Atypical phenylketonuria: 
Over 60 years from the discovery of tetrahydrobiopterin

Fenyloketonuria nietypowa
– ponad 60 lat badań po odkryciu tetrahydrobiopteryny

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Abstract
Phenylketonuria (PKU) was described and defined biochemically for the first time in 1934 by Norwegian Ivar Asbjørn Følling. This disease is a hereditary autosomal recessive metabolic disorder caused by a mutation of the gene encoding phenylalanine hydroxylase that normally converts phenylalanine to tyrosine. In the 1960s, a screening test for hyperphenylalaninemia was developed by Guthrie. Due to population-based newborn screening (NBS), PKU became the first disease to be diagnosed at an early, presymptomatic stage. Tetrahydrobiopterin (BH4) is an essential cofactor for the activity of various enzymes, including phenylalanine hydroxylase. Moreover, a heterogeneous group of congenital disorders of BH4 metabolism was presented with (atypical PKU) or without hyperphenylalaninemia. In some patients with classical PKU, the intake of BH4 restores the ability to metabolize phenylalanine. Kaufman’s discovery of BH4 6 decades ago resulted in the opening of novel and productive avenues of biological and medical research.

Key words: history of medicine, atypical phenylketonuria, tetrahydrobiopterin

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**Introduction**

Introduction of newborn screening (NBS) for classical phenylketonuria – PKU (MIM #261600) is considered one of the most important achievements of preventive medicine. It is often forgotten that this is still not a closed chapter. For instance, diet therapy is not fully effective and, for decades, a lot of effort has been put into learning about diseases that are hidden behind clinical symptoms and biochemical abnormalities similar to those found in PKU. It is surprising that even in the best journals there are thoughtless statements, unadjusted by reviewers, that a low-phenylalanine diet solves all problems of patients with PKU: “Currently, newborns with these conditions [i.e., those suffering from PKU; author’s note] suffer as much as those with PKU: “Currently, newborns with these conditions [i.e., those suffering from PKU; author’s note] suffer as much as those with PKU.” This statement has been sharply criticised in a special article in “The Pediatrics.”

The year 2018 marked the 60th anniversary of Kaufman’s discoveries concerning the essential nature of a non-protein cofactor during the conversion of amino acid phenylalanine to tyrosine via the PAH enzyme (EC 1.14.16.1).4,5 The gradually discovered cofactor – tetrahydrobiopterin (6R-L-erythro-5,6,7,8-tetrahydrobiopterin, BH4) – proved to be essential also for the hydroxylase activity, i.e., tyrosine hydroxylase (EC 1.14.16.3) and tryptophan hydroxylase (EC 1.14.16.4), participating in the synthesis of neurotransmitters such as adrenaline, norepinephrine, dopamine, serotonin (5-hydroxytryptamine), and all 3 nitric oxide synthases (NOS1–3; EC 1.14.13.39) as well as alkylglycerol monooxygenase (AGMO, EC 1.14.16.5). In vivo BH4 is either synthesized de novo or regenerated via the salvage pathway. The biosynthesis de novo is run with 3 enzymes: GTP cyclohydrolase I (GTPCH, EC 3.5.4.16), 6-pyruvoyltetrahydropterin synthetase (PTPS, EC 4.6.1.10) and sepiapterin reductase (SR, EC 1.1.1.153). The regeneration of BH4 from its oxidized forms of 7,8-dihydrobiopterin (BH2) and sepiapterin occurs in 2 independent reactions catalyzed mainly by SR, dihydrofolate reductase (DHFR, EC 1.5.1.3) and dihydropteridine reductase (DHP, ER 1.5.1.34). In 1967, Kaufman predicted the discovery of the abnormal synthesis and regeneration of BH4 with a more severe clinical course than in PKU (classical (mild) PKU, defined as hyperphenylalaninemia >1200 μmol/L resulting from a congenital defect in PAH and with a daily dietary tolerance of phenylalanine <20 mg/kg b.w.), including, in addition to hyperphenylalaninemia, severe neurotransmitter deficiencies.3

**Cofactor of the reaction converting phenylalanine to tyrosine**

Classical PKU, discovered and described by Ivar Asbjørn Følling in 1934, is the most common congenital amino acid metabolism disorder caused by reduced activity of cytosolic phenylalanine hydroxylase (phenylalanine 4-monoxygenase, PAH, EC 1.14.16.1), as demonstrated by George Amede Jervis, and, at the same time, the first specifically known disease resulting in mental impairment, for which a highly effective diagnosis and treatment scheme has been developed. Seymour Kaufman, a biochemist, summarized in his memoir book (“Overcoming a Bad Gene”): “It is fair to say that if Følling was the father of PKU, Jervis was the midwife.”3

Phenylalanine hydroxylase represents 0.1–0.3% of all proteins in the liver, and its activity in the kidneys reaches up to 45% of the activity recorded in the liver, which may determine the phenotypic heterogeneity of PKU.
and June Lloyd and Klaus Bartholome used the term ‘atypical phenylketonuria’. All 3 patients (aged 2–7 years) from the Hospital for Sick Children in London, UK, described in detail by Smith et al. in “The Lancet”, died of Mendelson’s syndrome, which, apart from failure to inhibit damage to the central nervous system, contributed to the exchange of names with a high emotional charge — ‘lethal PKU’ and ‘malignant PKU’.³

**Detailed identification of the biochemical background of atypical phenylketonuria**

In 1975, Kaufman et al. reported the first patient with atypical PKU and accompanying decreased folate concentrations, and identified the biochemical background of the disease (DHPR defect).³ The BH₄ treatment was considered from the onset of the disease; however, the very limited availability of this cofactor was an obstacle.³,8,10 Cheaper 6-methyl-tetrahydrobiopterin was initially considered as an alternative, but after the initiation of the therapy, its disqualifying hepatotoxicity was revealed.³

In 1976, David Miles Danks, Richard (Dick) Cotton (whose achievements were recently reminded by Nenad Blau) and Peter Schlesinger from the University of Melbourne, Australia, suggested a BH₄ loading test to be performed in every newly diagnosed patient with hyperphenylalaninemia (e.g., during NBS for PKU) in order to exclude/identify atypical PKU.¹² In his laboratory, Kaufman led to the first diagnosis of PTPS deficiency in 1977.¹² Twenty years after the discovery of BH₄, a collective study by Danks et al. (i.a., with Kaufman) concerning malignant PKU was published in the inaugural volume of “The Journal of Inherited Metabolic Diseases”¹⁴ (for many years, the co-editor of this scientific journal, which was the first ever periodical dedicated to congenital metabolic errors,¹⁵ was Professor Maria Barbara Cabalska — born on October 10, 1927, deceased on February 5, 2020), the head of the Holistic Pediatrics Clinic at the Institute of Mother and Child for many years), in which 9 patients with DHPR deficiency (2 patients with confirmed BH₄ deficiency and 6 cases considered underdiagnosed) were compared.

Recently, a biography (abounding in interesting memories) of an Australian professor of pediatrics and genetics, the abovementioned Danks (born on June 4, 1931, deceased on July 8, 2003) has been published.¹⁵ This researcher made, among others, an important observation about the association of the abnormal fur of sheep grazing in areas characterized by copper-deficient soil with the characteristic hair of patients diagnosed with Menkes disease (kinky hair disease). In 1972, he initiated — which is worth emphasizing — without being a computer geek himself, the creation of POSSUM (Pictures of Standard Syndromes and Unknown Malformations), i.e., a prestigious electronic dysmorphology database. Only in 1984, the first patient with a defect in GTPCH was described.¹⁶ The urinary excretion of pterins, dopamine and serotonin in a 4-year-old boy was very small, but the proportions of pterin fractions did not differ from normal. Further theoretically predicted by Kaufman variants of atypical hyperphenylalaninemia were identified in patients after the next few years.¹⁷ Detailed data concerning more than 1,000 patients from all over the world with atypical forms of PKU (in Poland, the number of all patients does not exceed 20, most of them have a PTPS defect), involving the molecular background and the descriptions of the clinical course of the disease as well as treatment results, is available in the BIODIF database.¹⁸

**Outline of the methodology of care for patients with atypical phenylketonuria**

Clinical symptoms of atypical PKU include mental impairment, convulsions and motor disorders, such as dysphagia, dystonia, dyskinesia, and enhanced reflexes. Diagnostics, often presymptomatic, uses the BH₄ loading test (the assessment of changes in phenylalanine and tyrosine blood levels), the assay of DHPR activity in blood and the analysis of the profile of bitopertin levels in urine and/or blood, carried out, among other things, as a differential diagnosis of hyperphenylalaninemia in newborns with abnormally high results of the determination of phenylalanine levels in blood in the screening test for PKU. The confirmation of the diagnosis is enabled by molecular diagnostics and enzyme assays. A low-phenylalanine diet (especially in DHPR defects), BH₄ (the intake of which may dangerously reduce tyrosine levels in patients, in both plasma and the cerebrospinal fluid, which justifies additional supplementation with this amino acid), L-dihydroxy-phenylalanine (L-DOPA), 5-hydroxytryptophan, enzyme inhibitors (such as carbidopa, selegiline – a metaphetamine derivative, entacapone, which decrease the demand for neurotransmitter precursors), folacin derivatives (especially in DHPR deficiency), dopaminergic receptor agonists (bromocriptine and pramipexole), and melatonin are used in the treatment. The therapy requires a thorough observation of the patient combined with monitoring the concentration and proportion of neurotransmitters in the cerebrospinal fluid, constant monitoring (drug-related) side effects, both neurological and psychological-psychiatric, including compulsive gambling, compulsive buying disorder or hypersexual disorder, e.g., due to pramipexole overdose.¹⁹ In phenylketonuria, the demand for drugs is highly variable. Clinical observations of patients in the Polish population,
initially made by Cabalska, and then mainly by Dr Maria Nowacka, confirmed the differential impact of treatment on psychomotor and mental development, regardless of the time of diagnosis/initiation of the treatment and a genotype (unpublished observations by Nowacka and Hozyasz). Detailed diagnostic and therapeutic algorithms for atypical PKU, including, among other things, the range of test results for the profile of biopertin, are available in specialist clinical guides.

**Latest discoveries about the nature of BH4**

The year 2017 brought first descriptions of clinically diverse forms of hyperphenylalaninemia, secondary to DNAJC12 deficiencies and characterized by autosomal recessive inheritance, manifesting mild neuro-psychological symptoms (such as attention deficit disorders, difficulties in recognizing emotions in faces, difficult social adaptation), non-progressive, early-stage Parkinson’s disease corresponding to DOPA treatment, or a syndrome of symptoms such as those typical of atypical PKU secondary to severe BH4 deficiency (despite normal cofactor concentrations) and autistic disorders. In the first diagnosed patients, a decrease in phenylalanine after BH4 loading was observed (this test was not performed in patients with non-progressive Parkinson’s disease). The gene DNAJC12 encodes a protein from the heat shock protein 70 (HSP70) family, functioning as a co-chaperone protein for phenylalanine, tyrosine and tryptophan hydroxylases. A consequence of DNAJC12 mutation is a deficiency of neurotransmitters at normal pterin concentrations. This gene is particularly associated with the processes of protein folding. Tetrahydrobiopterin, neurotransmitter precursors and a low-phenylalanine diet are used for the treatment of congenital deficiencies of the activity of DNAJC12. Kaufman could not theoretically predict the prevalence of hyperphenylalaninemia secondary to DNAJC12 deficiencies, but he did point to the possibility of identifying the mutation of a gene encoding phenylalanine hydroxylase, at which PKU treatment with BH4 would be possible (a useful synthetic form of BH4 was developed for oral intake – Kuvan sapropterin) – thanks to the response of the defective enzyme to the supposedly multi-directional care effect of the increased availability of this cofactor. Currently, we know that such a relationship exists, for example, in some complex parts of heterozygotes with classical PKU, and its use is a significant step toward personalized medicine, which is interestingly presented in Marta Kinga Danecka’s impressive online dissertation. Reviews of BH4 deficiencies, both with and without hyperphenylalaninemia, are continuously updated by specialized research teams.

The metabolism of BH4, present in breast milk in a relatively high concentration in contrast to infant formulas, is very complex and, as recent studies have shown, may be significantly related to the gastrointestinal microflora. A strain of *Lactobacillus reuteri* was developed to metabolize phenylalanine, which in a mouse model of PKU with reduced PAH activity created conditions for a less restrictive low-phenylalanine diet.

Papers concerning an experimental animal model of GTPCH deficiency have shown that bacterial flora is likely to be a significant source of BH4. *Bifidobacteria* provide an important part of the BH4 pool in infants, and it cannot be excluded that they affect the phenotypic expression of atypical PKU (at least in the first months of life). The relationships between BH4 availability from the gastrointestinal microbiota and, for example, infantile colic (or other functional gastrointestinal disorders) have not been analyzed yet. An interesting animal model of PTPS deficiency, useful in pharmacological studies, has recently been described.

A discussion concerning the role of BH4 in the treatment of PKU and in the pathogenesis of cardiovascular diseases, diabetes mellitus, leucoderma, neurodegenerative diseases with newly discovered mechanisms of pathogenesis, developmental disorders of autism spectrum, and pain perception, is beyond the planned scope of this paper. Attention should be paid to the links between the metabolism of phenylalanine/BH4 and mental disorders, especially schizophrenia, and the introduction of a breath test into clinical practice, after the oral loading of phenylalanine labelled with the $^{13}$C isotope.

**Short bio of Seymour Kaufman**

Kaufman, a prominent biochemist, was born in Brooklyn, New York, USA, on March 13, 1924. Observing the uniqueness of students’ achievements during his 4 years of studies at the prestigious New York High School for Music and Art, he felt the need for a professional redirection to another area of study. His choice was chemistry, followed by biochemistry (in 1949, he received a PhD degree at Duke University) with enzymology. With time, he started working at the National Institute of Mental Health (NIMH). After his internship (1960–1962) at Professor Ernst Hadorn (specializing in dyes, including pteridine ones, found in the pollen covering butterfly wings) at the Institute of Zoology of the University of Zurich, Switzerland, Kaufman finally identified a chemical compound (pteridine-like) that acts as a cofactor for the conversion reaction of phenylalanine to tyrosine. In 1969, he became the head of the Laboratory of Neurochemistry, at NIMH.

In his professional work, he was striving for final decisions, he was not satisfied with indirect support of inference. One of his students, Ephraim Levin, wrote: The one word which best comprises Seymour Kaufman’s approach to research is careful. He felt that to publish an er-
raneous datum or draw an unjustified conclusion would be a disaster, because it might deflect the progress of science. He once said that he spent 10% of his time being 90% sure, and the remainder being 99.9% sure.42

After his retirement in 1999, he remained scientifically active until a severe illness in 2005.

Kaufman’s achievements, in addition to learning about the nature of BH4, include the discovery of the role of ascorbic acid as a cofactor of the hydroxylation reaction of dopamine to norepinephrine, and numerous neuropsychopharmacological studies. In 1991, he won the prestigious Hillebrand Prize, awarded to chemists in the USA. Kaufman amassed an impressive collection of sculptures and paintings, including lithographs made by his favorite post-impressionist – Toulouse-Lautrec, which brings to mind his fascination with ephemeral colors from Professor Ernst Hadorn’s (the creator of developmental genetics) Swiss laboratory.44 He valued good food, but he did not make it by himself. He was probably inspired in this area of interest by his trip to Europe (1958) for several consecutive scientific conferences, during which, encouraged by Seymour Kety – the scientific director of NIMH, he celebrated meals for 4 consecutive days, each time in a different French restaurant distinguished in the “Michelin Guide” (there were only 12 at that time).42 Kaufman, like other most creative researchers of PKU, including Guthrie (the creator of NBS for PKU and other congenital metabolic errors) and Dent (the creator of paper chromatography, the author of the first insight into the maternal PKU syndrome, the founder of the London Clinic for patients with congenital metabolic errors – known as the ‘metabolic mecca’ – the current director of which is Charles Dent Metabolic Unit at the National Hospital for Neurology and Neurosurgery), was one of the sympathizers of oenology.32,45

He had 3 children with his wife Elaine and lived to see his grandchildren. His daughter Emily took on his artistic passions and gained recognition as a sculptor. He died on June 23, 2009.

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