In July of 1990, *The Journal* published an article with a novel message: ten subjects with classical phenylketonuria (PKU,[1] OMIM, 261600) and two with the allelic variant phenotype called non-PKU hyperphenylalaninemia (non-PKU HPA), on average, could hydroxylate L-phenylalanine to tyrosine in vivo at rates equivalent to those measured in six control subjects (1). *The Journal* now publishes contrary evidence (2): in vivo hydroxylation of phenylalanine to tyrosine, as predicted (3), is deficient in PKU subjects when measured by a method the same in principle, but slightly different in detail from the one described in the prior publication.

The conclusions drawn in the 1990 article were logically inconsistent: how and why would patients manifest the hyperphenylalaninemia of PKU if they had adequate hydroxylation activity? In comparison, the conclusions of the second article have the flavor of common sense. For many reasons, PKU and non-PKU HPA have made valid and major claims on our interest in human genetics and medical science, and these two articles are no exception.

PKU was the fifth disorder to be described in a new class called inborn errors of metabolism (3). In the decades after its discovery in 1934, PKU emerged as the first form of mental retardation to have an overt chemical explanation; the deviant metabolism and concordant HPA could be attributed to deficient activity of hepatic phenylalanine hydroxylase (PAH) enzyme, as measured in vitro on hepatic biopsy material. PKU became one of the first genetic disorders to have an effective treatment against its disease phenotype (mental retardation) and to catalyze systematic screening programs to facilitate early diagnosis and treatment in the newborn. After the human gene (*PAH*) was cloned, world-wide analysis of its mutations revealed extensive allelic variation, both disease-producing and neutral polymorphic. Over 384 alleles are now recorded in the locus-specific *PAH* mutation database (http://www.mcgill.ca/pahdb [4]). Crystallization and characterization of the catalytic core of PAH enzyme at the two-angstrom level (5) will soon permit virtual molecular modeling. Throughout the majority of the past six decades, it has been believed that deficient PAH enzyme function, caused either by primary catalytic dysfunction of PAH, or secondarily by impaired availability of its cofactor (tetrahydrobiopterin), would explain human hyperphenylalaninemia. We now know that *PAH* mutations account for most cases ($\sim$ 98%) of impaired phenylalanine hydroxylation (3).

Although PKU is a Mendelian disorder, it is also multifactorial, requiring both exposure to dietary phenylalanine and inheritance of a mutant PAH genotype. It also has aspects of a complex trait in that the genotype is not necessarily predictive of the HPA phenotype or ability to dispose of excess phenylalanine (6–8), implying that events other than phenylalanine hydroxylation contribute to phenylalanine homeostasis. In an important theoretical paper analyzing the effect of mutation on metabolic phenotypes (9), Kacser and Burns proposed that in vivo enzymes do not act in isolation, are kinetically linked to other enzymes by substrates and products, and also proposed that output (e.g., a flux or fluxes) of a metabolic system is a systemic property in vivo; thus, response to variation at one locus (e.g., *PAH*) acts over the whole system. The relative importance of a particular locus product (e.g., PAH enzyme), for function of the system as a whole, can be described by its sensitivity coefficient, which records the fractional change in flux rate in the mutant state relative to fractional change in enzyme activity. Whether there are many or few flux components in the system, their sum is always unity in vivo. Thus, the sensitivity coefficient for each component in the system must be a fraction of one (unity) when there are multiple components. Accordingly, when many components, in addition to phenylalanine hydroxylation, contribute to plasma phenylalanine homeostasis (3), it follows that a reduction to half-normal PAH enzyme activity in the PKU heterozygote need not be accompanied by a corresponding rise in the plasma phenylalanine level. And indeed this is the case (Fig. 1). Hence, the recessive nature of the mutant HPA phenotype. On the other hand, the mutant homozygous state (PKU) shows that PAH enzyme is a key component of the overall system, assuming it is nearly or wholly nonfunctional in PKU.

PAH mutations do indeed impair PAH enzyme function, as proven by both in vitro expression analysis (10) and a new way to measure hydroxylation-dependent flux rates in vivo (6). In the former approach, a recombinant DNA construct bearing a *PAH* mutation is expressed in an in vitro system where conversion of phenylalanine to tyrosine by the expressed PAH protein is measured. By this measurement, PAH enzyme function is impaired in various ways by various mutations in the *PAH* gene (10). Evidence also exists that *PAH* mutations impair the oxidative flux of phenylalanine in vivo; the most recent source is the van Spronsen et al. article in *The Journal* (2), but there is an earlier one as well. In a study by Treacy et al. (6), exhaled $^{13}CO_2$ was measured during the 90-min interval of initial hepatic uptake of substrate after oral ingestion of tracer doses of L-[1-^{13}C]phenylalanine by patients in whom the mutant *PAH* genotype had been characterized by DNA analysis. The expected gene dose effect for an autosomal recessive phenotype was observed in normal homozygous, heterozygous, or mutant homozygous persons (Fig. 1). The fact of gene dosage both validates the $^{13}CO_2$ method and demonstrates that in vivo phenylalanine hydroxylation is deficient in the PKU and non-PKU HPA patient. In brief, numerous observations are concordant with the hypothesis that phenylalanine hydroxylation is the principal, but not the only, metabolic pathway for disposal (runout) of phenylalanine in human subjects; furthermore, these observations agree that mutations at the *PAH* locus can perturb phenylalanine hydroxylation and cause hyperphenylalaninemia in the homozygous phenotype.

Why did the 1990 article (1) pass peer review almost a decade ago? Am I, as its reader (or re-reader), missing some-

1. *Abbreviations used in this paper:* HPA, hyperphenylalaninemia; PAH, phenylalanine hydroxylase; PKU, phenylketonuria.

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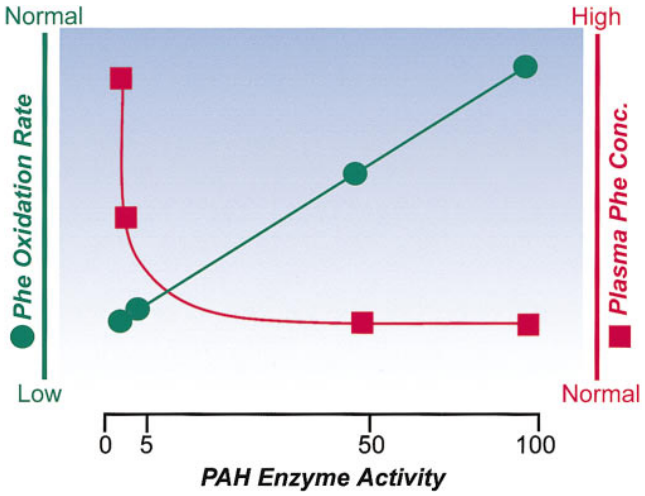

Figure 1. Relationship between plasma phenylalanine levels (*red boxes*) and PAH enzyme activity (*green circles*) in normal homozygotes (100% PAH enzyme activity), heterozygotes (half-normal activity), and mutant homozygotes (< 5% normal activity) for autosomal recessive classical PKU (left hand symbol) and non-PKU HPA (right hand symbol). The idealized diagram is based on actual data (6) where phenylalanine oxidation rates were measured in vivo from expired $^{13}CO_2$ expiration after ingestion of L-[1-^{13}C]phenylalanine. Plasma phenylalanine values were obtained in the same patients under standardized conditions. The mutant genotypes had been confirmed by DNA analysis. Gene dosage indicates that PAH oxidation (hydroxylation) is impaired in the mutant state; the variant plasma phenylalanine phenotype is recessive and behaves as a complex trait (8).

thing? Is there a truth that I should be accepting in that first article? I have asked several experts for enlightenment, and we all remain members of a mystified community. Is the van Spronsen et al. paper (2) a truthful correction of the message in the earlier report? I accept it as such, but the record shows once again that reviewers found the new paper a challenge.

The tools of science are hypothesis, measurement, experiment, and observation. Whereas the same basic set of tools was used by both teams investigating PAH enzyme activity in PKU patients (1, 2), the results of their inquiries a decade apart are different and we may never know why. But in an open society where science can flourish, both reports can coexist and readers will test and retest their validity. The peer review system (like democracy, a flawed system, but the best we have) will continue to arbitrate; and for the better if the alternative is, for example, Lysenko's way of doing science.

Science, as revealed in *The Journal*, is a rich culture. As an attack on ignorance, science is a well-spring of knowledge for society. In these two papers, and in their companions, medical science is seen working at three different levels: patient-oriented, disease-oriented, and basic. Goldstein and Brown (11) remind us that medical science, to remain healthy, needs all three types of research, and healthy debate. In the diversity, there is a unity from which we all benefit.

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