

IN BUILDING THE FIRST MAN-MADE PROTEIN, PANAYOTIS KATSOYANNIS REVEALED VOLUMES ABOUT INSULIN AND PROTEIN CHEMISTRY

BY SHARON TREGASKIS

OUT OF DARKNESS

Panayotis Katsoyannis was in his final year of high school in Athens when Italy invaded Greece in October 1940. Germany joined the fray on April 6, 1941. Three weeks later, a swastika-imprinted flag flew in front of the Acropolis.

Liberation, in the form of British troops, would arrive in October 1944. "For almost four years, it was hide and seek," says Katsoyannis, who still reels off the military dates as easily as that of his own birth, Jan. 7, 1924. "We had our way with the Italians, but not the Germans. They would kill you. That was it. There was not any forgiveness."

Four-hundred thousand Greeks would perish during the occupation. Another 158,000 would lose their lives in the subsequent civil war—the opening salvo of the Cold War. "We were very much restricted, in every aspect of life—work, living, eating," says Katsoyannis. He recalled machine-gunfire blasting through

Panayotis Katsoyannis developed synthetic insulin at Pitt. He went on to create 150 analogs of the protein, manipulating it in ways chemists didn't think were possible at the time. In a photo taken for *LIFE* in 1964, the Pitt prof is shown (left) with his first grad student, Andy Tometsko, and a model of insulin.

PHOTOGRAPHY | FRITZ GORO/TIME LIFE PICTURES/GETTY IMAGES



When Katsoyannis embarked on his audacious project, scientists weren't even sure whether you could build a biologically active protein outside of the body. Students and postdocs, including Tometsko (shown here), were critical to the success of the work.

the windows of his family home and youngsters felled just yards ahead of him on the sidewalk by ricocheting shrapnel. "Those were dark years."

And yet for Katsoyannis (pronounced CAT-so-yawn-nis), at least, grace prevailed. An aspiring civil engineer, he had failed his university entrance exams when the tranquilizers prescribed for a gallbladder attack left him too drowsy to put pen to paper. Not that it mattered: By the time he'd graduated from high school, the Nazis had closed the universities to students.

Katsoyannis' father, a scholar of ancient Greek literature and headmaster of a gymnasium (an upper-level secondary school), bemoaned his 17-year-old's academic hiatus to a friend, peptide chemist Leonidas Zervas, a PhD. An invitation soon followed: Would young Panos assist in the professor's University of Athens laboratory? Students had been barred from the institution, but as much as possible, the faculty continued with its research. "When I started working with him, I forgot about civil engineering," says Katsoyannis, who would spend most of the next decade training with Zervas. "My heart belonged to chemistry."

That passion would propel the young biochemist from Athens to Manhattan, and then on to Pittsburgh, where, as a member of Klaus Hofmann's Department of Biochemistry from 1958 to 1964, he would synthesize insulin, a globe-shaped protein composed of 51 amino acids—a strand of 21 and another of 30—bonded by two sulfur-based bridges. "First Man-Made Protein in History," a *LIFE* magazine article trumpeted in 1964 below a photograph of the Pitt professor—clad in his white lab coat. An array of brightly painted billiard balls illustrated the "200 delicate steps" by which he had constructed the hormone responsible for regulating metabolism.

"Insulin, with its long double chains of amino acids, is one of nature's most complicated compounds," the *LIFE* article explained, "and the job of putting it together in a laboratory was roughly equivalent to working a dozen jigsaw puzzles simultaneously while blindfolded."

The *LIFE* article's rhetoric was far from hyperbolic, says Antonios Trakatellis, an MD/PhD and longtime collaborator with Katsoyannis. Their friendship spans their tenures in Pitt's Department of Biochemistry, Brookhaven National Laboratory, and Mount Sinai School of Medicine, where Katsoyannis would serve as founding chair of the Department of Biochemistry from 1968 to 1998. "Of course today, insulin is made with biotechnical methods," says Trakatellis, who went on to serve as rector of the Aristotle University of Thessaloniki and later as vice president of the European Parliament. "But at that time, it was an enormous accomplishment to synthesize a molecule which is so big."

Not only did Katsoyannis synthesize the molecule, he proved its biological function, heralding a fundamental paradigm shift for organic chemistry. He then went on to create analogs of the molecule, swapping out an amino acid here or a bond there, then investigating the differences in functional activity between the variants.

"There was a question about whether you could actually use peptide synthesis to create a fully biologically active [protein], compared to what was made in the body by biological processes," says endocrinologist Jeffrey Flier, an MD, dean of Harvard Medical School, and a student of Katsoyannis' at Mount Sinai. (His

research investigates the molecular causes of obesity and diabetes.) "There were still questions about whether a protein hormone as complicated as insulin would be fully active when it was synthesized."

Despite those achievements, Katsoyannis' technique was never directly employed to treat diabetes—the production process simply took too long, and yields were too low for commercial viability. Until Genentech cofounder Herbert Boyer (who received his Pitt PhD in chemistry in 1963) developed the technology to engineer *E. coli* to produce a pure form of insulin in the late '70s, it was cheaper to extract the hormone from animal pancreata and tolerate the occasional side effect. Ultimately, Katsoyannis' persistence in crafting and testing the functional activity of his analogs laid the foundation for contemporary pharmaceutical engineering, says Richard DiMarchi, a PhD and retired group vice president at Eli Lilly and Company. DiMarchi is now the Standiford H. Cox Professor of Chemistry and the Linda and Jack Gill Chair in Biomolecular Sciences at Indiana University; his work was key to the development of Humulin, Humatrope, and Humalog (which he designed), biosynthetic insulin analogs widely used to treat both type 1 and type 2 diabetes.

"If you were forced to ID a single lab that contributed most to the structure-function relationship, that would be the Katsoyannis group," he says. "They made the insulin analogs that helped us understand the degrees of freedom in what you could change to create superior medicinal value."

In the 1920s, Katsoyannis' mentor, Zervas, had trained with chemist Max Bergmann in Germany. In 1932, the two had developed the Bergmann-Zervas method of synthesizing peptides (the short chains of amino acids that make up proteins) while preserving their function. Soon after, Bergmann fled Germany to do research at the Rockefeller Institute in Manhattan; he was followed a year later by Zervas.

By the time Katsoyannis came along, equipment was scarce. So, too, were reagents, mostly just leftovers from inventory bought before the occupation. Every six months or so, Zervas sent his young assistant to the barbershop and the fishmonger for raw materials from which he would extract the amino acids their work required. "Today, you just open a catalog and order as much as you want," says Katsoyannis. "In those years, there were not amino acids available; we would hydrolyze proteins such as hair to isolate cysteine. You couldn't just put a kilo of hair in the flask with hydrochloric acid; you were restricted by how much material you could make each time because we didn't have the appropriate glassware."

"It was up to you to find and learn," says Katsoyannis, who mastered English and German to read the papers his mentor had coauthored with Bergmann. "And if you couldn't find a Greek book, you had to learn French, English, German, Italian, and find a book to read—it strengthened your will to do things." By the time the University of Athens reopened and awarded the budding chemist his bachelor's degree—summa cum laude—in 1948, his dissertation research on phosphorylation, the process that activates and deactivates amino acids, was already well under way.

With their nation's economy—and its academic community—in tatters, young Greek scientists of the mid-century aspiring to academic posts on native soil completed their compulsory military service, then established their reputations abroad, relying on the international web of connections woven by their mentors. For Katsoyannis, Zervas' network would prove vital. In 1951, while his protégé devised chemical smoke screens and new lubricants for firearms at the behest of the Hellenic Army, Zervas wrote to colleagues working on the structure and chemistry of peptide synthesis in the United States and Switzerland. Vincent du Vigneaud, a Bergmann protégé in Manhattan, responded first. "Du Vigneaud said, 'If it is a student of Zervas', he will be very close to the work we're doing,'" recalls Katsoyannis. "He wrote right away, and I came to the U.S. without thinking anymore about Switzerland."

In du Vigneaud's laboratory at Cornell University, Katsoyannis would deploy the Bergmann-Zervas technique for stringing together amino acids as part of a five-member team attempting to synthesize two peptide hormones produced in the posterior pituitary. In 1953, they assembled oxytocin—implicated in labor and lactation—and, in 1954, vasopressin—central to the maintenance of blood pressure. Cysteine, the sulfur-based compound Katsoyannis had hydrolyzed using hair swept from Athenian barbershop floors, featured prominently in each of the ring-shaped proteins. In 1955, du Vigneaud would garner the Nobel Prize in chemistry for the "first synthesis of a polypeptide hormone."

"And if you couldn't find a Greek book, you had to learn French, English, German, Italian, and find a book to read—it strengthened your will to do things."

"In this work," du Vigneaud declared in his acceptance speech, "I should like to acknowledge the splendid collaboration of Ressler, Swan, Roberts, and Katsoyannis."

The next year, the Nobel laureate would promote his Greek research associate to assistant professor; the two would forge a fast friendship severed only by du Vigneaud's death in 1978. Yet for Katsoyannis—by then married to Frieda, an American-born Greek, and father to Miranda, their first-born—an independent research career beckoned. "If I had stayed," he says, "I'd have continued working for du Vigneaud because he didn't want independent investigators. I started looking around."

The particulars came into focus during a talk by Frederick Sanger, a PhD who had already published the sequence of amino acids in both chains of bovine insulin. (His subsequent publication of the sequence for human insulin would garner the 1958 Nobel Prize in chemistry for clearly establishing that each protein has a unique chemical composition.) "When he put the structure of insulin on the board," says Katsoyannis, "I said, *My God, it's full of cysteines! I know how to do that. That's the thing I'm going to work on.*"

Katsoyannis was 33. The literature on

insulin—by then a darling of protein chemists worldwide—was only three years older. In 1921, Canadian physicians Frederick Banting and Charles Best had extracted the protein from the pancreas of a dog and proved its therapeutic value in treating diabetes, a feat for which Banting would be awarded a Nobel. In 1922, Indiana-based Eli Lilly began commercial production of the hormone, using pancreata salvaged from slaughterhouses. The next year, Denmark's Nordisk did the same. Diabetes had been transformed from a death sentence into a chronic illness.

Basic science was benefiting, too. Commercial production of pharmaceutical-grade insulin gave researchers access to substantial quantities of a pure molecule with which to work. Insulin was crystallized by an American in 1926; its molecular weight was determined by Swedes in 1931. Even then, however, chemists speculated that proteins were changelings—variable, molecule by molecule, in their particulars. Sanger, an Englishman, devoted a full decade to decoding the order of insulin's components. By stringing together the amino acids, Sanger had detailed and then proved the resulting protein's functionality in a biological system. Katsoyannis would trigger a shift in the basic understanding of protein science. He confirmed that even the fundamental units of biology were chemically and structurally consistent, molecule by molecule.

He would also attempt to outdo the men who had trained him. "Going from oxytocin to insulin is like going from a mountain of 1,000 meters to Everest," says Trakatellis. "Many people can climb a mountain of 1,000 meters, but Everest, that's another story. Panos had an audacious vision."

When he revealed his ambition during interviews, says Katsoyannis, most prospective employers laughed. At Pitt they offered him an associate professorship. Chair of biochemistry Klaus Hofmann—a trainee of both Bergmann and Cornell's du Vigneaud—was just two years away from synthesizing a 23-amino acid version of ACTH, the anterior-pituitary hormone central to the preservation of brain function during stressful times. "You want to go in a place where the groups do a similar type of work that you are doing," says Katsoyannis, who moved to Shadyside in 1958, after a series of interviews at Greek institutions confirmed that his research was more likely to thrive at Pitt. "You have the time to talk about the things that you are interested in; they have similar equipment."

Katsoyannis spent many long days in his 400-square-foot laboratory in Scaife Hall, yet the professor dedicated a special time slot exclusively for his children. On Saturday mornings, from 8 to 9, he watched cartoons—*Bugs Bunny*, *The Magilla Gorilla Show*, and the like—with his young family: daughter Miranda, born in 1956, and son George, born in 1960. "We would all pile onto the bed, watch TV, and be laughing," says Miranda, now a Washington, D.C.–based legislative analyst for the CDC. "It was a ritual." Later in the morning, he would make the 5-minute commute from the family's Fifth Avenue apartment for departmental faculty meetings. ("Klaus told me years later that [Saturday meetings] would keep them from talking too much because they all wanted to go home," says Hofmann's widow, Frances Finn Reichl, PhD '64, who studied protein biochemistry with Katsoyannis and served as a professor of biochemistry at Pitt herself for three decades.)

After the faculty adjourned, Katsoyannis would head to his laboratory, drying, freezing, hydrolyzing, and rinsing to isolate this pentapeptide, that nonapeptide—tacking on one more amino acid here, condensing the desired fragment there. Just four months after Hofmann published his synthesis of ACTH, Katsoyannis submitted the manuscript "Insulin Peptides I. Synthesis of Cysteine-Containing Peptides Related to the A-Chain of Sheep Insulin." The *Journal of the American Chemical Society* published the report in October 1961.

Twenty-one installments would follow, spanning a decade of painstaking research with eight coauthors at Pitt, Brookhaven National Laboratory, and the newly established Mount Sinai School of Medicine. "The way he went about doing it was a *tour de force*," says Clyde Zalut, a one-time research tech at Brookhaven who earned his PhD under Katsoyannis' mentorship at Mount Sinai and coauthored seven of the articles. "Doing the work in a very thorough, careful way proved that the basic science that had been developed up to that point was capable of producing large proteins, not just small proteins."

At Pitt, Katsoyannis' meticulous attention to detail infused his lectures, as well. "He had a great knack for explaining things so you understood them very, very well," says Finn Reichl. "He was systematic, yet it wasn't boring. I think it was because he loved it. The medical students thought he was terrific. They called him Adonis."

"Going from oxytocin to insulin is like going from a mountain of 1,000 meters to Everest."

The feeling was mutual. "I had a teaching rotation I enjoyed very much," says Katsoyannis. "After the lecture was over, the students would applaud."

Yet among the faculty, tensions could flare. Finn Reichl recalls an epic battle over a laboratory exercise for the medical students involving ascorbic acid—vitamin C. Katsoyannis had devised an experiment featuring the volatile compound and charcoal. A colleague, an expert in vitamins, criticized the plan, warning that the ascorbic acid would oxidize. "Dr. Katsoyannis said, *If that becomes oxidized, I will cut my wrists and bleed to death in front of everybody.*" The other professor had a point, says Finn Reichl. "Ascorbic acid will oxidize if you look at it. But Panos won that one."

On another occasion, says the Pitt professor emerita, Katsoyannis got into a spat with Seymour B. Koritz, a PhD expert in steroid hormones. "He nicknamed him Seymour Oscar Benjamin Koritz," says Finn Reichl. "S.O.B. Koritz." The hard feelings didn't last. When Katsoyannis assumed the chairmanship at Mount Sinai in 1968, Koritz was one of his first recruits.

"He was very forceful in his thinking,

although he would not oppress other people," says Zalut, now retired from Harvard Medical School. Katsoyannis always let him speak his mind, debating laboratory tactics and suggesting alternative approaches. "He was outspoken about how he wanted to do the research, and he was usually correct," says Zalut.

In 1964, the *Journal of the American Chemical Society* published "Insulin Peptides. X. The Synthesis of the B-Chain of Insulin and Its Combination with Natural or Synthetic A-Chain to Generate Insulin Activity." In 1963, the ninth installment in the series had detailed the reverse: synthesis of the A-chain and its combination with a natural B-chain to generate activity. Teams in Germany and China published similar achievements, minus the thorough evidence of functional performance. *LIFE* sent reporters. The same year, Hofmann resigned as chair of the department to launch the Protein Research Laboratory at Pitt, and Katsoyannis accepted an appointment at Brookhaven. He took Trakatellis and two postdoctoral associates along with him. "Because Panos was in the same field with Klaus, they were good friends," says Finn Reichl. And it came as no surprise when Hofmann's first round of recruits departed, his friend Katsoyannis included. "If you hire good ones, it's what you expect to have happen," Finn Reichl notes.

When Mount Sinai came calling a few years later, Katsoyannis leapt at the opportunity to assemble a biochemistry faculty for the nascent medical school. Besides recruiting Trakatellis and Koritz, he hired Pitt's Gerald Schwartz (PhD '64) and Diana Beattie (PhD '61), who is now dean of premedical and pharmacy programs at Oman Medical College. She had been a trainee of Segal and Hofmann.

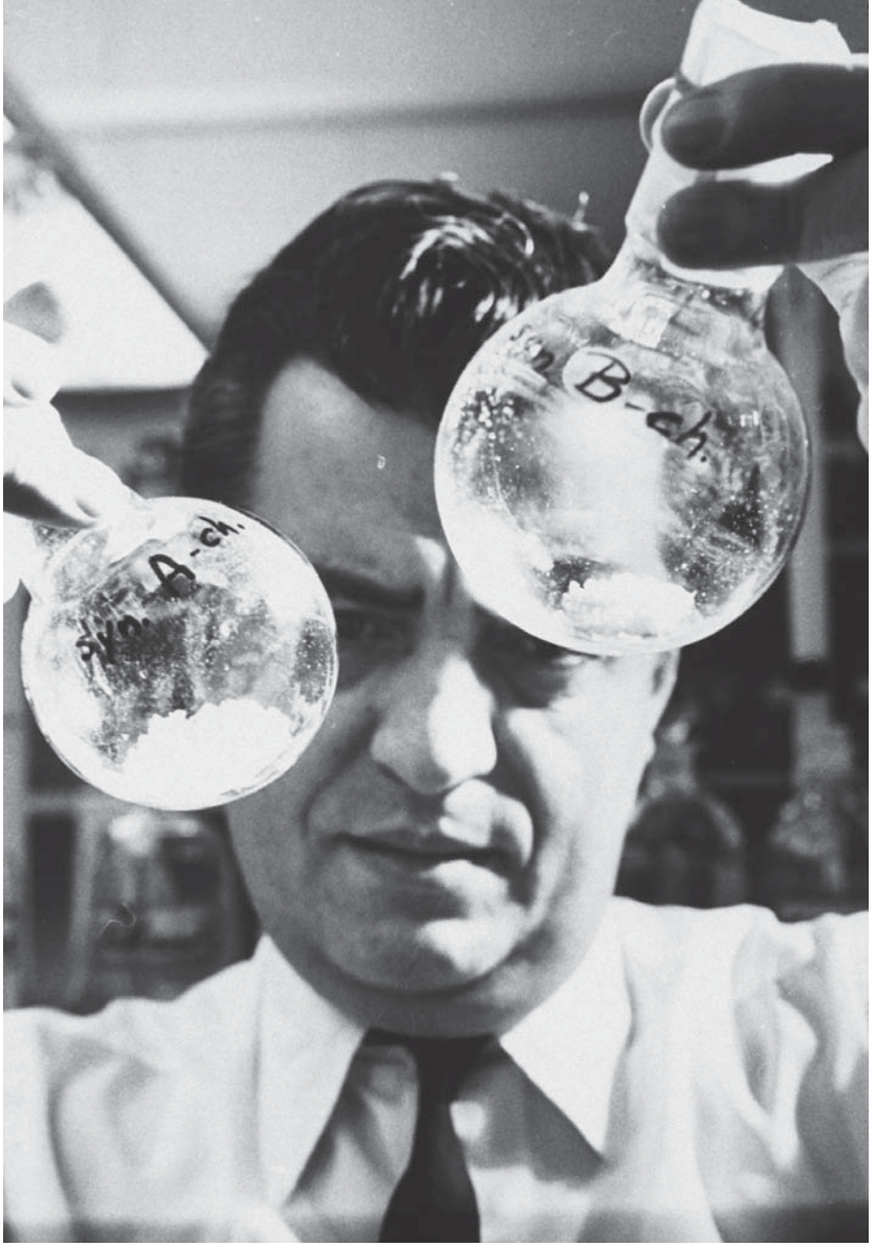
"Women had a hard time getting jobs in 1968," says Beattie, an expert in mitochondrial biogenesis and metabolism. "He paid me the same amount as the men he hired, promoted me to associate and then full professor, and certainly treated me well."

What else could he have done, muses Katsoyannis, now retired and living near Washington, D.C., just minutes from his daughter and her family and responsible for the care of his wife since a 2007 stroke. "I have a daughter, a daughter-in-law, and a wife," he says. "They would kill me if I didn't hire women." When West Virginia University—where Beattie would spend 21 years as chair of its biochemistry department—called for a reference, Katsoyannis did as Hofmann had decades earlier for his good friend. "She was a very good scientist and teacher, established in student affairs," he recalls. "It was with a heavy heart that I recommended her, because I could not hinder her career."

After his final installment on the synthesis of insulin in the *Journal of the American Chemical Society* in 1971, Katsoyannis would go on to craft some 150 analogs of the molecule, analyzing their interactions with insulin receptors for new insights into the relationship between structure and function.

"We started to play quite a lot with that," says Trakatellis, who visits his friend every time he returns to the United States. After Trakatellis moved back to Greece in 1972, Katsoyannis continued the effort, swapping out this amino acid, replacing that one, hinging and unhinging the chains in new locations, and in the process heightening and shifting the molecule's biological activity. The work began, says Trakatellis, when the pair started combining chains of the molecule from multiple species, making versions half animal and half human, joking to themselves about the mythological Greek heroes whose superpowers derived from their mixed parentage.

"Before we did our work, nobody would dare even think of manipulating a protein, changing its profile," says Katsoyannis. "People would think it was beyond the capabilities of chemistry." ■



In 1964, Katsoyannis holds beakers of synthetic A and B chains of insulin. The Pitt prof thought he wanted to be a civil engineer but later found his "heart belonged to chemistry."