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of phenylalanine alone. PKU management programs now take into account these "BH4-dependent" forms of HPA, the frequency of which varies among human populations.

However, we are identifying new forms of HPA that are "BH4-responsive." They seem to involve one of two response mechanisms: either the high dose of BH4 overcomes "unfriendly binding" of cofactor on the surface of the PAH enzyme; or the BH4 molecule itself stabilizes the mutant PAH enzyme protein and allows it to function.

The latter idea fits into an important new view: namely that 60% of PKU gene mutations produce their effect through "mispreading" of the PAH enzyme protein subunit, leading to its rapid intracellular removal. Anything that would stabilize the protein for a longer life in the cell would improve function and thus be "a treatment." BH4-responsive PKU thus becomes a model for thinking about such a form of therapy for other genetic diseases because 50% of all human mutations probably produce their effects through protein misfolding and instability. One anticipates a growth industry in the invention of soluble chemical "chaperones" to stabilize mutant proteins.

When we sequenced the PAH gene we anticipated that mutation analysis would predict phenotype (clinical expression) and thus the intensity of treatment for classical PKU and variant forms of HPA. While there is a broad correlation between mutation type and metabolic effect, the prediction is not perfect. PKU then becomes a reminder that "genotype" (the specific mutation) is not necessarily the "phenotype" (clinical expression) for any Mendelian disorder.

Thus PKU emerges as yet another prototype, showing that Mendelian diseases are indeed complex. Accordingly, scientists interested in PKU are learning on another level what parents and patients have always known: notably, that every patient has his or her own particular form of PKU/hyperphenylalaninemia. In other words, treat the patient not the genetic mutation!

New guidelines from Europe and a recent NIH consensus conference in the U.S. (see Winter 2001 issue) recommend treatment of PKU as early as possible, with greater stringency as long as possible, perhaps even for a lifetime. The effectiveness of treatment is particularly important for the female patient who wishes to become pregnant and protect the fetus from the intranteic effect of maternal hyperphenylalaninemia (see Fall 2002 issue). Because of the prospect for prolonged treatment we hope to see treatment modalities improved. This would include improving the properties of existing dietary products; adopting other metabolic approaches to prevent excess plasma phenylalanine from penetrating the brain compartment; and to provide alternative enzyme therapy (for example, there is research underway with the enzyme phenylalanine ammonia lyase to degrade phenylalanine in the intestine before it is absorbed into the body pool).

PKU in the 21st century will continue to illustrate how the intellectual discipline of genetics and the practice of genetics are different yet also come together. Mendelian genetics was a transforming paradigm to understand many hundreds of human diseases; but a solely Mendelian view is an oversimplification for most genetic problems. PKU has been a prototype for the growth of this knowledge about human genetics. The latter is important not only for people with PKU but also for those with any human genetic disease as simple or as complex as it may be.

PAH Enzyme Structural Studies
by Dr. Heidi Erlaenden, Dr. Sven and Dr. Raymond C. Stevens, The Searle Research Institute, La Jolla, California

We know that mutations in the gene that "encodes" for the enzyme phenylalanine hydroxylase (PAH) cause both phenylketonuria (PKU) and hyperphenylalaninemia (HPA). PAH converts the amino acid phenylalanine from proteins in the diet into another amino-acid, tyrosine. Dr. Sven points out, the enzyme needs a cofactor, called tetrahydrobiopterin (BH4), as well as oxygen from the air we breathe. We now know there are more than 400 PKU/HPA mutations in the PAH gene.

Most of these PKU/HPA mutations result in an enzyme with changed chemical and physical properties compared to the normal enzyme (often referred to as "wild-type PAH"). Scientists have studied extensively the effects of these mutations on the PAH enzyme in biochemical systems within a test-tube (also called in vitro systems) by using a cloned and biochemically-produced version of the enzyme.

To help in the interpretation of the mutational studies, our research group first determined a three-dimensional atomic structure of the phenylalanine hydroxylase wild-type enzyme in 1997 (see figure, p. 3). This structure of the PAH enzyme is a representation of the shape of the human protein. It tells us where the individual atoms of each amino acid that constitutes the protein are located, in three-dimensions. Based on this three-dimensional structure we can identify the sites of the mutations found in PKU/HPA patients, and start to explain why the mutation leads to non-functional protein (and therefore an accumulation of the amino acid L-phenylalanine, acquired through the diet).

The three-dimensional structure of the PAH enzyme shows that the enzyme forms three distinct domains (sections): one regulatory domain of the enzyme (which regulates the phenylalanine to tyrosine conversion), one catalytic domain (responsible for the actual phenylalanine to tyrosine conversion), and finally a tetramerization domain (responsible for putting together four molecules of the same kind into a functional large molecule called a "tetramer"). We have identified several three-dimensional structures of phenylalanine hydroxylase since 1997. We now know where the phe to tyrosine conversion occurs, and where the phenylalanine and BH4 is located in the structure. The exciting thing is that we can use this three-dimensional structure to predict the effects of newly discovered mutations on the PAH enzyme.

The future goal for our research is to use the structure to make a form of the PAH enzyme that can be taken orally (before a meal) along with the BH4 cofactor, as an enzyme replacement therapy (along the lines of the Phenylalanine—phenylalanine ammonia lyase—research mentioned in the Fall 1999 issue). Hopefully, this would mean ultimately that a patient can consume a "normal" diet instead of a low phenylalanine diet.

Investigation of Phe Transport into the Human Brain
by Dr. Harald E. Meller, Max Planck Institute of Cognitive Neurosciences, Leipzig, Germany

Magnetic resonance imaging (MRI) is a modern technology based on the extremely weak magnetism of certain atomic nuclei, for example, hydrogen nuclei (commonly referred to as "protons") in water molecules. By utilizing a strong magnet and radio-frequency signals, detailed images from the living human body are recorded entirely non-invasively and with no known risk. Further metabolic information about certain chemicals in different tissues can be obtained with the same equipment and a closely related method called magnetic resonance spectroscopy (MRS). Researchers at Yale University were the first to detect a phenylalanine MRS signal in the brain of
animals made hyperphenylalaninemic. Even more interesting, they observed that the amount of phenylalanine in brain differed from the amount in blood.

Our laboratory at the University of Münster, Germany, like a few others, uses MRS to measure brain free phenylalanine concentrations in patients with PKU. Because PKU can result in neurological and psychological problems for off-diet patients, we are interested in comparing brain phe and occurrence of such problems. Phenylalanine is not produced by the human body. When food is ingested, phe is delivered to the brain by circulating blood. However, like most other chemicals, phe cannot enter the brain cells directly. It must be transported across the "blood-brain barrier" by a specific carrier protein located in the capillary cell membrane. (First indications that the central nervous system is separated from the blood by a barrier of some kind arose about a century ago, when the German immunologist Paul Ehrlich observed that dyes injected into a vein of laboratory animals stained all organs except the brain.)

Phenylalanine shares its blood-brain barrier carrier protein with a number of other amino acids, all of which compete for available carrier sites. This concept might ultimately be put into use to support diet therapy in PKU: by ingesting high amounts of competing amino acids to block carrier sites for phe, brain phe could be reduced despite high blood phe (Editor's note: This is the idea behind the Danish pill, PreKUnil, described in the Winter 2002 issue).

We also study the rate of phe transport across the blood-brain barrier. After the patient takes a certain amount of phe we execute a series of MRS examinations to observe how rapidly it accumulates in the brain and then declines back to normal levels. Recently, we observed abnormally low brain phenylalanine concentrations in spite of high blood levels in three untreated women with classic PKU. Although they had never received any diet, they were almost unaffected clinically and had normal intelligence scores. Our hypothesis is that phe is not transported efficiently into the brain if it cannot bind well to the carrier protein. This would explain abnormally low brain phe levels and might provide some sort of "protection" of the brain against PKU in some individuals. Studies in a larger group of patients indicated that the blood-brain barrier phe transport properties vary to some extent among individuals, and correlate weakly with the patient's clinical status.

However, more questions remain to be answered before we can reliably ascertain that individual blood-brain barrier transport is a major factor or only one of subtle influence in typical PKU patients. We believe that other experiments on phe transport and its potential manipulation by therapy are indicated.

Researchers Collaborate to Produce Natural High Protein Diet for PKU
By Dr. Harald Trick, Department of Plant Pathology, Kansas State University

Previously, Dr. Bryan Hainline (Indiana School of Medicine) received a two-year NIH grant to evaluate the modification of a corn protein called gamma zein (his work is reported in the Winter 1999 issue). Gamma zein is a naturally occurring, common protein found in corn that contains only 2 phenylalanine residues out of 223 amino acid residues in the corn protein (0.9%) compared to the typical 4-5% of other food proteins. Thus this protein is a good candidate for a whole protein additive to the PKU diet. Using molecular approaches, Dr. Hainline was successful in changing the genetic structure of the protein by removing the two phe amino acid residues without altering the structure of the protein. Dr. Hainline performed his research in a bacterial expression system to prove this concept. The next step in the development of this protein as a food additive for PKU patients would be to mass-produce it at low cost. One method to do this would be to harvest the protein from a genetically modified plant. To do this, Dr. Hainline enlisted the help of myself and Dr. Subbaratnam Muthukrishnan from Kansas State University. We have been recently awarded a three-year USDA grant to express a phe-free protein in soybean. Soybean is an excellent choice for producing large amounts of protein because a large portion of the bean is composed of protein and its native proteins are biochemically different than the gamma zein protein, making separation of phe-containing proteins and the phe-free zein protein relatively easy. An added benefit is that soybean is an easy crop to raise and harvest.

The overall goal of this research is to produce the phe-free gamma zein protein in soybean, isolate the pure phe-free proteins, and then incorporate these proteins into the diet of PKU patients. We envision the protein to be a powder, but it could be formulated to have better texture, like tofu—or better yet, like ground beef! Over the next three years we plan to further modify the gamma zein gene to maximize the expression in soybean seeds, perform the genetic transformation on the soybean cultures, recover genetically engineered soybean plants, and develop and optimize extraction and purification schemes for the phe-free zein protein. A product is still several years away but using genetically engineered plants as "factories" to produce high quality, nutritive protein will be worth waiting for.