How Ketotic Hyperglycinemia Became Propionic Acidemia

Y. E. "Ted" Hsia
Emeritus Professor of Pediatrics and of Cell and Molecular Biology,
University of Hawaii

Eric—1957

Eric was born in St. Christopher's Hospital, Philadelphia. . He had poor mental development, perhaps due to some brain injury at birth, after a difficult pregnancy. Eric had pyloric stenosis surgery for persistent vomiting. He suffered many repeated attacks of severe illness with excess acidity and ketones in the blood, but responded to intravenous fluids. Using the latest technical tools of the time, especially amino acid analysis, Eric was found to have very high levels of the amino acid glycine in the blood. After the family moved to New Haven, his condition continued to fluctuate, often becoming worse with viral or other infections. Dr. David Clement of New Haven referred him to Dr. Robert Cooke at Johns Hopkins Medical Center for evaluation, where he became a frequent patient. Dr. Barton Childs found by a series of brilliant trial and error tests that Eric became worse if given any one of five specific amino acids [leucine, isoleucine, valine, threonine, and methionine]. He had less ketones, less acidosis and less glycine in the blood while he was taking a diet that was low in these toxic amino acids. The medical team at Johns Hopkins team published their findings about Eric in 1961, and called his disorder **ketotic hyperglycinemia.** Based on this report, a few other patients were found in various medical centers, who seemed to have the same disorder.

Glycine is the most common and most simple amino acid. It is converted to many other metabolic chemicals, and is involved in a host of body activities. At that time, no toxic effect was known to be caused by increased levels of glycine in the blood. In Eric, the glycine itself did not seem to be harmful. The team at Johns Hopkins did extensive investigations, but could not pinpoint a disturbance in any of the many known pathways converting glycine to other chemical products.

Eric's healthy parents, who were health professionals, cooperated bravely with many of the investigations proposed for their son, but watched closely over these investigations, and did not give their consent to all the tests that many doctors wanted to do, because of the large number that had been proposed, and because some involved more danger or discomfort than others. Despite the numerous investigations, the cause of Eric's disorder remained a mystery. Biochemical analysis of the ketones in his blood and urine by Dr. John Menkes at Johns Hopkins showed they were unusual in having odd-numbered carbon chains rather than the usual even-numbered carbon chains.

I started at Yale as a post-doctoral fellow under Dr. Ira Brandt in 1962 and left in 1965, during this time I became acquainted with Eric and his problems. Before I returned to Yale as a junior faculty member in 1967, Eric became severely ill and died.

Anne—1961

His mother had a second pregnancy, during which she was again quite sick. When Eric's sister, Anne, was born, Dr. Brandt immediately placed her on the same special diet, even before sending her blood to Dr. William Nyhan at Johns Hopkins for analysis. She was found to have the same chemical disturbances as her brother. Anne was then kept on the special diet, and did quite well, despite repeated episodes of ketoacidosis. These responded to prompt treatment at the earliest signs of any illness. Her treatment and management was based on the experience the doctors and her parents had gained from studying and treating her brother. Of course Anne was extremely precious in her parents' eyes, and they tried their best to bring her up as a normal girl, and shielded her from the many investigations they had agreed to let the doctors do on Eric. I had known her very briefly when I was a post-doctoral fellow.

Thanks to the early initiation of treatment, Anne had developed normally, and in fact at age 9 years her intelligence was found to be superior.

Another Boy

One night, I was called at home about a 5 month-old boy in the emergency room at Yale, who had recurrent pneumonia. I was called because the doctors were puzzled about his having unusually severe acidosis and ketosis. We discovered he had had a strange illness, presumably an acidosis, which relapsed several times, but nothing else seemed abnormal. His mental development was a little delayed. Amino acid testing revealed slightly raised blood glycine, and the ketones in his urine had odd-numbered carbons; he also had raised blood ammonia.

At that time Dr. Oberholzer and Dr. Stokke had reported a new disorder they had independently discovered, called methylmalonic aciduria, which could be detected by a relatively simple chemical test for methylmalonic acid [MMA]. The head of our Division of Genetics, Dr. Leon Rosenberg, considered this other boy might have methylmalonic acidemia, and

Continued from Page 9

arranged for a laboratory technician to set up the MMA test.

Sure enough, this other boy's urine was loaded with huge amounts of MMA. MMA is a four-carbon organic acid, in the metabolic pathway leading from the amino acids that are toxic in ketotic hyperglycinuria, to the formation of succinic acid, which enters into the very important energy-producing Krebs citric acid cycle.

We did many studies on this second boy. Because his chemical disturbances were very similar to those found in Eric, we assessed his response to the same five amino acids that were given to Eric, and found each amino acid worsened his status. Giving him a diet low in the same amino acids improved his condition. (When Anne's urine was tested, she had no rise in MMA.)

This was when it first became possible to grow [fibroblast] cells from a skin biopsy. We took a skin biopsy from this other boy, and I concocted a fairly simple but reliable apparatus to measure the breakdown of radioactively labeled MMA or of radioactively labeled succinic acid by cells. With this test, we confirmed that cultured cells in the skin biopsy from this other boy had a block in the breakdown of MMA, but not of succinic acid, so the defect would be in an enzyme between the breakdown of these two acids. This was confirmed when we eventually showed that one of the enzymes involved in the conversion of MMA to succinic acid, methylmalonyl-CoA mutase, was defective in his cells.

Dr. Charles Scriver of McGill University, a very competitive but friendly rival to Dr. Rosenberg in their studies of metabolic disorders, suggested we try giving this second boy some vitamin B12, because a derivative of B12 was a cofactor for methylmalonyl-CoA mutase. (In cells, these acids are present as their coenzyme A derivatives). Dr. Scriver had played a very pivotal role in showing that some patients with deficiencies in an enzyme which used vitamin B6, pyridoxine, as a cofactor responded to treatment with pyridoxine.

Sure enough, when we gave this other boy vitamin B12, his urine MMA fell dramatically.

His cells recovered much of the ability to break down MMA when grown with lots of extra vitamin B12 in the culture medium. We also showed that cells from his blood had the same block in MMA breakdown, which became normal after he was given B12. The findings in this other boy was a very important milestone in showing there was a group of enzyme deficiencies that are vitamin-responsive. Since then, anyone with an inherited defect in an enzyme that had a cofactor, was given the vitamin precursor of that cofactor, occasionally with very gratifying success.

After finding and studying other patients with raised blood MMA, researchers found that some patients with blocks in other enzymes, and even with blockage of the mutase enzyme, some were responsive to vitamin B12, and others were not.

This other boy had a very gratifying catch-up of his mental development over the next nine months.

Vitamin B12 Deficiency

An unusual anemia called pernicious anemia, which is also complicated by abnormal brain disturbances, had been thought to be a type of leukemia, because no known treatment would prevent the older adults who developed pernicious anemia from dying within two years. People who kept to a vegan diet [eating no meat or meat products, milk or eggs at all] were also prone to develop pernicious anemia.

While I was a medical student at Oxford, pernicious anemia was discovered to be cured by eating lots of raw liver! By the time I graduated from medical school, the tiny amounts of the curative substance in the liver had been extracted, its structure had been analyzed, and it was named vitamin B12. We tried testing blood cells from patients with pernicious anemia, using my concocted apparatus before and after they started treatment with B12, and showed these patients had the same block in MMA breakdown, which was corrected after giving B12.

Miniature Pigs

Because researchers had found that the biochemical metabolism of pigs was very similar to humans in many ways, we tried to do some research, using miniature pigs which were made vitamin B12 deficient. We soon discovered the chemicals in their urine were so complicated that urine testing for MMA was futile. With pig blood cells, I could do the same radioactive assay that I had concocted for MMA conversion to succinic acid. Getting blood from these struggling squealing pigs, however, was a very trying experience. We did find that the pigs on a vitamin B12 deficient diet had poor conversion of MMA to succinic acid, again corrected by giving B12.

What was the cause of Ketotic Hyperglycinemia?

Dr. Rosenberg was very intrigued about the many striking similarities between methylmalonic acidemia and ketotic hyperglycinemia. He was convinced that

the two disorders were related. From biochemical textbooks, we learned that propionic acid was a three-carbon intermediate chemical in the breakdown of amino acids and fatty acids which had odd-numbered carbons, including the five amino acids that were toxic in both these disorders. The four-carbon MMA was derived from the three-carbon propionic acid by an enzyme that added carbon dioxide; MMA was then converted to succinic acid. (When cattle and other ruminants eat hay, the microorganisms in their complicated stomachs convert all the carbohydrate from hay into propionic acid, the animals then derive nutrition from hay by using these enzymes. This is the major source of energy for these animals.)

Testing Anne.

When we surmised that ketotic hyperglycinemia might be due to a block in the same pathway as MMA, we proposed to use my concocted apparatus to measure the breakdown of propionic acid, as well as of MMA and succinic acid. Unlike MMA, the propionic acid was very difficult to detect, using the techniques available at that time.

We wanted to test cells from Anne, whose family was still living in New Haven. We approached her parents, and requested blood and a biopsy from her. The parents had protected Anne from all the many tests that the medical scientists wanted to do on her. We explained the reasons for our wanting to test their daughter. They reluctantly agreed to have the testing done, but told us that Anne was scheduled to have her tonsils taken out in a few weeks, so we could take our samples while she was under the anesthetic. On the day of her operation, I went to the operating suite, and took blood from Anne after she had been put to sleep, as well as a biopsy for tissue culture. We rushed to our laboratory with her blood, and found her blood cells could break down MMA and succinic acid, but not propionic acid! Our excitement was short-lived, because we then realized the abnormal test result might be due to some effect of the anesthetics she had been given. We went back to her parents, told them our findings, and they agreed to let me take some blood from her when she was awake. Anne was not too happy to be poked, but took it really quite well. When we tested this blood, we verified that the block in breakdown of propionic acid was still present. We also showed in a series of simultaneous tests that Anne had a block between the breakdown of propionic acid and of MMA, the other boy had a block in breakdown of both propionic acid and MMA, but broke down succinic acid normally, while blood cells from me and other "normal" people had no block in the breakdown of these three organic acids.

A Block in Propionic Acid Processing and Ketotic Hyperglycinemia.

After we had completed our tests, as we were preparing to report our findings, Dr. Fritz Hommes published an article from Scandinavia about a child with raised propionic acid in the blood. It may have been a great disappointment for us to have been scooped in this way, but I was quite satisfied with how carefully we had done our testing, and how we had a fairly complete story to publish, connecting propionic acid to ketotic hyperglycinemia.

In 1969, we submitted a brief manuscript, entitled "**Defective propionate carboxylation in ketotic hyperglycinaemia"** to the prestigious British journal, the Lancet. The editors of the Lancet agreed to publish our findings, but wanted to change our title to "Propionicacidaemia is the cause of ketotic hyperglycinaemia." We were reluctant to accept this change, as we felt it was too presumptuous of us to make this claim, because we had not yet shown that an enzyme involved with propionic acid breakdown was indeed the precise cause for ketotic hyperglycinemia. The editors might have thought we were too cautious, but agreed to keep the original title.

Propionyl-Co A Carboxylase.

We then set out to analyze the enzyme, propionyl Co A carboxylase, which converts propionyl CoA to methylmalonyl CoA. Sure enough, this enzyme had less than 1% of normal activity in the cultured cells from Anne. Cells from her parents had about half of normal enzyme activity, confirming that it was an autosomal recessive condition, inherited from the parents, who were both “carriers” for this defect. We reported this in 1971. The group at Johns Hopkins, and several other medical teams soon confirmed these findings in several other patients. A few years later, I met Dr. Menkes, who very graciously congratulated me on our discoveries. He said their team had many clues about a block in odd-chain fatty acid metabolism, but had failed to make the deduction that we had made.

This enzyme has turned out to be quite complicated. It is a complex of three subunits. The vitamin biotin is attached to one subunit, which swings to grab a carbon dioxide molecule from a second subunit, then swings over to the third subunit to attach the carbon dioxide to propionyl-CoA to form methylmalonyl-CoA, which is then released. This swinging back and forth is called "ping-pong kinetics." Of course we tried giving large amounts of biotin to Anne, but it did not improve her

Continued from Page 11

condition.

Dr. Roy Gravel, then working with us at Yale, was able to show that some patients with propionic acidemia had abnormalities in different subunits, while a few patients had an abnormality in the processing of biotin , or the assembly of the subunits by the enzyme holocarboxylase synthetase. The human body has four enzymes that use biotin as a cofactor; each one attaches carbon dioxide to a different precursor. Some patients with a block in attaching biotin to its subunit, causing multiple carboxylase deficiency, did respond to treatment with biotin. After this, Dr. Barry Wolf, also with us at Yale then, has worked out all the details about how biotin is normally processed and recycled in the body. He discovered the disorder called biotinidase deficiency, and other related disorders.

Chad

Many years later, when I had started a genetic clinic in Hawaii, I was asked to see Chad, who was very ill, and turned out to have propionic acidemia. As his mother has written, he has had many complications. Soon after we saw him, we arranged for him to be tested using a very new apparatus called "tandem mass spectroscopy." At first, the interpretation of his results could not distinguish between methylmalonic acidemia and propionic acidemia, but with Chad's sample, and experience with many more patients, this should no longer be a problem. Chad's remarkable survival is mainly credited to his intrepid mother's devotion and active, knowledgeable participation in his care. I am also grateful for the information and advice about Chad's care that I was given freely by some specialists who had much more experience than I had in some of the complications that Chad had.

Propionic acidemia Patients.

Since propionic acidemia was first studied, extensive experience has led to a much clearer understanding of the many different ways that things can go wrong in propionic acidemia, and how to treat them. Also, newer techniques have been developed, such as gas-liquid chromatography, to measure propionic acid levels in the blood, so that treatment can be followed more closely, and tandem mass spectrometry, which will provide a profile of all the organic acids in the blood, so that any newborn infant can be tested for the Organic acidemias, including propionic acidemia. Because of these advances, my concocted apparatus has lost its usefulness.

Each patient with propionic acidemia may have a different degree of severity and a different pattern of complications, but with early detection and improved treatment, it is hoped that more patients will do as well as Anne, the sister of the original patient.

Some Relevant Articles

Childs, B.; Nyhan, W. L.; Borden, M.; Bard, L.; Cooke, R. E. : **Idiopathic hyperglycinemia and hyperglycinuria: a new disorder of amino acid metabolism.** *Pediatrics* 27: 522-538, 1961.

Hommes, F. A.; Kuipers, J. R. G.; Elema, J. D.; Jansen, J. F.; Jonxis, J. H. P. : **Propionic acidemia, a new inborn error of metabolism.** *Pediat. Res.* 2: 519-524, 1968.

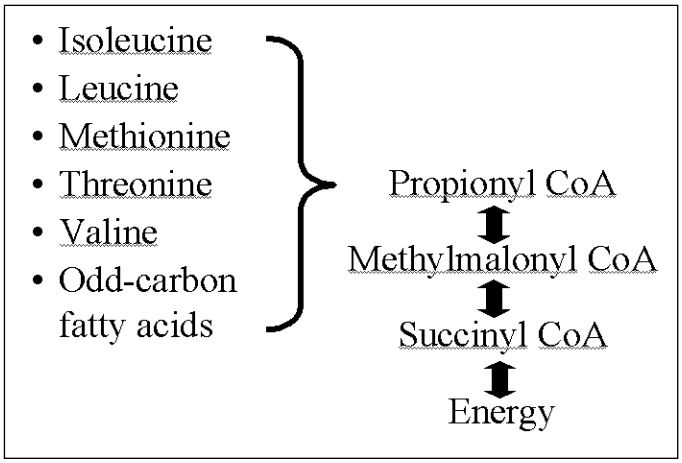
Hsia, Y. E.; Lilljeqvist, A. C.; Rosenberg, L. E. : **Vitamin B12-dependent methylmalonic aciduria: amino acid toxicity, long chain ketonuria, and protective effect of vitamin B12.** *Pediatrics* 46: 497-507, 1970.

Hsia, Y. E.; Scully, K. J.; Rosenberg, L. E. : **Defective propionate carboxylation in ketotic hyperglycinaemia.** *Lancet* I: 757-758, 1969.

Hsia, Y. E.; Scully, K. J.; Rosenberg, L. E. : **Inherited propionyl-CoA carboxylase deficiency in 'ketotic hyperglycinemia'.** *J. Clin. Invest.* 50: 127-130, 1971.

Rosenberg, L. E.; Lilljeqvist, A. C.; Hsia, Y. E. : **Methylmalonic aciduria: an inborn error leading to metabolic acidosis, long-chain ketonuria and hyperglycinemia.** *New Eng. J. Med.* 278: 1319-1322, 1968.

Rosenberg, L. E.; Lilljeqvist, A. C.; Hsia, Y. E. : **Methylmalonic aciduria: metabolic block localization and vitamin B12 dependency.** *Science* 162: 805-807, 1968



This account is from my memory, and I may have overlooked many details, so this account may contain some errors.

None of this would have been possible without the cooperation of these patients and their parents. — T. Hsia