resulted both in significantly improved whole body protein accretion and reduced fat deposition rates, when compared to higher glucose infusion rates.

37 NORMAL SMALL BOWEL HISTOLOGY DOES NOT EXCLUDE COELIAC DISEASE (CD).

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The diagnosis of CD includes flat small-intestinal mucosa which normalizes on gluten-free diet and relapses within two years on gluten-containing diet. However, there are several observations, e.g. late relapsers and late onset CD in monozygotic twins, indicating that CD may exist latent in patients having normal mucosa when eating a normal diet. We now report three patients having normal mucosa before the initial diagnosis of CD was made. Case no. 1 (HLA B 8, DR 3,4) was biopsied at 11 months of age because of positive IgG class gliadin antibodies (AGA) and the mucosa was found normal. The reticulin antibodies (ARA) were negative. At 3.5 years of age both IgA-AGA and IgA-ARA turned positive and mucosal atrophy was found. Case no. 2 (HLA B 8, DR 3) was biopsied at 11.4 years of age because of suspected dermatitis herpetiformis (DH). DH was excluded with skin biopsy and small-intestinal mucosa was normal. Mucosal atrophy was found at the age of 17.5 years. Case no. 3 (HLA B 8, DR 3,5) volunteered for biopsy in a family study at the age of 32 years. She was symptomless and the mucosa was normal. Another family study at the age of 41 years revealed mucosal atrophy. Permanent teeth enamel hypoplasia indicated that she might have had active CD also in young age. We conclude that latent CD does exist. Our observations change fundamentally not only the concepts of CD but also our understanding of the heredity of CD.

38 THE PEPTIDE DOMAINS OF AETIOLOGICAL IMPORTANCE IN COELIAC DISEASE MAY RESIDE WITHIN THE N-TERMINAL REGION OF ɣ-GLIADIN.

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We have previously demonstrated that patients with coeliac disease have a characteristic gliadin antibody pattern dominated by reactivity against a few polypeptides in the ɣ-fraction. Recent gliadin- and hordein- (the prolamin fractions of wheat and barley) immunoadsorbent studies have shown, that the prolamin reactivity originates from the same population of antibodies. The antibody reactivity against separated hordein polypeptides was therefore investigated by immunoblotting, but a characteristic pattern could not be connected to coeliac disease.

The combination of these observations on basis of detailed comparison of available amino acid sequences leads to the speculation, that distinct antigenic epitopes characteristic for coeliac disease may reside within the N-terminal region of gliadins.

The antibodies in patients with coeliac disease does however not react with a typical α-gliadin expressed by E.coli, which extends the indications that the antigenic epitopes are in the ɣ-gliadin fraction.

A determination of the aetiologically active peptide domains and an elucidation of the initial events of the pathogenesis should nevertheless be based on direct studies of the enterocytes under in vivo resembling conditions.

39 JEJUNAL LYMPHOCYTES IN COELIAC DISEASE BEFORE AND AFTER ORGAN CULTURE

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Subsets of lymphocytes of jejunal mucosa from children with active coeliac disease were measured before and after 24 hs organ culture to determine whether a normalization of the lymphocyte population will occur together with the restitution of epithelial cell morphology.
Methods: Organ culture of intestinal mucosa was performed according to Browning and Trier (J Clin Invest 48:1423, 1969). Lymphocyte subsets were counted on frozen sections using antibody technique with commercial monoclonal antibodies (Becton&Dickinson).
Results: Intraepithelially, CD8 positive lymphocytes dominated and this subset of cells decreased during culture from 50 to 37 per 100 epithelial cells (mean values). All biopsies with intraepithelial CD8 positive lymphocytes exceeding 40 per 100 epithelial cells showed a marked decrease. In the lamina propria CD4 positive lymphocytes were more prevalent and during culture the ratio CD4 positive to CD8 positive lymphocytes increased from 1.1 to 1.3. Thus during 24 hs culture the antigen expression of the lymphocytes alters towards that of lymphocytes in restituted mucosa. Gluten added to the culture medium inhibited this partial normalization.
Conclusion: The change of the lymphocyte population during organ culture of jejunal mucosa mimics that seen in vivo in coeliac disease during restitution. The technique should be of value in the study of immunological mechanisms in this disease.

40 "IN VITRO" PROLIFERATION OF ACTIVATED LYMPHOCYTES FROM SMALL BOWEL BIOPSIES IN COELIAC DISEASE

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The ability of interleukin 2 (IL 2) to stimulate cellular division of activated T lymphocytes allows us to propagate "in vitro" from the minute fragments of the biopsies the T cells infiltrating the inflammatory tissue. Previous studies on human allograft rejection (1), rheumatoid arthritis (2) and lung cancer (3) suggest that a high percentage of lymphocytes reactive against specific antigens should be present in the infiltrated small bowel mucosa of patients with gluten enteropathy. Therefore we developed a system that allows us to propagate relevant amounts of activated T lymphocytes from minute fragments of per oral small bowel biopsies performed for diagnostic purposes. In preliminary experiments on two different patients with gluten enteropathy, in challenge with gluten containing diet, the culture of bioptic tissue in presence of IL-2 allowed us to recover as many as 10^9 lymphocytes. The evaluation of surface markers with monoclonal antibodies showed that these cells were virtually all T lymphocytes (CD3+): the two different T cells subsets, helper/inducer (CD4+) and suppressor/ cytotoxic (CD8+) were in both patients almost equally represented. On the other hand in three other patients, one with coeliac disease in gluten free diet, one with asymptomatic coeliac disease, identified during a family study, and one with food allergy, the culture of bioptic fragments in presence of IL-2 failed to produce a substantial lymphocytes' proliferation. The ability of IL-2 to expand lymphocytes infiltrating small bowel mucosa in patients with active coeliac disease could allow us to obtain relevant numbers of cells that can be used for functional assays.

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2) Stamenkovic I, et Al. PNAS in press.
3) Kurnick JT, et Al. Clin Immunol Immunopathol 1986, 38, 367.