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Diet

DURING THE LATE 1980S, I WAS THE PHYSICIAN in charge of a primary care program for about 800 adults who were living at the Fernald State School in Waltham, Massachusetts. Built in the late 19th century at a time when society felt the best option for mentally retarded (as they were then called) children was to care for them in largely bucolic, institutional settings for life, Fernald—a lovely campus of red brick buildings shaded by stately oak trees—recalled a medical era long forgotten in the gleaming modern hospitals of Cambridge and Boston.

Among my patients were two sisters in their early 30s. Carol was born in the fall of 1958; Nancy was born in 1960. Both were tall, slender, attractive women with naturally blond hair and light-blue eyes. From a distance one might believe that they were twins. Both women were also profoundly mentally retarded, with IQ scores below 50. Neither one could speak or care for herself. Both were extremely anxious in the presence of everyone (including me), except for their elderly parents, who visited them occasionally on Sunday afternoons, and the poorly paid Haitian caregivers who dressed and fed them each day. Carol and Nancy are among the last few people born in the United States who bear the full burden of a rare single-gene disorder called phenylketonuria (PKU).

Starting in 1962, the states began to test all newborns for PKU. This was because a small group of researchers had shown that by immediately placing infants born with PKU on a special low-protein diet containing only trace amounts of the amino acid phenylalanine, they could avert the severe intellectual disability that would otherwise enshroud them. Because of this unprecedented intervention, in just a few years PKU virtually disappeared from the differential diagnosis of severe developmental delay in young children. I recall hosting pediatric residents from Boston Children's Hospital

who worked in its PKU clinic (even when they are adhering to their brain-saving diet, the blood chemistry of these children needs to be carefully monitored) so they could meet Carol and Nancy to better grasp just how profoundly devastating PKU is if untreated. The conquest of PKU is one of the great achievements in our quest to treat hundreds of rare, mysterious, genetic disorders, most of which do not receive the attention that will be necessary if we are to conquer them. But, before I recount the fascinating story of PKU, a brief explanation of inborn errors of metabolism is in order.

The most common genetic disorder in the world is probably a disorder of the red blood cells that, long before it carried the scientific name of glucose-6-phosphate dehydrogenase (G6PD) deficiency, was known as *favism*. That term derives from the Latin name given to the fava or broad bean plant, an ancient crop cultivated throughout southern Europe, the Middle East, and parts of Africa. Historians of medicine have long hypothesized that the admonition by Pythagoras, one of the first great physicians, to avoid eating the fava bean derived from observations that some men became acutely ill if they did. In its severe form, the disease causes sudden, massive destruction of red blood cells. The severely affected person develops abdominal pain, yellow eyes and skin (jaundice), and profound weakness. Usually, over time, he recovers (so long as he avoids the bean) as the body makes new red blood cells. However, the disease has a wide range of severity, and it can be fatal. Why men? Today we know that the gene responsible for G6PD deficiency sits on the X chromosome. Like other X-linked disorders, it is far more common in men because they do not have a second, protective X chromosome with a normal copy of the gene, whereas women do.

It is plausible that ancient people learned that some men should avoid the bean simply because so many were at risk for the severe consequences of eating it. In many parts of the world, around one in 10 persons carries a variation in the gene that results in a defective G6PD enzyme that, given the right environmental exposure, triggers the sudden destruction of red cells. In the world today, literally millions of men carry one of hundreds of different mutations that predispose to favism.

How can deleterious mutations become so common among humans? The answer, as you might have guessed, is that the mutations that cause G6PD deficiency also confer a benefit. For more than a century, physicians have realized that favism is a disease of the tropics, which means it is found mostly where malaria is endemic. In the late 1960s and 1970s scientists showed that mutations in the *G6PD* gene conferred some resistance to infection from the malaria parasite.

Our understanding of the molecular basis for G6PD deficiency is closely connected with the long-running battle against malaria. Beginning in the late 1920s, scientists who were studying an antimalarial drug called pamaquine noticed that a few days after they ingested it, a few of the prison "volunteers" developed dark urine, turned yellow, and had a rapid fall in the hematocrit. Over the next two decades, physicians realized that sensitivity to the drug was familial, and that some persons from some populations were more at risk than those from others. About 1948 it became clear that persons sensitive to drugs like pamaquine and its newer version primaquine also were sensitive to eating fava beans. By then scientists knew enough biochemistry to infer that both the food and the drug sensitivity could be caused by a defect in the pathway by which cells produce a compound called glutathione, which is essential to maintaining the integrity of the red cell membrane. In 1958 a team at the National Institutes of Health (NIH) led by Dr. Paul Marks, who would go on to become the longest serving director of Memorial Sloan Kettering Cancer Institute in New York, published a paper delineating the precise nature of the biochemical deficiency.

About 1865 Gregor Mendel, a Moravian monk and autodidact, deduced from his study of sweet pea plants that there must be discrete (but completely unknown) particles, inherited without change through generations, that programmed the elements of physical existence. But his work, published in an obscure journal, was barely noticed. It was not until 1900, when three European scientists independently rediscovered the laws of Mendelian inheritance—the rules that explain how single-gene disorders are inherited as dominant or recessive conditions—that an intellectual scaffold permitted organized study of genetic disorders. But it was the flimsiest of scaffolds, and with a few exceptions many decades would elapse before scientific progress permitted the first meaningful therapy for these rare disorders. Even today, the immense scientific advance in understanding human genetic diseases that was catalyzed by the success of the Human Genome Project—the decade-long effort to decipher human DNA—must still be regarded as no more than an early success in the long journey to develop treatments for genetic disorders.

Black Diapers: Archibald Garrod and Inborn Errors of Metabolism

No one more deserves the title of the *founder* of human biochemical genetics than Archibald Garrod, the son of a professor of medicine, who won a *first* at

Oxford, and then earned his degree in Medicine at St. Bartholomew's Hospital in London in 1886. Although his mentors singled him out early as a future professor of medicine, many other able young physicians were in line ahead of him, so in 1892 Garrod moved from St. Bartholomew's to the Great Ormond Street Hospital for Sick Children. There, during the period 1898–1902, building on his astute observations of children with unusual syndromes, he developed the theory that these disorders were caused by "inborn errors of metabolism," a phrase in wide use today.

To appreciate the immensity of Garrod's contribution, we must grasp the theory of disease that was prevalent in 1890. In essence, physicians, enthralled with the discoveries made by Pasteur, Koch, and other progenitors of the germ theory, had come to believe that nearly all disease (excepting alcoholism and trauma) was caused by the action of some invisible external agent or an imbalance of several in the body. This was not so very different from the ancient Greek view that health and disease were determined by various "humours." The idea that the absence of a particular chemical in a cell could lead to a discrete, well-defined disorder was largely out of reach. Organic chemistry was the province of the dye industry and the understanding of biochemistry was growing mainly out of studies of fermentation.

Garrod's name is forever linked with a rare single-gene disorder called alkaptonuria, which had been known to physicians for many years. If one is aware of the disorder, the diagnosis of alkaptonuria is extraordinarily simple. Mothers bring their infants to doctors because they are alarmed to see diapers stained with urine as "black as ink." It was also recognized that although these children were relatively healthy, that in adulthood they would often mysteriously develop severe back pain and incapacitating arthritis. In the 1890s, the few academic physicians who studied the matter believed that there must be some species of *bacteria* living in the gut of these patients that interfered with the metabolism of tyrosine, leading in turn to the excretion of a metabolite called homogentisic acid, a pigment that caused the black urine. Garrod, who earlier in his career (along with Frederick Hopkins who would in 1929 win a Nobel Prize for proving the existence of vitamins) had studied the excretion by the kidneys of several organic compounds, was not so sure.

One day, after seeing a child with alkaptonuria, he made the inspired guess that the disease arose as a consequence of a malfunction of some chemical in the body that led to a failure to properly metabolize tyrosine. As luck would have it, the child's mother was pregnant. When that baby was born, Garrod asked the nurses to examine each of her diapers. Fifty-seven hours later, a

nurse brought him the first black diaper. By combing through hospital records Garrod discovered two facts about alkaptonuria that fit perfectly with the newly discovered laws of inheritance: (1) the disease often appeared in two or more siblings, but *never* in the parents, and (2) the disorder was often seen in the offspring of marriages between first cousins. Thus, he became one of the first physicians to infer that a disorder was due to the inheritance of recessive mutations in the same gene from each parent.

It is said that after this discovery, Garrod became obsessed with the study of human urine. Over the next few years he established that three more rare disorders—cystinuria, albinism, and pentosuria—were also recessively inherited, and that they could be best explained by the malfunction of some chemical in the body that, when normal, properly metabolized some other chemical. In two of the three—cystinuria and pentosuria—patients excrete unusually large amounts of a metabolite in their urine. In classic cystinuria, patients lack a proper version of a protein that transports several amino acids. The disease is so named because patients are at high risk for having cystine kidney stones. Pentosuria is a clinically benign condition in which patients who lack the normal form of a certain enzyme intended to metabolize a sugar (L-xylulose) to an alcohol, instead excrete a large amount of that sugar in their urine. It is usually discovered by accident.

Garrod was awarded his post at the prestigious St. Bartholomew's Hospital just 1 year after publishing the first of his two papers in human biochemical genetics. From that moment his career was meteoric; just 7 years later he was made a Fellow of the Royal Society of Medicine. In 1908 he delivered the famous Croonian Lectures to the Royal College of Medicine under the title, "Inborn Errors of Metabolism." Although Garrod's career led him on many different diagnostic odysseys, he always taught his students "to have a love for the unusual and for rarities in medicine, because they so frequently lead to discoveries." Garrod's lectures, published first in *The Lancet* and shortly thereafter as a book of the same title, constitute a true paradigm shift in conceptions of disease causation. Today, a first edition of his book is a much sought after prize among those eccentric folks (myself included) who collect rare medical books. Just a few days before writing these lines I attended an antiquarian book fair in Boston where I was tempted by a pristine copy, but could not justify the $2250 price tag!

During the next few decades, many basic scientists, especially Beadle and Tatum in the United States (who shared a Nobel Prize for their work on mapping enzymatic errors) pursued ideas that Garrod had discussed

in his lectures, and established the "one gene–one enzyme theory." They used organisms such as *Drosophila*, corn, and yeast to prove that each protein in the body was the product of a single gene that coded for it. But the first great advance in *treating* rare human inborn errors of metabolism began because of the persistence of a heartbroken, but dedicated, *mother* and the curiosity of a young physician-scientist.

PKU

In the early 1930s in Norway, Borgny Egeland was struggling to understand why both her children had failed to acquire language, had never learned to walk, and were now profoundly retarded. She went from physician to physician telling the same story: Both children had seemed healthy at first, but had fallen far behind their developmental milestones by their second birthdays. In addition, both had a constant musty odor in their urine and about their bodies, both were of lighter complexion than their parents, and both had a seizure disorder. The first three physicians that she approached did not become interested in trying to decipher such an obviously rare disorder, but the fourth, Asbjørn Følling, did.

As luck would have it, before going into medicine Følling had studied chemistry and had been a professor of nutrition. After he found that available tests to look for abnormal amounts of carbohydrates or proteins in the urine of Borgny Egeland's children gave normal results, he tested their samples with a compound called ferric chloride, which was known to change the color of urine in the presence of ketones. When added to the urine of these two children, the liquid turned dark green—an unexpected finding. This provided a hint that the children had a disorder involving proteins. Følling now confronted a question that it seemed he had been training throughout his professional life to answer: What was the abnormal substance and how was it linked with the children's profound disability?

Drawing on the help of several other chemists, but mostly working alone, Dr. Følling was able to show that the urine in these children contained a vast excess of phenylpyruvic acid. After repeating the test several times, he wisely decided to screen the urine of other mentally retarded children. Among 430 such children, he found eight that also had very high levels of phenylpyruvic acid. He then studied their families and was able to show (much as had Garrod with alkaptonuria) that a recessively inherited gene caused this mysterious condition, making it one of the first orphan diseases to be deciphered. Based on current knowledge of biochemistry (which

had vastly increased over the three decades since Garrod's work), Følling guessed that the children's cells could not perform some chemical step in the conversion of phenylalanine to tyrosine. This would explain why they were light skinned; tyrosine is needed to make melanin, the key skin pigment.

Følling next sought a means to quantify the amount of the chemical in the urine so that he could screen widely for the disorder and understand variation in its concentrations. He asked bacteriologists at the university where he worked if there was a strain of bacteria that could be harnessed for this purpose. From one he learned about a strain of *Proteus vulgaris* that could not break down phenylalanine, the very amino acid that Følling thought was elevated in this new disease and the growth of which would be inhibited in the presence of high levels of that amino acid. By using a bacterial inhibition assay (a semiquantitative way to determine if the levels of phenylalanine were so high as to block bacterial growth), he developed the first version of a simple test to screen individuals for this disorder. Although *P. vulgaris* could grow in blood from healthy children, it could not grow in blood from the children that Borgny Egeland had brought to him.

In the 1930s there was no treatment for phenylketonuria, or PKU as the disease is now commonly called, but a positive test in an affected child did at least alert the parents to the one in four risk in each pregnancy of having another affected child. Even more important, however, was that Følling's great discovery suggested a treatment. If excess blood levels of phenylalanine or one of its metabolites caused the mental retardation by crossing the blood–brain barrier and poisoning brain cells, then perhaps by sharply reducing dietary intake of phenylalanine from birth it might be possible to prevent or ameliorate the mental retardation. Unfortunately, the cataclysm of the events leading up to and through World War II caused most biomedical research that was not related to the war effort to halt, including this one.

Shortly after the war ended, Horst Bickel, a German pediatrician who was one of the first physicians in the world to dedicate his career to developing treatments for orphan inborn errors of metabolism, made the next big leap in the quest to cure PKU. From 1949 to 1955, Bickel worked at the Children's Hospital in Birmingham, England, where he directed one of the world's first pediatric metabolic units (the forerunner of today's genetic clinics). In that era when pediatricians evaluated children with relatively normal features who had severe developmental delay, they routinely used Følling's urine test to screen for PKU. Usually the disease was not diagnosed in affected children until about age 2, at a stage when physicians thought

it was too late for dietary therapy to help an already badly damaged brain. In 1953 Bickel examined a 2-year-old girl with newly diagnosed PKU. He proposed to the child's mother that even though it would probably not correct her mental retardation, that a low-phenylalanine diet might prevent her from deteriorating further and might improve her behavior. It was well known that children with untreated PKU often had severe behavioral problems. The mother agreed to try.

Bickel was an expert biochemist. In addition to instructing the mother on what phenylalanine-rich foods (including all meats and dairy products) that her daughter should not eat, he developed a slurry of amino acids that could be given as a drink and that would ensure that she would have adequate amounts of protein while ingesting very little phenylalanine. The mother adhered rigorously to the dietary therapy and reported regularly to Bickel on the child's progress. By 9 months she was convinced that her daughter's interest in the world had increased and that her behavior had improved.

Next came the crucial step. Bickel asked the mother to stop the special diet and feed the child as she had done in the past. Now on a normal (but for her very high) phenylalanine diet, within 2 days the child took an obvious turn for the worse. A few days later Bickel placed the child back on the low-phenylalanine diet, and in a matter of days, her behavior obviously improved. He was able to correlate changes in behavior with blood levels of phenylalanine. Although the experiment involved a single patient, because the biochemistry of the disease was so clear (often, as we shall see, the case with orphan disorders) Bickel thought it highly likely that other PKU children would benefit as well. But in 1954 there was not yet newborn screening of infants for PKU, so even if a commercially available diet could be developed, it could not be used early enough to avert mental retardation, only at most to improve the ease of managing the child's behavior.

Nevertheless, by 1956 experts were optimistic that the devastation wrought by PKU could be prevented. To do so, two challenges would have to be overcome: (1) the development of safe and effective, commercially available low-phenylalanine foods, and (2) the creation of a low-cost accurate mass screening test that could identify babies with PKU shortly after they were born. Without early identification to reduce exposure of the brain to toxic levels of phenylalanine, the diet would likely be of only minimal value. Both tasks were difficult. Although it was possible to develop a safe and nutritious low-phenylalanine infant formula, nutrition experts worried it would not be palatable and that it would be difficult to convince a food

company to make it because the product would be consumed by only about 400 newborns a year in the United States. As for the screening test, it posed a financial and logistical nightmare. For such a program to work, about 12,000 tests would have to be performed to identify just *one* child with PKU. Would the cost of screening a population justify the expense? Would society even accept such a screening program? By 1956 researchers had already shown that, when used too early, the urinary test developed by Følling resulted in too many false negative and false positive test results to use as a tool to screen all babies (Følling's test was much more accurate at about 8 weeks of life when levels of phenylpyruvic acid became high in the urine, but by then the brain was *already* badly harmed). If deployed too early, the test threatened to do more harm than good—by causing babies who received an erroneous diagnosis of PKU to be put on a low-phenylalanine diet that was *not* good for them. Also, there was little reason to deploy a screening program until doctors could be sure that they could quickly place infants who had been correctly diagnosed with PKU on a special low-phenylalanine diet.

In 1949 a scientist named Louis Woolf developed the first crude low-phenylalanine supplement, which he created by hydrolyzing (breaking down) proteins. But no good formula was easily available until the late 1950s. Fortunately, about 1957, Mead Johnson, an Indiana-based infant food company that was founded in 1900 by a man who was desperately concerned about his son's poor health, agreed to create Lofenalac, the first widely available baby formula for infants and children with PKU. The product was based on the discovery that by passing casein (a major component of cheeses) hydrolysates over charcoal filters, food scientists could remove most phenylalanine from the milk protein. The resulting material could be used as the base element in an infant formula and for making low-phenylalanine foods. Lofenalac is essentially a low-protein, high-caloric powder that is supplemented with certain amino acids, vitamins, and minerals. Researchers were able to show that when young PKU children were put on a Lofenalac diet, their blood levels of phenylalanine fell into a range that was close to normal. This suggested that, started early enough, the diet might permit the brain to develop normally. With the launch of Lofenalac in 1958, the *medical food* industry was born.

The next hero in the history of developing a treatment for PKU is Dr. Robert Guthrie. Born in 1916 in Marionville, Missouri, Guthrie grew up in Minnesota where he developed a lifelong passion for sailing on Lake Minnetonka. He earned his MD at the University of Minnesota in 1942 and he

stayed on to earn a PhD in bacteriology in 1946. Guthrie spent the next 12 years working at the Roswell Park Institute for Cancer Research in Buffalo, New York. A devoted father of six children, Guthrie's professional life changed when his second son was born with mental retardation. It took an even more dramatic turn in 1957 when his niece was diagnosed with PKU.

Guthrie became active in local groups interested in helping children with disabilities and he became friends with Dr. Robert Warner, a pediatrician at the Children's Hospital of Buffalo, who was trying to promote research on the causes of mental retardation. Warner persuaded Guthrie to move from the cancer institute to the hospital and to redirect his research efforts to understanding rare genetic disorders in children. Deeply affected by his niece's condition and knowing the limits of the Følling urine test, Guthrie set out to develop a test that could diagnose PKU at birth—possibly in time to avert or greatly reduce the then inevitable mental retardation.

Drawing on his training in bacteriology, Guthrie reasoned that he could use a strain of bacteria that could only grow in the presence of high levels of phenylalanine as a method to identify children with excess levels. In a remarkably short time, he developed what became known as an automated bacterial inhibition assay test. Simply put, a strain of the bacteria *Bacillus subtilis* was grown on gel agar plates that were infused with a chemical called β-2-thienylalanine, which competed with phenylalanine and prevented the bacteria from growing. However, if a sufficiently large amount of phenylalanine was added to the medium, the bacteria would grow, overcoming the inhibiting factor. A technician glancing at the gel would see a whitish growth halo of the bacteria.

Once he had tinkered with the system so it would respond to the levels of phenylalanine typically found in the blood of a patient with PKU, Guthrie, who knew that the relevant metabolites were stable in dried blood, had the transformative idea of developing a universal PKU screening test. In the newborn unit a nurse could quickly prick the heel of a newborn and spread a drop of blood on a filter paper that could then be sent to a central laboratory. There a technician could punch out a tiny circle of dried blood and place it on a particular spot on the gel. A spot in which bacteria grew would indicate the sample came from an infant with very high levels of phenylalanine. Technicians could scan hundreds of wells quickly and easily pick out the positive tests.

In 1960 Guthrie tried out his new method by obtaining blood samples from scores of mentally retarded residents living in the nearby Newark,

New York state school. In blinded testing, he correctly identified all the patients who were known to have PKU and also found four other patients who had never before been diagnosed! Knowing that the medical establishment and state departments of public health would demand more proof than his current research offered, Guthrie quickly obtained funding to conduct a large pilot study of PKU screening. In little more than a year, hospitals in 29 states collected more than 400,000 samples from newborns and mailed them to a laboratory he set up to manage the huge sample flow. To compare the new blood test with the Følling urine test, Guthrie asked the hospitals to give each mother a urine collecting kit and to send him a sample taken when the baby was 3 weeks old. Among the 400,000 babies who underwent the heel stick, Guthrie found 275 who tested positive. Repeat testing confirmed that 37 of them had phenylketonuria; the rest had only transient elevations that he could not fully explain. He also found that four of the 37 truly affected babies did not test positive on the *urine* test. It produced too many false negatives. Thus, he proved that the blood test he had developed was superior.

Most of the participating hospitals continued to screen. The leaders of the National Association for Retarded Children quickly began lobbying for state-based funding for screening programs, and in 1964 the federal Children's Bureau urged universal screening. In an unprecedented series of events, state legislatures, often after hearing lectures by Guthrie—who had become an indefatigable champion of mass newborn screening—began to enact mandatory testing laws. By 1968 virtually every child in the United States (as well as children in the United Kingdom and other European nations) was being tested for PKU. To ensure accuracy, each baby who tested positive was retested. If testing confirmed high levels of phenylalanine, the baby was immediately put on a special diet. At newly created specialty clinics, moms of the affected kids were educated as to why they should not breast-feed and why they must feed their baby a special low-phenylalanine formula and that their babies would need to be on a rigorously controlled diet for life. If they adhered to the diet, there was a very good chance that their infants would escape the severe mental retardation to which two tiny mutations in their genomes had predestined them.

Shortly before Asbjørn Følling began his effort in Norway to understand the cause of mental retardation in a Borgny Egeland's little girl, another mother was confronting the same sorrow in Nanjing, China. In 1917 Pearl Comfort Sydenstricker, the daughter of American missionaries, who had grown up in China and attended college in the United States, returned to China, and married John Lossing Buck, an agricultural

economist. In 1921 the woman, now Pearl S. Buck, gave birth to her only biological daughter, Carol. A few months later the family moved to Nanjing, where Pearl taught English literature at the University of Nanking. Like all mothers, she was bursting with happiness about her little daughter. She later recalled her pride as "people spoke of her unusual beauty and of the intelligence in her deep blue eyes."

But as the months passed, and Carol did not develop normally, Pearl became increasingly fearful. In 1925 she asked a child psychologist who was visiting the university to examine Carol. When he told her that he thought her daughter had a serious medical problem, Pearl, already growing estranged from her husband, took Carol to the United States. A series of expert evaluations, concluding with a visit to the Mayo Clinic in Rochester, Minnesota, confirmed her worst fears. Doctors there advised her that although they could not tell her the cause, they were sure that Carol's intellectual deficits were permanent and (in keeping with the times) that the best course was to have her placed in a residential institution that cared for persons with severe mental retardation. Years later Pearl Buck recalled, "I don't know of any blow in all my life that was as rending. It was as if my very flesh were torn. It was beyond belief, and yet I knew I had to believe it, and to shape my life around the fact."

Pearl and Carol returned to China, and the mother threw herself into the effort of trying to educate her daughter. Over the next 5 years she saw some small successes, but it was clear that Carol would never be able to care for herself. In 1929 Pearl traveled with Carol to the United States and placed her in the Training School in Vineland, New Jersey, one of the most forward-looking residential institutions of the time. She returned to China, but it was already clear that her marriage was going to end. With her husband unwilling to shoulder the expenses at Vineland (preferring to move Carol to a less expensive facility), Pearl soon returned to the United States, moving to Perkasie, Pennsylvania to be close to her.

In 1931 Pearl was a near-penniless, single mother responsible for Carol's expensive care. Having dreamed from childhood of being a writer, she approached the Presbyterian Mission Board in New York, asking for support. The board offered her $500 to write a children's story about missionaries, and one of the board members was so moved by her story that she personally loaned her $2000. Pearl returned to China and wrote the story she had contracted to produce (called "The Young Revolutionist"), but only after she wrote a book that had long been taking shape in her mind, *The Good Earth*. When it was published in the United States, this tale of

peasant life in China stayed on the best-seller list for 2 years, earning Pearl more than $1,000,000, some of which she used to endow lifetime care for Carol at Vineland. The debut novel won the Pulitzer Prize for fiction in 1932. It was the first of many awards that Pearl would win over 40 years for her many publications. Just 6 years after Pearl won the Pulitzer, in 1938 she became the first woman to win the Nobel Prize for Literature.

Though millions of people read *The Good Earth* over the ensuing decades, virtually no one realized that the mentally retarded baby daughter cared for so fiercely by the protagonists, the farmers Wang Lung and his wife, O-lan, was modeled on Carol. As one of Pearl Buck's biographers wrote, "The nameless child, who serves throughout the novel as a symbol of humanity's essential helplessness, is Pearl's anguished, barely disguised memorial to Carol."

Until the 1960s, families usually shrouded the existence of a mentally retarded child in secrecy. For 25 years Pearl Buck (who adopted many children with both her first and second husband) refused to mention Carol. The topic was off the table for the many journalists who interviewed her. Then in 1950, now 58 years old, Pearl S. Buck, Nobel Laureate, finally coming to terms with her shame and sorrow, wrote an article for *The Ladies Home Journal* called, "The Child Who Never Grew." Her frank disclosure about her daughter's retardation and her regrets about how she dealt with it, elicited such a tsunami of response from readers that an expanded version was published in book form a few months later. Thus, Pearl Buck became one of the first formidable voices to speak out about the needs of people with disabilities.

Twelve years later, when Eunice Kennedy Shriver, an older sister of President John F. Kennedy, first wrote about her sister Rosemary (who was mentally retarded and living in a private institution) in *The Saturday Evening Post*, she cited Buck's earlier writing as having given her the courage to speak out in support of people with disabilities. The wife of French President Charles de Gaulle, who had a disabled child, also credited Pearl Buck for giving her the courage to go public with her sorrow. Sometime in the early 1960s, doctors at Vineland told Pearl that Carol's problems were due to phenylketonuria. They had used the test based on Asbjørn Følling's research to make the diagnosis. The beautiful deep blue eyes that Pearl had so admired in infant Carol were caused by the genetic disorder, which among other things causes a lack of dark pigments in the eyes.

From 1985 to 2000, I worked at the Eunice Kennedy Shriver Center for Mental Retardation in Waltham, Massachusetts. For five of those years I was in charge of the medical team that cared for about 800 institutionalized mentally retarded persons at the Walter E. Fernald State School (the Shriver

Center was located on its campus). Among my patients were the two young women with PKU who I mentioned earlier. They were tall, thin, blue-eyed, blond women who from across the room appeared normal. But, when I performed their annual physical examinations, they emitted frightened, whining noises. They could not speak; they seemed never at ease, and all their movements were awkward. They had lived at Fernald for almost their whole lives. I met their parents once. I still remember the father's tears. About his wife and himself, he said, "We live with unending sorrow."

Although I did not know Eunice Kennedy Shriver well, I did meet with her on a number of occasions. What I remember best was the way she greeted the persons who lived at Fernald. When she visited with a group, she would look each person squarely in the eyes, firmly shake their hands, and speak to each just as she would to potential donors at a political fundraiser. She refused to acknowledge that they were "different." I also remember a Board of Directors meeting in the early 1990s when Eunice nominated her brother, Senator Ted Kennedy, to be president of her foundation. Her eyes twinkled as she said, "He really deserves to be president of something." As I write these words, I feel a tiny connection to that young woman in Nanjing, China who gave birth to a child with PKU in 1920, a birth that drove her in 1931 to write a book to earn money to support that child, a book that made her rich and earned a Nobel Prize. Twenty years later she would write another book that would help lift the curtain hiding orphan genetic disorders.

The advent of newborn screening for PKU came at an auspicious moment; it strongly reinforced policies being developed by President Kennedy, who had a sister with mental retardation and who, along with his sister, Eunice, had decided to support advances in understanding this mysterious collection of disorders, making it a health priority. In 1962 President Kennedy created a national award to recognize pioneering work in the field. Fittingly, the first recipient was Dr. Asbjørn Følling. According to his son (also a physician), when Dr. Følling, who did not like to travel, received the notice of the award and an invitation to accept it in Washington, D.C., he did not even reply, apparently not understanding the prestige associated with being selected. It took a second call from the White House to get him on the plane in Norway! Also fittingly, Dr. Guthrie won the same award in 1977.

When mass screening for PKU was launched in the mid-1960s there were still many important unanswered questions. Were all children with elevated blood phenylalanine destined for mental retardation? How low must dietary therapy drive blood levels to prevent disability? Could phenylalanine levels

be driven too low, resulting in another form of mental retardation? At some point in life—perhaps after the brain completed its development in adolescence—could the patients go off the diet? Today, thanks to much research, we have good answers to these questions. To maximize brain development all children with persistently elevated levels of blood phenylalanine must be placed and rigorously kept on a low-phenylalanine diet at least through adolescence. Even when dietary compliance is nearly perfect, the IQ scores of well-treated patients run 5–10 points lower than those of their unaffected brothers and sisters. If adults with PKU reject their diet, they experience noticeable behavioral and cognitive problems, albeit not nearly as severe as the impact of the disease on untreated infants. It is not necessary or wise to put infants on a diet with no phenylalanine; after all, it is an amino acid that the cells need to make most proteins. The task is to determine the lowest safe level.

Forty years of successful PKU screening has created a new medical challenge. All over the western world there are young women with treated PKU who wish to marry and have children. If they become pregnant and do not adhere to the special diet they are highly likely (nearly 90%) to bear children with severe mental retardation, heart defects, and other problems. This terrible outcome results from the fact that the high levels of phenylalanine and its metabolites cross the placenta and have toxic effects on the developing fetus. It is an ongoing challenge to clinical geneticists to keep track of these women and make sure they get the very special prenatal care they need. Canada created a national registry to track these women, a course of action that the privacy-sensitive United States probably will not tolerate.

Today there are about 40,000 persons with PKU living normal lives—except for adhering to special diets—in the United States and Europe. Each year about 400 babies born in the United States are diagnosed with PKU. Not surprisingly, as the decades have passed and infants have outgrown special formulas, the challenge of keeping children, teenagers, and adults on diets that are not too palatable has become ever more important.

After discovering the cause of PKU and developing a highly accurate newborn screening test, the next great task in conquering this orphan disease was to develop a highly nutritious, very low protein (the source of most phenylalanine), reasonably priced formula for infants. As I have mentioned, in 1958 Mead Johnson launched the first baby formula for infants with phenylketonuria, Lofenalac, which has long dominated that niche market in the United States. Parents purchase the formula as sets of packets that one mixes with water and a measured amount of evaporated milk just before feeding the infant. Hungry babies seem not to mind the taste, which

almost all children and adults find slightly unpleasant—a sort of chalky, slightly bitter, yet rather bland taste. During the 1980s and 1990s most PKU children continued to derive most of their calories from Lofenalac, despite the fact that as they grew older, it became more difficult to persuade them to consume it.

The advent of dietary therapy for PKU initiated a sustained debate about how to regulate such products and how to inform patients about risks in other products. The Food and Drug Administration (FDA) recognized a different regulatory framework was needed for approving the creation of an infant formula that was low in protein (and very low in phenylalanine) than for a new blood pressure drug. In 1973 when Abbott Pharmaceuticals launched Ensure, the first widely used lactose-free nutritional supplement for persons of all ages with a wide variety of clinical problems, the need for a new regulatory framework became even more urgent.

The FDA created a new class of substances that it called "medical foods." In essence, a medical food is an orally consumed product that has been formulated and processed to serve particular patients with a defined metabolic problem for which the patient is under a physician's care. The formal definition of a medical food was incorporated into law in 1988 with the passage of the "Orphan Drug Act" (which we will discuss later).

One of the first things that parents who discover that they have a child with PKU do is to become experts in nutrition. Fortunately, there are some foods—mostly vegetables—that PKU children can eat, but the list of forbidden foods is formidable. Meat, fish, and milk products top the list. Imagine managing the life of a child who cannot eat pizza, ice cream, or hamburgers! It sounds simple enough to say that to avert mental retardation, you merely have to make sure your child adheres to a special diet, but what if there are few palatable foods to provide?

During the 1980s and 1990s several small companies, recognizing the growing market for PKU foods, began to market low-phenylalanine foods (breads, frozen dinners, desserts) directly to families. These products are more expensive than regular foods, which led many states to enact laws that mandated that (depending on family income) PKU families could receive several thousand dollars a year to offset some of the extra costs. But the real problem was not the cost; it was the taste. Even the best of this first generation of nonformula PKU foods often had an unappealing texture and a slightly bitter taste. I have tasted some that I could just barely make myself swallow. Imagine battling with a 7 year old to consume such products two or three times a day!

Not surprisingly, the parents of two children with PKU finally decided to do something about this problem. In 1992 David and Lynn Paolella were a young, successful, happily married couple. David was a rising star in architecture in the Boston area and Lynn, a jeweler, was eagerly awaiting the birth of their second child. When they learned on his third day of life that their newborn son had PKU, they, like any parents, delved deeply into the disease. Over the years they adhered closely to the prescribed diet and worked closely with the staff at the PKU clinic at Boston Children's Hospital to monitor their son's blood phenylalanine levels. But as their son reached school age and began to rebel against the formula and the poorly tasting commercially available foods, a rebellion with which—given the taste—they were strongly sympathetic, they took a dramatic leap.

Lynn, who had given up her career to ensure that her children (by this time they had three children—two with PKU) adhered to the strictest possible diet (consider the challenge posed by sleepovers and birthday parties), began to devote her time to creating new recipes for low-phenylalanine meals. By trial and error, Lynn, who told me that a lot of her early efforts were mostly enjoyed by the family dog, gradually developed meals that tasted far better than did prepackaged commercially available PKU products. Painfully aware of how dissatisfied the families were with what was available in the marketplace, in 2000, David ended his career as an architect, and he and Lynn set out on the potentially quixotic task of building a company that would produce a line of meals for all children and adults with PKU. I met David a few years ago when Dr. Harvey Levy, a professor at Harvard Medical School and one of the nation's leading experts on PKU, suggested he would be a great person to teach me about how treating PKU affects everyday family life.

Today, Lynn and David's company, Cambrooke Therapeutics, offers a wide range of low-phenylalanine prepared foods that can be purchased online. Over the last few years they have been pioneering the creation of a wide variety of protein substitute drinks (PKU formulas) that rely on a new method for processing cheese (appropriately developed at the University of Wisconsin) to yield a more palatable and functional protein source. Cambrooke's medical foods constitute a major advance in taste along with improved nutrition. David and Lynn have been tireless advocates for PKU families and have taken tremendous financial risks to do everything they can to help improve the life experience and outcomes for PKU patients and their families. From Borgny Egeland to Lynn and David Paolella, the history of PKU has many heroes.

Since the early 1960s, the standard of care for treating infants born with PKU has been the prompt and unrelenting use of a low-phenylalanine diet coupled with periodic blood evaluations to make sure that blood phenylalanine levels are at a safe level. The results have been astounding. About 40,000 persons in the United States and Europe, ranging in age from infancy to 50 are—thanks to Følling's curiosity, Guthrie's test, and Bickel's diet—living regular lives. Children born with PKU are schooled in regular classrooms; teenagers compete in high school sports and edit school newspapers; affected young adults excel in college and build solid careers. They look normal, act normal, and are normal, except for one aspect of their lives—their diet.

When universal newborn screening for PKU was introduced, there was still much to learn about both the disease and the novel dietary therapy. One of the big unanswered questions was whether or not patients would have to be maintained on the diet for life. It now appears that to achieve and maintain the best possible cognitive function, persons with PKU do indeed have to be forever on the diet. Over the last four decades there have been many studies of the cognitive development of persons with PKU. Studies of affected adults show that if they go off diet for even a few weeks, they experience harmful behavioral changes and a discernible decline in cognitive skills. For obvious ethical reasons, no one is going to conduct an experiment in which they pull the patients off the diet for a lengthy period of time to see how much damage ensues.

Despite the risks of doing so, many persons with PKU, especially teenagers and young adults, frequently cheat on their diets. Imagine a 12-year-old girl at a birthday party who cannot have any cake or ice cream or a 16-year-old boy hanging out with his friends on a Saturday night who cannot—ever—join them for burgers or a pizza. Just like we do, they love the tastes of these foods, but they are forbidden. No one really knows how quickly brain damage accrues when a young adult goes off the diet. But the best evidence says that noticeable behavioral changes such as easy irritability happen within a few days.

The need to stay on diet is reinforced by the fact that among persons with the most severe form of PKU (as with most diseases there is a range of severity that is only partially understood), after strict adherence for months on end, blood levels of phenylalanine are still quite a bit higher than they are in unaffected persons. Thus, although the PKU diet has long and justly been trumpeted as a great victory in the fight against an orphan genetic disorder, it falls well short of a cure. Diet has changed PKU from a devastating form of mental retardation to a chronic disorder

that can be kept at bay only if the patient commits to living in a special environment. This raises the question: Can we develop an even better therapy, perhaps one that frees the person with PKU from his dietary chains? In the case of PKU, as well as for other inborn errors of metabolism for which dietary therapy is an option, there is an obvious strategy to try.

Like many other enzymes, phenylalanine hydroxylase, the enzyme that is defective in PKU, depends on a cofactor (a small molecule like a vitamin) to help it work. If the PKU is attributed to a genetic mutation that greatly lessens, but does not eliminate, production of that enzyme, leaving it partially functional, it might be possible to squeeze more action out of it by providing a lot more of the cofactor. Over the years, scientists have studied the relevant PKU cofactor—tetrahydrobiopterin (BH_4)—in an effort to understand this question. Fortunately, BH_4 can be safely given to humans. In 1999 Japanese researchers undertook studies in which they gave unusually high doses of BH_4 to persons with PKU. They showed that in nine of 37 PKU subjects, treatment with BH_4 lowered blood phenylalanine levels by 30% in just 8 hours. When the group was treated daily for several weeks, blood levels dropped 30% or more in 17 out of 37 (46%) of patients. This impressive drop in nearly one-half of the subjects prompted major interest in developing a new therapy to treat PKU. It catalyzed one of the most rapidly successful drug development programs in the history of the pharmaceutical industry.

In the same year the Japanese reported their findings, BioMarin, a young biotech company devoted to orphan genetic disorders, raised $67 million in its IPO (initial public offering). BioMarin had been founded in 1997 to develop a new drug called Aldurazyme to treat an orphan genetic disease called Hurler syndrome. Its then medical leader, a physician named Emil Kakkis, is an expert in a class of childhood-onset genetic diseases called lysosomal storage disorders. In 2004, the year Aldurazyme won FDA approval, BioMarin geared up a new program to develop a pharmaceutical version of BH4 to improve the treatment of PKU.

The company goal was to develop a compound called sapropterin hydrochloride and show that it sharply lowered blood phenylalanine levels in PKU patients. Unlike most drug development projects, BioMarin did not have to spend many millions searching for a novel chemical compound nor did it face the daunting challenge of manufacturing a new drug with acceptable purity in large quantities. Although BioMarin would have to run safety studies, prior experience virtually guaranteed the compound could be safely administered to humans. The company really faced a single, albeit difficult, hurdle—proving that the drug was efficacious.

In a negotiation that is almost without precedent in the annals of the drug industry, the FDA agreed with the company in advance that an approval "end point" for the study would be if the drug could be shown to lower blood phenylalanine levels by 30% in a specified fraction of patients who were maintained on their regular diet. The FDA did not demand that the company show any neurological or other benefit to the patient. Both parties essentially agreed to accept the premise that if the blood levels of phenylalanine achieved with diet could be further lowered by giving a safe cofactor, then the patient might benefit in two ways: maintain consistently lower phenylalanine levels and suffer less damage if he or she deviated from a rigid adherence to the diet.

In just less than 3 years, BioMarin, working with academic experts, completed several clinical trials of its experimental drug. First it showed that among a large group of PKU patients aged 8 to 48 that sapropterin at a dose of 10 mg/kg a day for just 8 days resulted in a drop of blood phenylalanine levels of 30% or more in about one in five persons. As suspected, some PKU patients benefited much more from cofactor therapy than did others (with the variation being largely a consequence of the specific nature of the many different possible mutations in the gene). In the next trial, which focused on the responders in the first trial, the scientists randomly divided 88 patients into two groups: one to receive sapropterin and the other to receive a placebo. At the end of 6 weeks of therapy the treated group showed a dramatic decrease in blood levels, whereas levels in the placebo group rose slightly. The third and final study focused on 90 PKU kids aged 4 to 12 who were on diet and ran blood levels above a certain value. The children were all given sapropterin at 20 mg/kg each day for 8 days. At the end, 56% of the children had experienced at least a 30% reduction in their blood levels from that they had maintained on dietary restriction.

Although it was concerned that the company had not linked lowered blood levels to clinical benefit and required that BioMarin conduct a number of follow-up studies, the FDA approved the drug (marketed as Kuvan) for use in December of 2007. The drug was launched at a price that, depending on the weight of the patient, would cost between about $50,000 and $150,000 a year. In 2011, just 3 years after product launch, BioMarin reported net product revenues of $117 million from the sale of Kuvan. From these numbers, it appears that about 15,000 persons in the western world with PKU are now regularly taking Kuvan. It will, however, be many years before we will know for certain how much Kuvan is helping. If patients wind up taking it largely to let them deviate from diet and if that means the net

change in median blood phenylalanine levels is not lowered, then in the long run patients taking Kuvan may have no better clinical status than patients who just stick to their diets. Still, Kuvan does promise to help those patients with PKU who are most responsive to cofactor therapy; further it will make eating a more pleasant experience for the rest of them.

Today, a little more than a century since Archibald Garrod's great insight about the cause of alkaptonuria and 50 years since Robert Guthrie launched the test that became the foundation for universal newborn screening, there is no question that dietary therapy for PKU is one of the great achievements in the struggle to help children with inborn errors of metabolism. But, it would be wrong to believe the battle against PKU is over. Even with the innovative work of David and Lynn Paolella, lifelong adherence to an expensive and relatively unpalatable diet is an imperfect therapy. Kuvan constitutes an important adjunctive therapy, but it only helps about 30% of PKU patients. Work on two innovative therapies offers some hope for the future.

The first involves harnessing an enzyme made by some bacteria called phenylalanine ammonia lyase (PAL), which breaks phenylalanine down into two harmless metabolites, as a drug to treat patients with PKU. Scientists have been working on this project for nearly a decade; they have found that although the enzyme dramatically reduces blood levels of phenylalanine, when given repeatedly it evokes a potentially harmful immune response. Efforts are under way to block or reduce that response by "masking" the drug with a technique called "pegylation" (creating a compound affectionately called PEG-PAL). The second and more daring approach (discussed more fully in Chapter 7) is gene therapy. In essence, the idea is to use certain viruses that are known *not* to cause human disease, as vectors to carry the DNA sequence coding for the normal phenylalanine hydroxylase gene and to use those vectors to transduce (invade) liver cells. Once in those cells, the vector will use the cell's normal machinery to make the normal version of the enzyme. Theoretically, a single appropriate dose of such a viral vector could control PKU for several years (depending on how frequently liver cells are replaced through natural turnover). Recent advances using adeno-associated viral (AAV) vectors offer promise, but there is much work to be done. I am optimistic; I believe that children with PKU who are today following a very strict low-phenyalanine diet will in 10 years have less demanding and more efficacious treatment options.

Phenylketonuria is the best known of several rare genetic disorders that can be partially ameliorated by rigorous adherence to a diet tailored to circumvent the problems posed by a defective enzyme. These include, for

example, several forms of tyrosinemia (a disorder of tyrosine metabolism), galactosemia (a disorder of carbohydrate metabolism), and a very rare disorder called maple syrup urine disease (in which a failure to metabolize the branched chain amino acids is associated with a sweet odor in the urine). In each case the histories—from characterizing the disease to developing an ameliorative dietary intervention—are congruent.

One of the most underappreciated victories in public health in the last half third of the 20th century was the steady expansion of universal newborn genetic screening. Here, too, we owe a great deal to Dr. Robert Guthrie. Starting about 1962, he became a tireless advocate before state legislatures and departments of public health for the enactment of mandatory newborn screening laws. Several clinical geneticists who knew him have regaled me with anecdotes concerning how Guthrie traveled from state to state with a bottle of bourbon in his suitcase, a beverage that he found effective in getting his message across in evening discussions with some elected officials. He was helpful. Between 1962 and 1968 *every* state in the United States enacted laws requiring that newborns be tested for PKU.

Most of these laws were constructed to allow scientific experts in the states to decide whether or not to add other disorders to the testing menu. For a few years in the 1990s, I was a member of the advisory group that evaluated proposed genetic tests for the state of Massachusetts. Today, pursuant to these laws many states screen for about 40 rare genetic disorders, and the number should grow dramatically in coming years. In many cases the first line of response to discovering a child with an inborn error of metabolism is immediately to place him or her on a special diet that averts mental retardation and, not infrequently, death. These disorders are individually rare, but collectively common. About one in 1000 children is born with one of them. Over the last 50 years mental retardation has been averted in tens of thousands of children with orphan genetic diseases thanks to a combination of scientific advance and wise public policy.

Neural Tube Defects

In Ireland in the 1960s about one in every 150 newborn children was afflicted with a neural tube defect (NTD)—at the time the highest incidence of this severe birth defect anywhere in the world. NTDs include a variety of disorders that arise because of the failure of the tissue that is destined to form the spinal cord to form a completely closed *tube*. As gestation continues, a fetus with a NTD may develop one of several different structural

problems ranging from a very small, protected lesion at the base of the spine to a profoundly devastating lesion that causes major brain malformations. A critically important fact to understand if one is struggling to prevent these birth defects is that the embryonic neural tube normally closes at about 21 days after conception—*before many women realize that they are pregnant.* Depending on where the failure of the tube to close occurs and the amount of nerve tissue exposed to the outside world, affected children may be diagnosed either with *anencephaly* (in which the brain is severely damaged) or *spina bifida* (in which the lesion is limited to a portion of the spinal cord, the lower the better). About 40% of affected infants have anencephaly (and usually die soon after being born) and about 60% have spina bifida. In the 1960s NTDs were so common and so severe that in some countries they constituted a national public health problem, one that demanded vast resources for treatment and care.

Although there are a few chromosomal and rare single-gene disorders that cause NTDs, in the vast majority of cases, we do not yet understand why a particular fetus develops one of these malformations. But epidemiological data show that powerful genetic risks exist. Although the background risk that any healthy woman will give birth to a child with a NTD is about one in 1000, if that woman has given birth to one child her risk of bearing an affected child in the next pregnancy jumps to about 1:25, a 40-fold increase. If she has given birth to two affected children, the risk for the next pregnancy is about 1:10. If a man or woman who was born with spina bifida procreates, the chance that the fetus will also have a NTD is also ~4%, a huge step-up in risk. Over the last 40 years, scientists have documented a number of other risk factors. Hispanic women are at higher risk for bearing children with NTDs than are white women; African-American women are at lower risk than are white women. Obese women, women with diabetes, and women who must take antiseizure medicines are at increased risk. Each of these factors might reflect underlying genetic or environmental risks, probably both.

As is often the case with birth defects, the incidence of NTDs varied widely among countries, from as much as one in 1000 across the United Kingdom to only one in 10,000 births in parts of Asia. Generally speaking, it was most common among northern Europeans, especially those of Celtic origin. Nobody knew what caused it, but everyone recognized that there must be genetically disposing factors because the single most powerful predictor of risk was a positive family history for the disorder. Part of the mystery of NTD is that throughout the world it affects more girls than boys with a ratio

of about 1.2:1. If we assume that about one in 1000 children is destined to be born with a NTD that means that each year in the United States and Europe about 10,000 babies are born with anencephaly or spina bifida, about 100,000 babies in a decade. Of course the worldwide numbers are far higher.

Over the last four decades two remarkable developments have significantly reduced the number of children born with NTDs in the United States. The first was the discovery by the British scientist Nicholas Wald in 1974 that a biomarker called α-fetoprotein (AFP) that could be easily and inexpensively measured in a woman's blood was higher than normal in pregnancies in which the fetus was afflicted with a NTD. Especially in the United Kingdom, several groups quickly undertook large studies of AFP in maternal serum (in affected fetuses this protein can leak through the lesion into the mother's circulatory system) and were able to show that elevated levels of AFP (often double the normal range) were strongly associated with a markedly increased risk (but not a certainty) that the fetus was afflicted. The actual diagnosis is usually confirmed by repeat testing and by ultrasound studies that can visualize the lesion. During the mid-1970s proponents of population-wide maternal serum AFP testing published arguments in British medical journal *The Lancet*, favoring what is essentially a eugenic approach—that affected fetuses should be identified early enough that the mothers might be given the option to terminate the pregnancy.

In 1977 a major U.K. collaborative study unequivocally established the value of a national screening program. Less than a decade after this discovery, largely because the National Health Service could efficiently introduce the screening test into routine prenatal care, there was a sharp drop in the number of births of children with NTDs. In the United Kingdom, most women who were offered the AFP test took it, and most women who learned that they were carrying an affected fetus did terminate the pregnancy. In 1999 Wald reported that in just 20 years the annual number of live births of infants with NTDs in the United Kingdom had decreased by 95%!

In the United States screening for fetuses with NTDs lagged behind the British program until May of 1987 when the American College of Obstetricians and Gynecologists (ACOG) issued a medical liability alert, warning that a physician who failed to offer the test could be sued if a child was born with a NTD. In effect, ACOG was declaring a new standard of practice that its members would ignore at their peril. The alert had a profound impact on obstetric practice. By 1990—just 3 years later—the vast majority of pregnant women in the United States were being routinely offered maternal serum AFP (MSAFP) screening. Although the data in the United States are not

as comprehensive as those in the United Kingdom, it appears that over the last 20 years the number of children born with NTDs in the United States has declined by >30%. The mandatory fortification of the food supply with folate is almost certainly the major cause of the decline. Many young pregnant women do not opt for NTD screening and among those who do that learn the fetus is affected, many do not terminate the pregnancy.

The implementation of MSAFP screening was not without its problems, the most important of which is that the screening algorithm was set so that the test would miss few affected pregnancies. Because the variability in the test results, for every true positive result that the test reported, it also called out about 10 that would turn out to be *false* positive results. Almost every woman who tested positive had to be promptly retested. Fortunately, on subsequent testing about 90% of those women turned out *not* to be carrying a fetus with a NTD. Even though the odds favored them, many of the women who were initially informed that the result was positive lived in a state of profound anxiety during the 10–14 days that it took to repeat the test and obtain the results. I well recall some of them telling me that the fear generated by a positive result on the initial screen cast a pall over the rest of the pregnancy. Over the years the quality of the test has improved, but it still generates many false positive results.

The second major development that contributed to the decline in births of children with NTDs was the discovery that women who regularly ingested the B vitamin folic acid (folate) could reduce their risk of bearing an infant with an NTD by about 50%. In the early 1960s Bryan Hibbard, an epidemiologist working at the University of Leeds, began to study pregnancy outcomes in poor women with the hope of identifying environmental risk factors that might be corrected with simple public health interventions. In 1964 he reported the results of his detailed analysis of 1484 low-income women in Liverpool with an unusually high level of problems in their pregnancies, noting that among other dietary deficiencies, the women had low levels of folate in their urine. This led him in 1965 to hypothesize that folate (which is an essential cofactor for many biochemical reactions in human cells) deficiency could cause birth defects, especially those involving the central nervous system. During the late 1960s and 1970s an increasing number of retrospective studies and uncontrolled trials were conducted that seemed to confirm Hibbard's hypothesis. In 1968 Hibbard and his colleagues showed that by providing folate supplementation throughout pregnancy to women who had in the past given birth to a child with an NTD they could reduce the expected recurrence risk by ~70%. But, it was not possible to generalize

from this happy finding to make a claim about the benefits of folate to all pregnant women.

It was not until the Medical Research Council in England and the Public Health Service in the United States conducted prospective trials that physicians fully grasped the magnitude of the preventive benefits of folate. In 1991 Wald and others showed with prospective studies that women who were consuming folate before and when they got pregnant had a sharp reduction in risk for bearing a child with a NTD. In 1992 the U.S. Public Health Service (USPHS) recommended that all women who were planning a pregnancy or who could become pregnant consume 400 μg of folate a day.

In the United States about one-half of pregnancies are not planned. Study after study showed that despite major educational efforts, many fewer than half of the women of child-bearing age were consuming folate *supplements* (although they were easily available in multivitamins) and that among those that were, many were taking only about half the folate needed to reduce the risk of NTDs should they become pregnant. This disappointing fact generated prolonged, often passionate debate that reached to the highest levels inside the FDA.

After years of study, in 1998 the FDA took the extraordinary step of mandating that cereals be *fortified* with at least 140 μg of folate per 100 g of grain (essentially a serving of breakfast cereal), in effect altering the food consumed by everyone to ensure that they got the vitamin to fertile women. Of course, it was not the first time that a vitamin has been routinely added to the food supply. Since the 1920s, many nations have added iodine to the food supply to prevent thyroid disorders. During World War II, disturbed by the nutritional deficiencies of many army recruits, the United States pushed for supplementing flour with thiamine, niacin, and riboflavin. Most commercially sold milk is fortified with vitamin D (to prevent the now rare bone disease called rickets).

The decision by the FDA to mandate the fortification of the nation's flour supply with folate—a decision that Commissioner David Kessler called the most difficult of his tenure—satisfied neither its proponents nor its critics. The proponents rightfully pointed out that a steadily growing body of evidence argued for fortification at much higher levels than the FDA recommended. Critics argued that the decision to fortify was in effect an uncontrolled experiment on the American people. Because fortification affects foods that almost everybody eats almost every day, it was possible that the program was creating a new health risk for the entire population.

That is why the new *fortification* rule only mandated that cereal makers provide about 25% of the folate intake recommended to avert NTDs. This reflected an uneasy compromise. No one knew whether regular exposure to high levels of folate might pose other kinds of risks. When one fortifies the nation's food supply with a chemical, even a tiny increase in risk for some serious disorder such as cancer could more than offset the public health gains of averting the births of about 1000 children with NTDs. Further, it was already known that taking high levels of folate could mask signs of an uncommon blood disease caused by vitamin B_{12} deficiency. Part of the logic for limiting the mandatory fortification to relatively modest levels was that women also consume folate when they eat green, leafy vegetables and some fruits. But experts estimated that the mandate resulted in most young women consuming about one-half the daily intake needed to avert NTDs should they become pregnant.

Soon after the FDA made its decision, Chile and Canada adopted a folate fortification policy, but, remarkably, most nations, including those in Western Europe, did not. This is especially troubling in the face of powerful evidence of efficacy, as evidenced by public health efforts in Chile. From 1967 to 1999, the incidence of births of children with NTDs in Chile was steady at 17 cases per 10,000 births. One year after Chile adopted a folate fortification program, the blood levels of folate in young women tripled. In the years since then the incidence of live births of infants with both anencephaly and spina bifida decreased by about 50%! Put another way, many children who would have been born with a NTD have been born without the congenital defect. Prenatal screening and abortion played only a small role in this sharp reduction in incidence. Over the last few years many cereal makers in the United States have increased the levels of folate to those (400 μg per serving) that scientific studies suggest could deliver significant prevention from NTDs, but some still do not. As of 2012 about 50 nations have decided to require that flour or cereals be supplemented with folate. Inexplicably, no European nations have yet mandated folate supplementation of food.

Although the government regulations were issued about 15 years ago, the fortification debate has continued. Among the leading proponents for increasing the levels of fortification is Dr. Godfrey Oakley, former director of the Birth Defects Division of the United States Centers for Disease Control and Prevention in Atlanta. In 2006, Oakley coauthored an editorial in *Pediatrics*, in which he asserted that in the 15 years since the emergence of a scientific consensus in 1991 that folate supplementation averted NTDs, failure

to aggressively push fortification and supplementation programs had resulted in the births of about 3 million babies throughout the world with congenital malformations that could have been easily prevented. Although the overstated claim reflects the passion of the advocate, the surprisingly slow adoption of a public health policy that offers immense benefits at reasonable cost surely has led to the birth of many thousands of affected babies in nations that have the resources to educate and supply folate to young women.

To this day, scientists remain uncertain as to why increased levels of folate in pregnant women's blood reduces the incidence of NTDs, but the evidence that it is does is irrefutable. Although the magnitude of the benefit varies considerably across populations (possibly because of differing genetic backgrounds or unknown environmental factors), dutiful and consistent consumption of 400 μg of folate a day on average reduces the background risk of bearing a child with spina bifida or anencephaly by at least 30%–50%.

Let us make sure we grasp how profound a statement this is. Consider the United States. If there was no screening and the general risk in the population for giving birth to a child with a NTD before the use of folate was one in 1000, some 4000 affected babies would be born each year. If proper folate consumption reduced that number by 50%, then only 2000 affected babies would be born each year and 2000 babies who would have developed a NTD would be born without that disability. Over a 20-year period that means that the simple act by women who could become pregnant of taking a daily supplement would result in the birth of 40,000 normal babies who might otherwise have had burdensome birth defects. Because the United States is one of the countries that requires the *fortification* of cereals with folate, it has achieved significant reductions in NTDs, but nowhere near 50%. Only the regular ingestion of supplements guarantees that a woman will maintain a protective level of folate. If all women in the United States who were able to become pregnant took 400 μg of folate a day, the number of children born with these defects could be cut substantially below current levels.

Our approach to averting the births of children with NTD is maddeningly contradictory. Few would oppose having women who may become pregnant take a daily vitamin supplement to reduce by half the risk of a serious birth defect in the fetus should they become pregnant. But many will be uncomfortable with a screening program that is intended to identify affected fetuses early enough to permit an abortion. This is especially so because many children with an NTD other than anencephaly are of normal intelligence (although often burdened with serious medical problems such as

the inability to walk and poor bladder control). It is not possible during pregnancy to predict the future intellectual status of a fetus with a low spinal lesion, so the mother and father confront a troubling existential uncertainty. In deciding to terminate, they must acknowledge that a significant fraction of fetuses with similar lesions would be born destined to have normal or near normal intellect. Women in the United States terminate pregnancies in which the fetus has been found to have spina bifida at a sharply lower rate (about 60%) than do women in Europe (about 80%). Of course this difference reflects the influence of many different factors. But one can surely infer that in the United States we should redouble our efforts to educate women about the protection that consumption of folate confers on the fetus and strive to convince as many women who could become pregnant as possible to consume 400 μg of folate each day.

Despite the incomplete success of the folate fortification programs, they still constitute a great victory in the war to conquer orphan disorders. Just as a low-phenylalanine diet averts mental retardation after birth, a high-folate diet averts congenital malformations before birth. Because neural tube defects occur about 10 to 20 times more often than does PKU, a highly effective folate supplementation program prevents about fivefold to 10-fold as many cases of NTDs than does a newborn screening program designed to avert mental retardation due to PKU.

Although they represent two of the great early successes in the field of clinical genetics, neither the increase of population-based newborn screening to detect inborn errors of metabolism nor the use of folate to reduce the risk of spina bifida is closely tied to the rise of that new medical discipline. To trace the trajectory of what today has become an immensely powerful set of technologies orchestrated to develop therapies for hundreds of orphan disorders, it will help to offer some historical context. No medical discipline arises because of the efforts of a single person. Many factors—the growth of a research mindset at a nation's medical schools, the availability of governmental and private funds to support that research, discoveries in basic science that open the door to new therapeutic possibilities, and lobbying efforts by families affected by genetic disorders are among the most obvious—contributed to the rise of clinical genetics. Still, in this field, the contributions of a few people certainly drove it forward. In providing some historical background, I have chosen mainly to highlight the work of several scientists who virtually everyone working in clinical genetics would agree played critical roles.