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Altered enzyme kinetics in fibroblasts of patients with maple syrup urine disease (MSUD).

MSUD results from an inherited defect in oxidative decarboxylation of branched chain α-keto acids (BCKA). Kinetic data for the BCKA decarboxylation were obtained with cultured fibroblasts of normal individuals and 9 MSUD patients with different clinical pictures. The liberation of $^{14}CO_2$ from (1-^{14}C)BCKA was determined by a micro-enzyme assay for substrate conc. ranging over three orders of magnitude. Of two kinetically distinct decarboxylase components for each BCKA present in normal controls the one with higher substrate affinity was affected in all cases of MSUD investigated: for α-ketoisocaproate (KIC) the normally hyperbolic substrate curve was changed to sigmoid shape, for α-ketoisovalerate (KIVA) and α-keto-ß-methylvalerate (MEVA) this componentwas not detectable at all. In one patient's cell strain, additionally, the component with lower substrate affinity was altered. Reaction rates of normal and mutant decarboxylases differ widely at low substrate conc. and do not at high conc. of BCKA in 8 cases of MSUD. Kinetic studies of BCKA decarboxylation appear suitable for classification in some cases of MSUD.

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Determination of Phe-hydroxylase in PKU and Hyperphe-aemia.

The activity of phe-hydroxylase was determined in liver needle biopsy material from patients with elevated phe levels. The reaction mixture for the enzyme assay was: phosphate buffer 150mM, phe 0.1mM, phe 14C 0.2µCi, dithiothreitol 2mM, biopterin 0.025mM. The extract was preincubated with lysolecithin (1mM) for 10min, the reaction stopped by boiling. Phe and tyr were separated by TLC. 14-C-activity was determined by direct scanning and counted in a liquid scintillation counter. So far we have observed 4 types of enzyme activity: 1) Control liver samples of non-metabolic disorders transformed 100µmoles/g^{-1}protein/60$min^{-1}$from phe to tyr. 2) Socalled hyperphe-aemia (phe levels around 10 mg%) had 10-20% normal activity. 3) A third group had 1-6%. 2 pairs of siblings also showed this residual activity. One sib in each family had not been treated (late discovered) and was mentally retarded, the other developed normally under treatment. 4) "Classical" PKU had no detectible hydroxylase activity. -This study intends to differentiate PKU from other hyperphe-aemias which perhaps may remain untreated.

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New aspects of tryptophan metabolism in untreated PKU and the urinary indole excretion in relation to Phe content of semi-synthetic diets.

The urinary Try metabolites from the kynurenine pathway and from indolic metabolism were analyzed in ten untreated PKUs.The results suggested a devision of the patients into groups A (higher serum Phe,reduced oxydative Try metabolism) and B (lower serum Phe and excessive excretion of oxydative metabolites and of N-acetyl-Try).One patient of group A was put on four semi-synthetic dietary regimes (70% free synthetic L-amino acids) with varying Phe content.On a low Phe diet the excretion pattern of the oxydative and indolic metabolites became similar to that in group B.No reciprocal relationship between serum Phe and 5-OH-indoleacetic acid was found.Numerous unidentified Try metabolites,specific for each group and certain dietary phases,were detected.The new analytical methods applied make it necessary to reaprise the previously published quantitative data on tryptophan metabolism in PKU.

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Methylmalonic acidemia (MMA):Enzymatic studies in 5 non-related patients.

MMA may be caused by at least three different enzyme defects:1)Defect of methylmalonyl-CoA(MMCoA)mutase-apoenzyme;2)Defective metabolism or transport of the coenzyme of MMCoA mutase,5'-deoxyadenosylcobalamin(dA-B_{12}),or of its precursor,vitamin B_{12};3)Deficiency of MMCoA racemase.-We have studied methylmalonate metabolism in leucocytes and cultured skin fibroblasts of 5 p.with MMA(1 p.B_{12}-responsive, 4 p. clinically non-responsive). Intact fibroblasts of all 5 p.failed to metabolize 2-methyl-^{14}C-malonate(MM-^{14}C)to $^{14}CO_2$,whether or not vitamin B_{12} or dA-B_{12}(10^{-5}M)were added;whereas in intact leucocytes of the 3 p.so far investigated, $^{14}CO_2$ formation from MM-^{14}C was always present and similar to controls. Disrupted fibroblasts were studied using a specific enzyme assay with propionyl-CoA and $NaH^{14}CO_3$ as substrates.Methylmalonate metabolism was absent or severely impaired in all p. as revealed by methylmalonate accumulation and succinate formation.While addition of vitamin B_{12}($10^{-11}$to 10^{-5}M) had no effect in any of the p.,addition of dA-B_{12}(10^{-7} to 10^{-5}M) did not enhance succinate formation in 2 p.,but fully restored enzyme activity in 2 other p.These data suggest a defective metabolism rather than a defective transport mechanism of the B_{12}-coenzyme in the latter 2 p.