Pediat. Res. *5: 83–99* (1971)

ABSTRACTS

Meeting of the European Society for Pediatric Research

Stockholm, Sweden, August 25–29, 1970

1. Evidence for two different biochemical defects causing Sanfilippo syndrome. U. N. WIESMANN and E. F. NEUFELD. *Univ. of Bern, Switzerland and NIH, Bethesda, Md., USA.*

The Sanfilippo syndrome is characterized by storage of heparitinsulfate (HS) and chondroitinsulfate (CH-B) in liver and spleen, excretion of large amounts of HS in urine, moderate skeletal involvement, and severe mental retardation. Cultivated skin fibroblasts from patients accumulate abnormal amounts of CH-B intracellularly. A defect in lysosomal degradation of mucopolysaccharides (MPS) is shown by intracellular incorporation of $S^{35}O4$ into MPS and by the rate of disappearance of the label from prelabeled cells. The foulthy degradation can be normalized either by mixing the cells from the patient with normal fibroblasts or by addition of preincubated media-fraction (precipitated at 80% NH_4SO_4) collected from normal cell lines or cells from other types of mucopolysaccharidosis. It is concluded that individual cell lines can substitute each other with specific factors they lack originally. In case of Sanfilippo cells the missing factor is not dialyzable and is heat-labile. Crosscorrection was not obtained by mixing cell lines from some patients; other cell lines, however, did mutually correct each other. Thus, two different types of the clinically identical Sanfilippo syndrome could be established. Cell lines of the same type do not substitute each other for the factor, but their MPS metabolism is corrected by any other cell line, including the other Sanfilippo type. Affected members of the same family show the same type, indicating the presence of true genetic variants. Equal distribution of the two types was found in a larger group of Sanfilippo patients. There is evidence that at least two different biochemical defects in MPS metabolism can cause the clinical picture of the Sanfilippo syndrome.

2. Enzyme replacement in Fabry's disease. W. KRIVIT, R. J. DESNICK, C. A. MAPES, and C. C. SWEELEY. *Univ. of Minn. Med. Sch. Minneapolis, Minn. and Mich. State Univ., East Lansing, Mich., USA.*

Fabry's disease was originally conceived diagnostically as a dermatological disorder (angiokeratoma). The systemic manifestations due to blood vessel involvement of renal, cardiac, and neural tissues widened the spectrum of clinical disease. The earliest manifestations occur in childhood and are a confusing diagnostic problem. The scope of the disease has been enlarged and manifested by the female heterozygote and by the several "forme fruste" genetic variants in males without skin lesions. Recent studies have provided explicit knowledge about the metabolic abnormality. Increased levels of galactosyl-galactosyl-glycosyl ceramide (GL-3) in blood, urine sediment, and most tissues have been consistently noted (J. Lipid Res., *10:* 188, 1969 and *11:* 31, 1970). These are correlated with the absence of a specific galactosyl hydrolase in hemizygotes. Heterozygous females have intermediate levels of the lipid and partial deficiency of the enzyme. Recently, the same galactosyl hydrolase which is absent in patients with Fabry's disease has been found in normal blood plasma. This suggested the possibility of direct enzyme therapy by plasma transfer. Six hours after the plasma infusion an "enhanced" or "induced" level of enzyme was noted. This level was 20-fold greater in the recipient than could be accounted for by the amount from the donor. Therapy with plasma infusion is under active investigation at present.

3. Carnosinase deficiency related to development of carnosinase activity. J. M. F. TRIJBELS, P. J. VAN HEESWIJK, P. J. J. VAN MUNSTER, and E. D. A. M. SCHRETLEN. *Univ. of Nijmegen, Nijmegen, The Netherlands.*

A patient suffering from a progressive neurological disorder with severe mental retardation excreted increased amounts of carnosine (β-alanyl-histidine) in urine. A strongly decreased activity of carnosinase was found in the serum of this patient, who is the first child of healthy consanguineous parents. Two patients with identical abnormalities have been described by Perry *et al.* [1]. In order to detect other patients with such a, presumably genetically determined, deficiency of serum carnosinase activity, a new method has been developed for the determination of the enzymic activity using L-carnosine-(β-alanyl-1-^{14}C) as a substrate. The serum carnosinase activity was calculated from the ratio disintegrations per minute β-alanine: total disintegrations per minute. The carnosinase activity had been determined in the serum of newborns, older children, and adults. During the first 10 years of life a striking correlation was established between the measured serum carnosinase activity and the age of the human subject. Newborns possess only very low activities (maximally 0.1 unit/liter during the first 50 days of life whereas children above the age of 10 years have activities between 20 and 70 units/liter, a range found for the enzymic activities in serum of 40 adults, varying in age between 20 and 41 years. This relationship between age of the subjects and the measured enzymic activities has to be considered in view of detection of more patients with the described dipeptiduria, which probably can be classified as a no-threshold type.

1. PERRY, T. L., HANSEN, S., TISCHLER, B., BUNTING, R., AND BERRY, K.: New Engl. J. Med., *277:* 1219 (1967).

4. Hyperpropionic acidemia in ketotic hyperglycinemia. L. M. CORBEEL, C. HARVENGT, R. EECKELS, E. EGGERMONT, and R. VAN DRIESSCHE. *Univ. of Leuven, Belgium.*

The patient, a 4-week-old female infant with vomiting, anemia,

leukopenia, thrombopenia, and failure to thrive had the biochemical abnormalities of ketotic hyperglycinemia: metabolic acidosis (pH 7.2; $^{-}HCO3$ 7 mEq liter); hyperglycinemia (8.2 mg/100 ml); increased serum levels of glutamine, glutamic acid, and the ketogenic amino acids as well as a positive urinary Acetest and diphenylhydrazine reaction. Gas chromatography of the serum volatile fatty acids (VFA) showed a considerable increase of propionic acid up to 70% of the total VFA content (normal value 3.2%) and a concomitant decrease of acetic acid to 24% (normal value 88%).

The results indicate that the acidosis of ketotic hyperglycinemia is accompanied by increased propionic acidemia and allow some speculation on the metabolic block of the disorder.

5. β-Hydroxyisovaleric aciduria and β-methylcrotonylglycinuria in a newborn child, caused by a new inborn defect in the leucine degradation pathway. O. Stokke, E. Jellum, and L. Eldjarn. *Rikshospitalet, Oslo, Norway.*

The patient was a 4.5-month-old girl, whose parents are related. She suffered from neurological symptoms similar to those of Werdnig-Hoffmann's disease. Clinical data are presented in the abstract of H. Pande *et al.* In periods the urine had a peculiar odor, like that of cat's urine. Urine and serum samples were sent to us for further examination. Several screening procedures for abnormal metabolites were used. Analyses of urinary extracts by gas-liquid chromatography, using polar columns, showed large quantities of two components normally not present. Mass spectrometric analyses revealed spectra compatible with β-hydroxyisovaleric acid (β-OH-IV) and β-methylcrotonylglycine (β-MCG). Analyses of synthesized authentic compounds verified these structures. Daily amounts excreted were about 400 mg β-OH-IV and 100 mg β-MCG. Neither the metabolites nor any short chain fatty acids, including isovaleric acid, could be demonstrated in the serum, the levels of these compounds must therefore be below 0.5 mg/100 ml. Both the mother, father, and the two elder brothers, all healthy, excreted 15–40 mg β-MCG daily in the urine, indicating that the condition is genetically determined. Both β-OH-IV and β-MCG stem from the leucine degradation pathway, and the accumulation is probably due to a defective β-methylcrotonyl-CoA carboxylase. Biochemically, as well as clinically, our findings are distinctly different from previously described errors in the leucine metabolism.

6. Symptoms of infantile spinal muscular atrophy combined with a hitherto not described error in leucine metabolism. H. Pande, P. E. Waaler, O. Stokke, E. Jellum, and L. Eldjarn. *Univ. of Bergen, and Rikshospitalet, Univ. of Oslo, Norway.*

A 4½-month-old girl with clinical signs of Werdnig-Hoffmann's disease (infantile spinal muscular atrophy) had in addition a peculiar smell from her urine. Large amounts of two abnormal metabolites were found: β-methylcrotonylglycine (β-MCG) and β-OH-isovaleric acid. (See separate paper by Stokke, Jellum and Eldjarn.) The urinary smell resembled that of cat's urine or black currant leaves. The metabolites were not found in the blood. She had no episodes of acidosis. By giving a diet low in leucine the urinary excretion of β-OH-isovaleric acid dropped from 400 mg to about 50 mg/24 hr, and β-MCG from 100 mg to about 50 mg/24 hr. The clinical course however, did not change during 3 months on diet, and it is doubtful whether her clinical condition was due to the error in leucine metabolism. She died when 9½ months old from pneumonia.

7 Celiac disease sans diarrhea. B. McNicholl and B. Egan-Mitchell. *Regional Hosp. and University College, Galway, Ireland.*

Three infants presented around 1 year of age with failure to thrive, vomiting, and anorexia and were shown to have severe jejunal mucosal lesions characteristic of celiac disease before or at the time of onset of mild diarrhea. Two infants had fecal retention, none had steatorrhea, and all have thrived on gluten-free, milk-containing diets. Other investigations were confirmatory, including some sugar tolerance tests, and assays of mucosal lactase, glutamyl-tyrosine, γ-glutamyl-β-napthylamide, and glycyl-leucine; the dipeptidase assays were by a zymographic technique. Immunoglobulins were measured by single radial diffusion and showed high IgA in each child, low IgM in one, and variable IgG levels. The mucosal lesion of gluten-induced celiac disease can be demonstrated before diarrhea or steatorrhea occur.

8. Studies of intestinal fructose absorption in the rat. M. Gracey, V. Burke, and A. Oshin. *Univ. of Birmingham, England.*

Fructose is an important dietary sugar, and its intake is increasing. However, the mechanism of its absorption is unknown. It is generally accepted that active transport does not occur and it is thought that transport is either passive or facilitated. We studied fructose uptake by the small intestine using rat jejunal segments 1.5 cm long, fixed in Plexiglas chambers [1] and incubated in Krebs-Henseleit buffer containing C^{14} fructose. A double incubation technique was used [2]. A base line tissue concentration is thus achieved during the first incubation, and subsequent absorption of the sugar from the second medium can then be studied. This method consistently showed accumulation of the sugar against a concentration gradient. This effect is more easily demonstrated and more marked in young rats, just after weaning. We have therefore shown active transport of fructose in the small intestine of the rat. This process is disrupted by lithium and so is sodium-dependent; it is also depressed by dinitrophenol. Moderate inhibition occurs with galactose, lysine, and proline. These findings suggest that active transport of fructose occurs via a sodium-dependent, energy-dependent mechanism which may be shared by other small, water-soluble molecules. These findings are relevant to clinical situations seen in childhood, particularly temporary monosaccharide malabsorption and glucose-galactose malabsorption.

1. Semenza G.: Biochim. Biophys. Acta, *173:* 404 (1969).
2. Alvarado F.: Biochim. Biophys. Acta, *112:* 292 (1966).

9. Peptidase activities of brush border membrane of rat intestine. S. Auricchio, M. Pierro, and M. Orsatti. *Univ. of Naples, Italy.*

A new method for the assay of peptidase activities is developed based on the oxidative deamination of L-amino acids with L-amino acid oxidase. This method measures the products of the enzymatic hydrolysis, without any interference from the peptides.

Brush border membrane hydrolyzes very rapidly tri- and tetra-L-alanine, L-leucyl-glycyl-glycine, L-leucyl-glycine, L-phenylalanyl-L-alanine, and L-leucine amide.

The enzymatic activities of the brush border membrane hydrolyzing L-phenylalanyl-L-alanine and L-leucine amide were characterized with regard to pH-activity curves, ion activation, and intracellular distribution.

The results suggest that intrinsic enzymes of the brush border membrane play a role in the terminal digestion of proteins.

10. Effect of saliva and serum of cystic fibrosis on alanine *in vitro*