CHAPTER 1

THE WISDOM OF THE BODY

> The living being is stable. It must be so in order not to be destroyed, dissolved, or disintegrated by the colossal forces, often adverse, which surround it.
>
> **–CHARLES RICHET, NOBEL LAUREATE (1913)**

The snapping of tree limbs jolted me out of a deep sleep. Peering through the front screen of our large tent, perched on a wooded bluff over the Tarangire River in northern Tanzania, I could not see anything outside in the pitch-black, moonless night. Maybe the wind had toppled a tree? I checked the clock—4 a.m.—and rolled over, hoping to get a couple of more hours of rest.

Then I heard heavy footsteps, crunching at first in front of the tent, then on all sides of us, accompanied by occasional low rumbling, almost purring noises. They were *really close.* My wife Jamie was now awake.

A family of elephants had hiked up the slope from the riverbed to browse on the trees and shrubs on top. With no natural predators, the animals walked wherever they desired, and at 8,000 pounds or more with strong, forklift-like tusks, they simply bulldozed their way through any thicket. As we heard branches and trunks splinter, I wondered about the thin canvas that separated us. With utter disregard for the resting humans nearby and, thankfully, no interest in

FIGURE 1.1 Elephant! Bull moments after a bluff charge, Tarangire National Park.

Photo courtesy of Patrick Carroll.

our rectangular refuges, they munched past dawn before heading back down the hill to drink.

As daylight came, we stepped carefully outside to photograph one straggler. Boy, elephants look even bigger when there is nothing between you and them. This bull was *huge*, more than ten feet tall at his shoulders, with giant ears. Stripping branches and leaves off of small trees, while ignoring the paparazzi peering around the corners of several tents, he seemed content. [Figure 1.1]

Until some noise from a tent spooked him. He trumpeted, pivoted to his left and took some quick steps in our direction.

There is more than one account of what happened next.

In my version, we dashed for the nearest tent, barreled inside, and instantly closed the zipper behind us (because four-ton elephants can't open zippers). We then just stood inside trembling and muttering, trying to regain our composure.

In the biological version of those few seconds, a remarkable number of things happened in my brain and body. Before my mind could even form the thought "Mad elephant! Run!" a primitive part of my brain, the amygdala, was signaling danger to my hypothalamus. This almond-sized command center just above the amygdala promptly sent out electrical and chemical signals to key organs. Through nerves, it signaled the adrenal glands that sit on top of my kidneys to release norepinephrine and epinephrine, also known as adrenaline. These hormones then circulated quickly through the bloodstream to many organs including: my heart, causing it to beat faster; my lungs, to open up airways and increase breathing rate; my skeletal muscles, to increase their contraction; my liver, to release stored sugar for a quick supply of energy; and smooth muscle cells throughout my body, causing blood vessels to constrict, skin hairs to stand on end, and blood to shunt away from the skin, intestine, and kidneys. The hypothalamus also sent a chemical signal, corticotropic releasing factor (CRF), to the nearby pituitary gland that triggered it to release a chemical called adrenocorticotropic hormone (ACTH) that traveled to another part of the adrenal gland and triggered the release of another chemical—cortisol, which increased blood pressure and blood flow to my muscles.

All these physiological changes are part of what is known as the "fight-or-flight" response. Coined and described a century ago by Harvard physiologist Walter Cannon, these responses are aroused by both fear and rage, and quickly prepare the body for conflict or escape. We opted for escape.

SCAREDY CATS

Cannon first became interested in the body's response to fear while conducting pioneering studies on digestion. X-rays had just been discovered when Cannon was a medical student; a professor suggested that he try to use the new gadget to watch the mechanics of the process. In December 1896, Cannon and a fellow student successfully obtained their first images—of a dog swallowing a pearl button. They

soon experimented with other animals including a chicken, a goose, a frog, and cats.

One challenge to observing digestion was that soft tissues, such as the stomach and intestines, did not show up well on X-rays. Cannon found that feeding animals food mixed with bismuth salts made their digestive tracts visible, because the element was opaque to the rays. He also explored the use of barium; it was too expensive at the time for research work but was later adopted by radiologists (and still is used in gastroenterology today). In a classic series of studies, Cannon was able to observe for the first time in living, healthy, nonanesthetized animals, as well as in people, how peristaltic contractions move food through the esophagus, stomach, and intestines.

During the course of his experiments, Cannon noticed that when a cat became agitated, the contractions promptly stopped. He jotted in his notebook:

> *Noticed sev times very distinctly (so absol no doubt) that when cat passed from quiet breathing into a rage w struggling, the movements stopped entirely. . . . After about ½ minute the movements started again.*

Cannon repeated the experiment again and again. Every time, the movements resumed once the animal calmed down. The second-year medical student now had another finding to his credit. In what would become the second classic paper of his budding career he wrote, "It has long been common knowledge that violent emotions interfere with the digestive process, but that the gastric motor activities should manifest such extreme sensitiveness to nervous conditions is surprising."

Cannon's knack for experiments soon derailed his plans to become a practicing physician. His talent, rigor, and work ethic so impressed the distinguished faculty of the Department of Physiology at Harvard that he was offered an instructorship on graduation.

THE NERVOUS STOMACH

In his own laboratory, Cannon aimed to figure out how emotions affected digestion. He observed that emotional distress also ceased digestion in rabbits, dogs, and guinea pigs, and from the medical literature that also seemed to be true of humans. The connection between emotions and digestion suggested some direct role of the nervous system in controlling the digestive organs.

Cannon knew that all the outward signs of emotional stress—the pallor caused by the contraction of blood vessels, "cold" sweat, dry mouth, dilation of pupils, skin hair standing on end—occurred in structures that are supplied by smooth muscle and innervated by the so-called sympathetic nervous system. The sympathetic system comprises a series of neurons that originate from the thoracic-lumbar region of the spinal cord and travel out to clusters of nerve cells (called ganglia). From there, a second set of generally much longer neurons extend to and innervate target organs. Most of the body's organs and glands receive sympathetic input, including the skin, arteries, and arterioles, the iris of the eyes, the heart, and the digestive organs. These same organs also receive input from nerves originating in the cranial or sacral parts of the spinal cord. [Figure 1.2]

To figure out what stopped the activity of the stomach and intestines under emotional stress, Cannon and his students conducted a series of simple but fundamental studies. One approach was to sever the nerves leading to the digestive organs. Cannon found that when the vagus nerve (originating in the cranial system) was severed but the splanchnic nerve (part of the sympathetic system) was left intact, the inhibition of peristalsis could still be induced by fear. In contrast, when the splanchnic nerves were cut and vagus remained intact, there was no response to fear. These results showed that the inhibition of peristalsis induced by emotion required the sympathetic splanchnic nerves.

Cannon had noticed that the inhibition of gastric activity often long outlasted the presence of whatever provoked the response. This suggested to him that there might be a second mechanism beyond direct nervous impulses that might prolong the agitated state. It had been reported that adrenalin, a substance extracted from the central portion of the adrenal glands, when injected into the bloodstream could produce some of the effects produced by stimulation of the sympathetic nervous system. Cannon wondered whether the adrenal glands might be involved in the body's response to fear and anger.

To test this possibility, Cannon "made use of the natural enmity" between dogs and cats. He and a young physician, Daniel de la Paz,

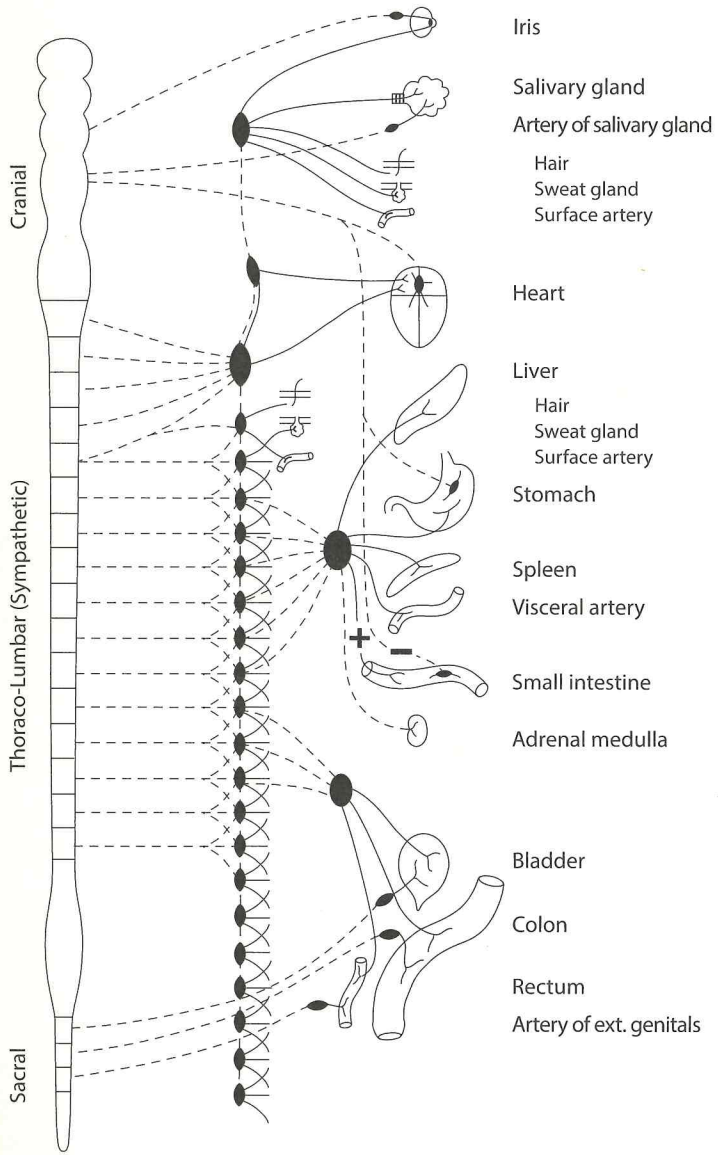


FIGURE 1.2 The sympathetic nervous system. This branch of the autonomic nervous system connects to various glands and smooth muscles to maintain homeostasis and to mediate the flight-or-fight response. Nerves emanating from the cranial and sacral regions generally act in opposition to those emanating from the thoraco-lumbar region (trace, for example, the innervation of the small intestine).

Figure adapted from *The Wisdom of the Body* by Walter B.Cannon (1963), modified by Leanne Olds.

compared blood samples of cats taken before and after they had been exposed to the stress of barking dogs. They discovered that the blood of frightened cats contained a substance that when applied to a small strip of isolated intestinal muscle stopped it from contracting. This was the same effect observed when adrenalin was applied to the muscle strip.

Epinephrine was one of the components of "adrenalin" produced by the adrenal glands. Cannon and his colleagues also found that epinephrine sped up heart rate, the release of sugar from the liver, and even blood clotting. These same effects were triggered by pain, as well as by fear or anger. None of these effects occurred when the adrenal glands were removed, or when the nerves leading to the adrenal glands were cut. Thus, the sympathetic nervous system and adrenal glands worked in concert to modulate other body organs in stressful conditions.

Cannon suggested that the responses induced by epinephrine reflected the "emergency" function of the adrenal glands in preparation for fight or flight, or in response to pain. A firm adherent to Darwin's principle of natural selection, Cannon interpreted the roles of the adrenal system through that lens:

> The organism which . . . can best muster its energies, can best call forth sugar to supply the laboring muscles, can best lessen fatigue, and best send blood to the parts essential in the run or fight of its life, is most likely to survive.

Cannon's student Philip Bard subsequently demonstrated that the hypothalamus is the critical part of the brain for control of the so-called involuntary (autonomic) functions of the nervous system, including digestion, heart rate, respiration, and the fight-or-flight response. Both this part of the brain and these emergency responses are ancient. This same set of responses helped our ancestors avoid lions and hyenas on the savannah, just as they help pedestrians to dodge taxis in New York today, or tourists to run from elephants.

A SCIENTIST-SOLDIER

Cannon was an Ivy League but not an Ivory Tower scientist. In 1916, three years into World War I, as the battlefield in Europe turned into a horrific stalemate that produced enormous casualties, it seemed

increasingly likely that the United States might be drawn into the conflict. Cannon was asked to chair a special committee of physiologists to advise the government on ways to protect the lives of soldiers and civilians. He learned that one of the most serious problems in battlefield medicine was the development of shock in wounded soldiers. Cannon recognized some of the shock symptoms—rapid pulse, dilated pupils, heavy sweating—from those he had observed in his experimental studies of animals under stress. Wounded soldiers who exhibited these symptoms often went downhill quickly and died. "Are there not untried ways of treating it?" he asked a fellow physiologist.

Cannon was so taken with the problem of shock that he began some animal experiments to see whether he could figure out ways to mitigate the syndrome. When the United States did finally enter World War I in April 1917, Cannon was forty-five years old and the father of five, and could have easily been excused from service. Instead, he volunteered as a member of a Harvard Hospital Unit that was one of the first American medical teams to go to Europe. Cannon requested to serve in a shock ward near the front lines in northern France.

Cannon said goodbye to his family in Boston, took a train to New York, and boarded the troopship *Saxonia* bound for England. The voyage overseas would take eleven days. To avoid detection by German submarines, the ship was blacked out at night, with all of its portholes closed. While ships usually have lights at both ends so as to avoid collisions, the *Saxonia* lit only its stern, to help draw any torpedo off target. Eight days into the voyage, as the ship drew nearer to the English coast, the orders came to sleep in one's clothes; if hit, it was better to jump into the lifeboats fully dressed. As the ship hit choppy seas in rain and fog, Cannon was relieved, "not a favorable condition, I should say, for good hunting," he wrote to his wife Cornelia. The appearance of a British destroyer escort further eased anxieties.

After arriving safely in England, Cannon continued on to the first of several field hospitals. A wave of casualties soon arrived from a major British offensive. Although Cannon had not practiced any medicine since his graduation from medical school seventeen years earlier, he asked to assist in the operating room, dressed wounds, and worked in the wards.

Cannon then moved to a hospital nearer to the front. He watched helplessly the heartbreaking, rapid decline of scores of soldiers. Why the soldiers died was a mystery that Cannon and several other American and British physiologists were hell-bent to solve.

One important clue to shock came from the then-novel approach of measuring soldiers' blood pressures, not just their pulses. Healthy soldiers had pressures of about 120–140 (mmHg; the abbreviation stands for millimeters of mercury), while shock patients had pressures below 90. It was learned that if this fell to 50–60, the patient did not recover.

A low blood pressure meant that vital organs would have difficulty obtaining sufficient fuel and disposing of waste. Early in his time in France, Cannon decided to measure the concentration of bicarbonate ions in the bloodstream of shock patients, a critical component of the blood's buffering system. He discovered that the patients had lower levels of bicarbonate, which meant that the normally slightly alkaline blood had become more acidic. And he found that the more acidic the blood was, the lower the blood pressure and the more severe the shock were. Cannon proposed a simple possible therapy: administer sodium bicarbonate to shock victims.

Cannon reported the first results in a letter to his wife Cornelia in late July 1917, just two months after his arrival in Europe:

> Well, on Monday there was a patient with a blood pressure of 64 (the normal is about 120) millimetres of mercury and in a bad state. We gave him soda [sodium bicarbonate], a teaspoonful every two hours and the next morning the pressure was 130. And on Wednesday a fellow came in with his whole upper arm in a pulp . . . such cases usually die. At the end of the operation he had the incredibly low pressure of 50; soda was started at once and the next morning the pressure was 112.

Cannon described three other soldiers who had been treated that same week and also had been "snatched from death," including one who was given the sodium bicarbonate intravenously and whose rapid respiration and pulse eased quickly.

Cannon and the Allied medical command were thrilled by this innovation. Since shock was often brought on by surgery, the use of bicarbonate was adopted as a standard preventative measure in all critical cases. Cannon and his colleagues also advocated other procedures for warding off the development of shock, including protecting wounded soldiers from exposure by wrapping them in warm blankets, giving warm fluids, transporting on dry stretchers, and using lighter forms of anesthesia during surgery.

To promote these methods, Cannon organized the training and deployment of "shock teams" to treat shocked soldiers on or near the battlefield. To see how the teams performed in battle, he went on an inspection tour close to the front.

In mid-July 1918, he was visiting a hospital near Chalons-sur-Marne, in eastern France. After spending an evening socializing with other doctors, Cannon retired to bed. He could hear guns firing in the distance, but that was typical. Just before midnight, Cannon was jolted awake by "the most stupendous, the most terrific, the most inconceivably awful roar . . . like thousands of huge motor trucks rushing over cobblestones." He jumped to his window and saw entire horizon lit up with gunflashes and shellbursts. He heard the zip-sish sound of a shell passing nearby, which exploded near the hospital. Shells continue to hit within a mile of the building, one about every three minutes for four straight hours.

In the middle of the massive German assault, Cannon was called to the shock ward as the first few casualties were brought in. Then came a flood of wounded—eventually more than 1,100 would arrive that day. As the shock ward filled, Cannon heard a deafening crash—a shell struck the next ward, just twenty feet away, blowing off the roof and sending shrapnel through the walls of his ward. Dust, smoke, and gasses from the explosion filled the air, but Cannon and the rest of the teams stayed at their stations until all patients had been attended to and moved to safer quarters behind the front.

The battle ended up being a turning point in the war. The German drive stalled, and the Allies pushed eastward over the following weeks and months. Cannon followed the leading front into formerly German-held territory. He saw French towns in complete ruins, desolate landscapes denuded of all greenery, and longs columns of enemy

prisoners. At last, the streams of Allied wounded coming into the hospitals slowed to a trickle, then stopped altogether; the war was over. Cannon wrote to his wife, "There is satisfaction now in knowing . . . that we were serving the wounded close to the center of the struggle which changed the whole history of the world."

Cannon's exemplary performance during the war was recognized by a series of promotions. In a span of just fourteen months, he went from first lieutenant to captain, then to major, and finally to lieutenant colonel. He was awarded the Order of the Bath by the British and cited by General Pershing, the leader of the American forces in Europe: "*For exceptional meritorious and conspicuous services as instructor in shock treatment.*" After a joyous celebration in Paris, he sailed back home to the United States, his wife, children, and Harvard laboratory in January 1919. [Figure 1.3]

THE WISDOM OF THE BODY

Cannon's experiences in France had a profound impact on the physiologist. They gave him a poignant, first-hand understanding of the important parameters for the maintenance of human life. Combined with his knowledge of the control of digestion, respiration, heart rate, and the responses to stress in animals, Cannon was provoked to think about the body's ability to react to disturbances and yet to maintain critical functions within fairly narrow ranges.

To Cannon, it appeared that many activities of the nervous and endocrine systems served to prevent wide oscillations and to hold the internal conditions of the body—temperature, acidity, water, salts, oxygen, and sugar—fairly constant. He knew too well that if these narrow limits are breached, serious illness or death often follows. For example, blood pH, a measure of acidity, is maintained near 7.4; if it drops to 6.95, coma and death result, and if it rises to 7.7, convulsions and seizures occur. Similarly, calcium levels are maintained around 10 milligrams per 100 milliliters of blood; half that level causes convulsions, double that level causes death.

Cannon began to speak of the innate "wisdom of the body" in lectures and papers. "Our bodies are built to take very effective care of themselves, in many ways which we have became aware of only in

FIGURE 1.3 Walter B. Cannon in his Army uniform.

Photo from Family Photograph Album. Walter Bradford Cannon papers, 1873–1945, 1972–1974 (inclusive), 1881–1945 (bulk). H MS c40. Courtesy of Harvard Medical Library, Francis A. Countway Library of Medicine, Boston, Massachusetts.

recent years," Cannon wrote. One of the recent breakthroughs was in understanding the role of insulin in controlling blood sugar. Cannon noted how when sugar levels rise after a meal, the vagus nerves stimulate the pancreas to secrete insulin, which causes excess sugar to be stored. Conversely, if sugar levels fall, other nerves in the autonomic system trigger the adrenal glands to liberate sugar from the liver. In this way, Cannon said, "the organism automatically restricts the range over which the percentage sugar in the blood may shift."

Cannon emphasized how most organs received dual nervous inputs that, as a rule, opposed each other. With this wiring, organ activity can be increased or decreased depending on conditions. Impressed by the body's ability to adjust to disturbances, Cannon coined a new term to describe the steady states maintained in the body: *homeostasis* (from the Greek *homeo* meaning "similar" and *stasis* meaning "standing still"). This was not some lofty or abstract philosophical notion; Cannon's concept was firmly rooted in three decades of physiological research. Homeostasis was fundamentally a matter of regulation. That is, physiological processes existed in the body that operated to maintain—to regulate—body conditions within certain ranges.

Cannon first elaborated his ideas in the scientific literature, then in a popular science book titled *The Wisdom of the Body*. He offered several lines of evidence to support his claim that stability was due to active regulation. First, he stressed that the constancy exhibited by body functions in the face of all sorts of external disturbances and variables indicated the presence of regulatory mechanisms that maintained steady states. Second, he argued that states remain steady because factors exist that resist change in either direction, positive or negative. Third, he pointed out that there was substantial evidence that multiple cooperating factors often act either simultaneously or in succession to maintain a state, such as the acid-base balance of the blood. And fourth, he suggested that the existence of some regulatory factor acting in one particular direction implied the existence of factors acting in the opposite direction, as had been shown for blood sugar.

In short, Cannon asserted that everything in the body is regulated. And so he concluded, "regulation in the organism is the central problem of physiology."

Grounded in Cannon's body of work on digestion, thirst, hunger, fear, pain, shock, and the nervous and endocrine systems, and made accessible by his lucid writing, homeostasis became a fundamental concept in physiology and biology. Some compared it to Darwin's principle of natural selection as one of the seminal integrative ideas in biology.

Cannon believed that the implications of homeostatic mechanisms to medicine were far reaching and very positive. He shared his "Reasons for Optimism in the Care of the Sick" in an address to Boston-area physicians that was subsequently published in the *New England Journal of Medicine*. He began his presentation with typical modesty:

> That you, a group of physicians who are daily confronting the practical problems of sick men and women, should ask me, a physiologist, a laboratory recluse, to address you is a surprising fact. Perhaps my presence here calls for some explanations from you—and for some apologies from me! . . . All that I propose to do as a physiologist is to draw forth some suggestions from years of research and reading and thinking about the workings of the organism . . . that may be useful as laying a basis for optimism in medical practice.

Cannon then recounted how when some factors

> tip the organism in one direction or the other, internal adjustments have promptly been called into service which have prevented the disturbances from going too far and have tipped the organism back to its normal position. Note that these are not processes which [sic] we manage ourselves. They are automatic adjustments.

In light of these marvelous powers of self-regulation Cannon asked, "If the body can largely care for itself what is the function of the physician?"

He explained that doctors' services are called for when these mechanisms are overwhelmed or malfunctioning. Cannon emphasized how many of the newer therapies available to physicians—insulin, thyroxin, antitoxins—were natural components of the body's

self-regulatory system. The physician's role was thus to reinforce or to restore the natural homeostatic mechanisms of the body. Cannon suggested that the power of these mechanisms, and the increasing ability of physicians to bolster them, were cause for optimism in medicine.

Cannon had the powerful ideas that regulation is the central matter of physiology, and that abnormal regulation is the central issue of medicine. Coincidentally, at the very same time that Cannon was expressing these pivotal ideas, another biologist was reaching the conclusion that regulation was the central issue in nature on a much larger scale.

But just before we do, there is one more impact of Elton's book to mention—in fostering the myth of suicidal lemmings. According to Elton's reading of Collett's book, a lemming year occurred "when a whole lot of lemmings apparently went crazy and walked downhill." He wrote in *Animal Ecology*: "The lemmings march chiefly at night, and may traverse more than a hundred miles of country before reaching the sea, into which they plunge unhesitatingly, and continue to swim until they die." This description, however, was based on yarns collected in Collett's book. Elton had never seen a lemming, nor a migration, let alone a mass suicide.

The myth of lemming suicide received a considerable boost from the 1958 Walt Disney film *White Wilderness*, which depicted lemmings leaping to their demise. After the narrator explained, "A kind of compulsion seizes each tiny rodent and, carried along by an unreasoning hysteria," viewers saw lemmings leaping into the water from a high cliff. The scene was faked; the animals were flung off the cliffs by the filmmakers.

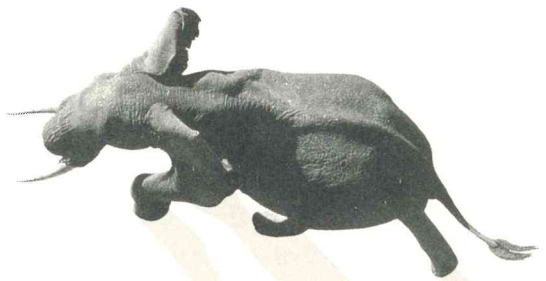
The movie won an Academy Award.

PART II

THE LOGIC OF LIFE

Anything that is found to be true of *E. coli*
must also be true of Elephants.

—JACQUES MONOD AND FRANÇOIS JACOB



Elton described how the regulation of animal numbers was enormously important both in nature and in applied fields, and Cannon explained how the regulation of physiology was crucial to animal and human health. By their own admissions, however, neither could say much in detail about how the quantity of anything was regulated in ecosystems or bodies.

The challenges in deciphering the rules of regulation were a bit different for ecologists and physiologists. For Elton and his tribe, the "players" were generally visible to the naked eye—the animals and plants in a given place. But the ecologists lacked ways of getting at the rules of the game; ecology was largely an observational and descriptive enterprise, not an experimental one.

Cannon and his clan, in contrast, were very good at conducting experiments, but they were handicapped because the study of physiology in the 1930s was largely restricted to phenomena observable at the level of body organs and tissues. The players that regulated those phenomena were invisible molecules that were difficult to isolate and identify.

In the next three chapters, I tell stories of how both general and some specific rules of physiological regulation were discovered. Funny enough, the first breakthroughs came from studying creatures without bodies—the tiny bacteria found in our digestive systems (Chapter 3). This pioneering work was important, because, although deciphered in bacteria, the rules turn out to be general ways of regulating all kinds of processes in all sorts of creatures, including ourselves. It was by following the trails blazed by these pioneers that the specific rules for important processes in humans—such as the regulation of cholesterol metabolism (Chapter 4) and cell growth (Chapter 5)—were cracked open. The result of identifying the specific players and rules of those games has been a biomedical revolution that has gone far beyond Cannon's greatest optimism, or imagination.

The discovery of these general rules was important for two additional reasons. First, they underpin what the pioneering molecular biologist François Jacob has described as a "logic" of life. The term is used in both its formal meaning (if A regulates B, and B regulates C, then A regulates C), and in the informal connotation that the regulatory logic makes "sense" for the organism—the same connotation

as Cannon's "wisdom of the body." I believe that understanding this logic greatly enhances one's appreciation for how life works.

And the second reason these general rules are important, and one major reason for my writing this book and structuring it as I have, is that analogous rules and logic operate on the ecological scale. I will get to specific ecological rules later in Part Three, but alert you to the importance of and similarity in logic now so that you might pay as much attention to it over the next few chapters as to the particulars of each story.

CHAPTER 3

GENERAL RULES OF REGULATION

The cell thus adapts its work to its wants.
It produces only what it needs when it needs it.

–FRANÇOIS JACOB

Great Britain was not the only nation interested in exploring the poles. Driven by economic and strategic considerations, sometimes by national glory, and occasionally by scientific curiosity, many countries sent expeditions north and south in the first part of the twentieth century.

On July 11, 1934, the three-masted French ship *Pourquoi-Pas? IV* ("Why Not?") left Saint-Malo on the Normandy coast for the icy shores of Greenland. In command was the celebrated polar explorer Jean-Baptiste Charcot. Trained as a physician, Charcot abandoned medicine and made his reputation on two government-sponsored French Antarctic expeditions: on the *Français* in 1903–1905 and the first *Pourquoi-Pas?* in 1908–1910. Braving ice, storms, temperatures that plunged to more than forty degrees below zero, and the long polar night, Charcot discovered new lands, charted over 1,800 miles of coastline and islands, became a national hero, and earned the genuine admiration of fellow explorers. After World War I, he turned his attention to the Arctic. This voyage was the sixty-seven-year-old's twenty-fifth polar expedition and his tenth to Greenland.

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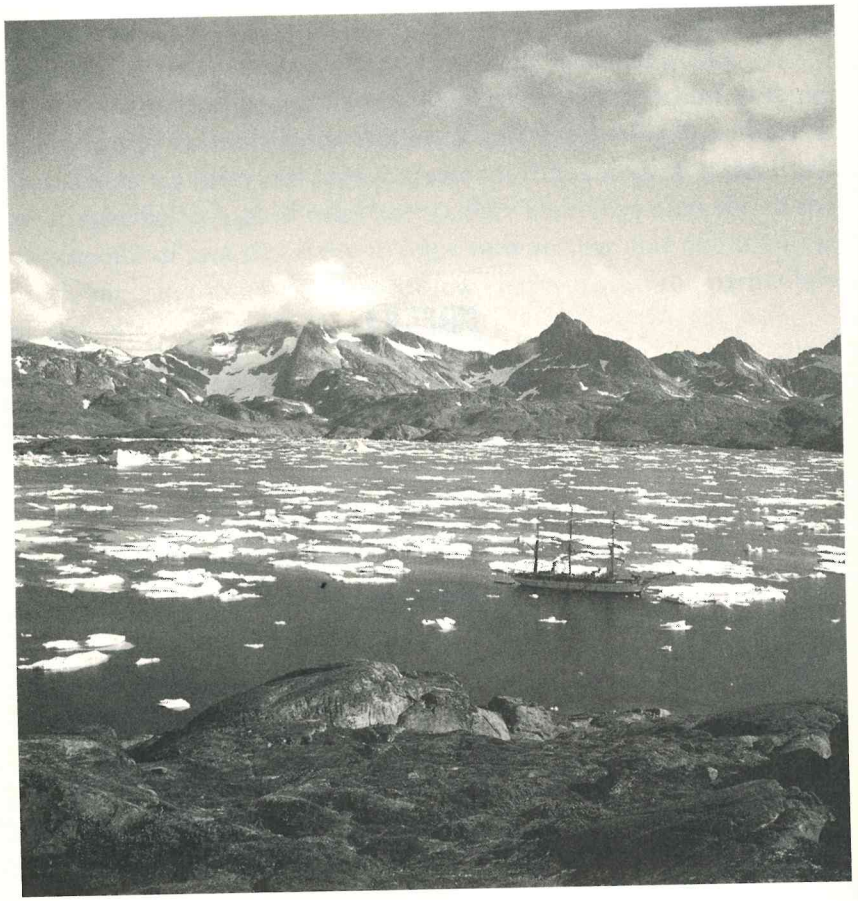

FIGURE 3.1 The Pourquoi-Pas? in Greenland, 1934.

Photo by Jacques Monod, © Institut Pasteur/Archives Jacques Monod.

The ship carried a crew of thirty-three men, all volunteers. Also on board were six university students, four of whom were to be dropped off at the village of Angmagssalik to live among the Inuit for a year as part of an ethnographic study. Two others were to conduct scientific studies on board and on shore, including twenty-four-year-old Jacques Monod.

Raised near the famous seaside resort of Cannes, Monod was an experienced sailor but an amateur in comparison to Charcot's team. He had no previous experiences in seas such as those he was about to encounter. The young zoologist had set aside his research at the Sorbonne in Paris for the privilege of joining Charcot's team and the two-month-long adventure of sailing to the Arctic. His duties were similar to Elton and his compatriots—to collect specimens.

Twelve days after leaving France, the ship stopped in the fog-enshrouded Faroe Islands. After making some repairs to a damaged boiler, the *Pourquoi-Pas?* sailed on to Iceland, took on coal, and steered for Greenland. All along the way, Monod collected plankton—small crustaceans, marine worms, and larvae—by dragging a net overboard. With each degree of latitude, the air grew colder, and more and more ice began to appear. As the ship approached Scoresby Sound on Greenland's east coast, ice fields blocked her entry. For five days, she inched forward, with a lookout in the crow's nest spotting gaps in the ice that often closed as quickly as they appeared, and another crew member at the rear pushing away blocks of ice that could smash the propeller.

After finally reaching shore, Monod had just three days to collect samples of the coastal marine life before the ship had to leave for Angmagssalik (today Tasiilaq). To collect rock and mineral samples, Monod and a companion set off to climb mountains around the fjord. An avid climber, he was enthralled by all he spied. "I saw so many beautiful and extraordinary things!" he wrote to his parents. "My dears, if you knew in what state of wonder and breathlessness I am!"

With its coal reserves dwindling, the ship had to depart to resupply in Iceland. It was promptly pounded by hurricane force winds. Navigating around icebergs, and with poor visibility, Charcot decided that the ship had to continue at all costs, as its coal supply was nearly exhausted. The crew was able to force their way to Reykjavik, to refuel, and to sail home without further incident.

Monod published a preliminary account of his collections and observations but, alas, he did not become a polar biologist. Two years later he was invited again to join the *Pourquoi-Pas?* on its next voyage to Greenland. Monod was leaning toward going, but at the last moment he decided instead to go to the California Institute of Technology to study genetics in the laboratory of Nobel laureate Thomas Hunt Morgan.

That turned out to be a remarkably fortunate decision. On September 15, 1936, after delivering supplies to Greenland and waiting out a storm, the *Pourquoi-Pas?* stopped once again in Reykjavik before resuming her voyage home. But within hours, she was caught within a fierce storm. Early on September 16, her fore and aft sails were shredded, and the jib smashed the radio antennae. The crippled ship drifted, was pushed onto its side, then smashed on a reef. Charcot and all but one of the forty-four men aboard perished in the cold, roiling seas.

The sparing of Monod's life also turned out to be a fortunate event for biology. Although Monod discovered nothing while in California, he would later become a co-founder of the new field of molecular biology. He and his collaborators would decipher some of the first general rules of the regulation of life at the molecular level, discoveries that would send him north once again—to Stockholm to collect a Nobel Prize.

But he would first have to endure a long and very lethal storm.

GROWTH . . . INTERRUPTED

After his stay in California, Monod returned to Paris to resume research at the Sorbonne and to search for a problem that he could sink his teeth into that would merit a doctorate.

This was a time for simple questions in biology, as so little was known about the processes going on inside living cells. One of the behaviors characteristic of all cells is the making of more cells by cell division. The questions were very Eltonian: What nutrients did cells require? What determined their numbers?

Before he had left for California, Monod had begun experiments into these matters. But the first organisms he studied were a group of single-celled protozoans that grew very slowly in the laboratory —a poor choice as a research subject. André Lwoff, a microbiologist at the nearby Pasteur Institute, suggested that Monod try bacteria instead, which were easy to grow in culture and multiplied very quickly.

Previously, researchers had largely used ill-defined food for bacterial broths, made, for example, by grinding up cow brains. Monod's

first advance was to use carefully defined ingredients that allowed him to undertake a series of experiments in which he systematically varied almost every ingredient and measured the impact on bacterial growth. One of his first clear-cut results was to show that the amount of bacterial growth was directly proportional to the amount of the carbon energy source provided (a sugar, such as glucose or mannitol). This observation implied a very simple relationship between nutrition and growth: bacteria converted whatever food was available into more copies of themselves.

By the summer of 1939, Monod was making progress, but the winds of war were once again blowing across Europe. Like many Frenchmen, Monod did not see what was coming. On August 31, 1939, Monod wrote to his father: "[T]here will be no war. Hitler . . . knows what it would cost him." The very next day, Germany invaded Poland, prompting a declaration of war from France and Great Britain.

War did not break out immediately between France and Germany—days, weeks, and then several tense months passed without combat. Monod became concerned that if fighting did erupt, he would be drafted into some menial administrative work on account of his age (thirty). He wanted to be able to use his scientific talent in some capacity, so he decided to leave the Sorbonne and enlist in the Army to be trained as a communications engineer.

Just after Monod completed his initial training, war finally came when Germany launched a massive assault on the Netherlands, Belgium, and Northern France on May 10, 1940. The French Army was overwhelmed in a matter of days; Monod's regiment never made it off their base until the war was all but over. Monod was again lucky in not being taken as a prisoner of war. After France surrendered, he returned home to resume his research in German-occupied Paris.

WHAT DO BACTERIA LIKE TO EAT?

At thirty years old, Monod was relatively old for a graduate student. He was desperate to find a problem to study that would enable him to finish his doctorate. He had carried out a large number of experiments examining the growth properties of bacteria in broths containing different individual sugars. In the fall of 1940, he decided to

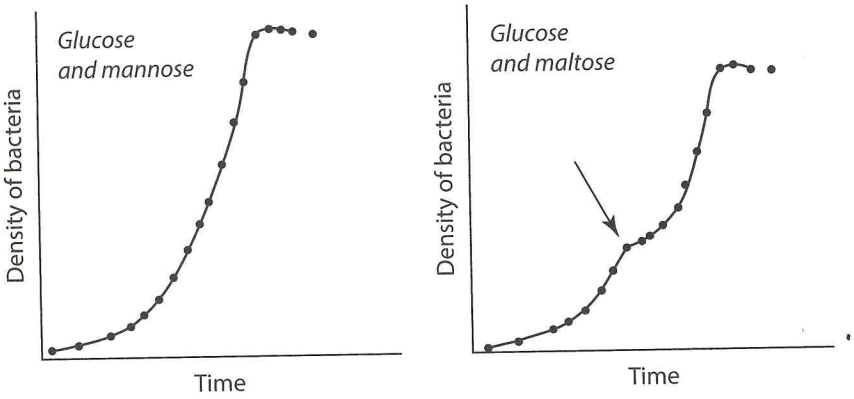

FIGURE 3.2 Monod's double-growth curve. When bacteria were grown in glucose and mannose, a single curve was observed (left), but when bacteria were grown in glucose and maltose, the bacteria grew exponentially, then paused briefly (arrow) before resuming exponential growth (right). That pause and second growth curve became the basis of Monod's thesis and eventual Nobel Prize.

Figure drawn by Leanne Olds based on original data in Jacques Monod's laboratory notebooks.

explore what happened when bacteria were offered different combinations of sugars.

Plotting the concentration of bacteria over time, he saw some familiar-looking growth curves, identical to those he obtained with single sugars. Those curves had three distinct phases: a short lag phase before the growth phase when the bacteria grew exponentially, doubling every thirty to sixty minutes, then a stationary phase when the concentration of bacteria did not increase further. But with certain combinations of sugars, the curves were different. They appeared to have two growth phases, separated by a second lag phase. [Figure 3.2]

Puzzled, Monod showed what he called his "double-growth" curves to Lwoff. The senior scientist hesitated and then said, "That could have something to do with enzyme adaptation."

"Enzyme adaptation? Never heard of it!" Monod replied.

Lwoff gave Monod a few older papers that had reported a phenomenon in which bacteria or yeast cells adapted to the presence

of a nutrient by making an enzyme that broke it down. The lags in Monod's curves could be the time needed for the microbes to adapt to each sugar. How a simple microbe "knew" to make an enzyme in response to a specific chemical was a complete mystery—Monod decided on the spot that solving it would be his quest.

Monod discovered that the appearance of a second growth curve depended on which specific sugars were provided. That suggested to him that bacteria preferred to eat certain sugars over others. They were ready to eat some sugars, but needed time to adapt to other, less preferred sugars and to make the enzymes necessary to digest them. He suspected that the explanation for the double-growth curve was that the bacteria were first using up one preferred sugar, then, after a pause, switching to using the second, less preferred sugar.

To test this idea, he had the simple notion of varying the ratios of the amounts of each sugar in the experiment. He reasoned that, if he was correct, the length of the growth phases when each sugar was being consumed would shift accordingly. That was exactly what he saw. [Figure 3.3]

Lwoff was impressed by Monod's gift for designing experiments that zeroed in on each point that he wanted to test. The Sorbonne awarded Monod his degree, although a member of his committee said, "What Monod is doing is of no interest to the Sorbonne."

Monod hoped to study the enzymes the bacteria produced in response to certain sugars, but before he made any headway, his work was interrupted yet again. As the German occupation continued, the atmosphere in Paris grew increasingly tense, and its streets more dangerous for Monod's Jewish wife Odette. She left the capital and took their children to a safer area in the south of France. Anticipating that there would be another battle for France when the Allies reinvaded Europe, Monod decided to join the most militant of Paris' Resistance groups, the Francs-Tireurs et Partisans (FTP).

Monod's responsibilities included gathering intelligence and coordinating arms drops from the Allies. For months, Monod juggled a double life as a Sorbonne scientist and Resistance operative, even hiding incriminating documents in the leg of a stuffed giraffe outside his laboratory. But as German pressure on the Resistance

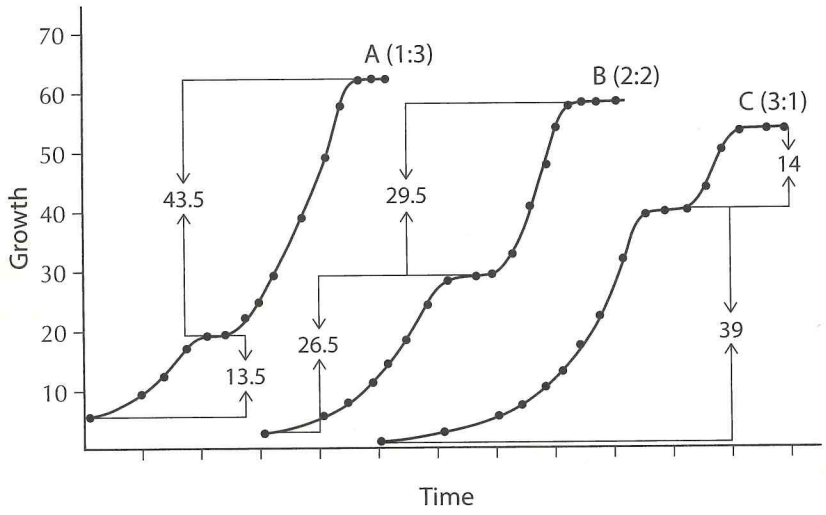

FIGURE 3.3 The proportion of each part of the double-growth curve depends on the ratio of the two sugars. As Monod mixed the two sugars in 1:3, 2:2, and 3:1 ratios, the first growth curve lengthened and the second growth curve shortened proportionately. This revealed that the bacteria used one sugar first, then the second.

From Monod (1942), modified by Leanne Olds.

increased, and some of Monod's superiors and colleagues were arrested and tortured, it became too dangerous for Monod to work at the Sorbonne or to sleep at home. Lwoff offered Monod refuge at the Pasteur, where he was able to continue doing experiments for a few months. Eventually, Monod had to abandon working in the lab. He went completely underground, wore a disguise, and stayed at networks of safe houses. [Figure 3.4]

Monod rose to become a senior officer in the national Resistance organization, the *Forces Françaises de l'Intérieur* (French Forces of the Interior or FFI), where he was involved in coordinating sabotage and even ordering the execution of traitors who collaborated with the enemy. Monod was one of the commanders who helped coordinate the battle for the liberation of Paris in August 1944. He then served as an officer in the French Army until Germany's surrender.

FIGURE 3.4 Jacques Monod's identity card, French Resistance, 1944. Monod held the rank of Commandant in the FFI. His alias "Malivert" is given because Resistance members could not use their real names.

Image courtesy of Oliver Monod.

SEARCHING FOR THE RULES OF ENZYME REGULATION

The war had occupied Monod, his family, and his country for six years. When it finally ended, he was eager to put that those dark times behind him and to hurl himself back into research. Lwoff offered, and Monod accepted, a permanent post at the Pasteur Institute.

Monod picked up where he had left off during the war. The logical appeal of enzyme adaptation was irresistible: How did a bacterium, so tiny it was barely visible in a microscope and without any nervous or endocrine system—just a bag of chemicals inside a membrane—"know" to make the right enzyme for whatever sugar was available?

Enzymes are proteins, and cells make thousands of different proteins. Monod recognized that his question was fundamentally a question of regulation: How did a cell "decide" to make one particular enzyme under certain conditions but not others?

Monod believed that there was much more at stake in his research on the regulation of bacterial enzymes than merely questions about the sugar-eating habits of microbes. He understood that what made the different types of cells in more complex creatures distinct from one another was similarly a matter of regulation. For example, red blood cells made hemoglobin proteins that carried oxygen, and white blood cells made antibody proteins that fought infections. Monod believed that understanding why and how a bacterium made a particular enzyme could shed light on the profound general mystery of how different cell types were made.

To try to crack open that mystery, he decided to focus on just one sugar, the milk sugar lactose, and one key player, the bacterial enzyme that cleaves it into galactose and glucose, called "β-galactosidase." Bacteria prefer to use the simple sugar glucose for energy. To use lactose, a compound made of the two sugars glucose and galactose, they have cleave it into its two halves.

The late 1940s and early 1950s were the dawn of molecular biology, and there was very little precedent for how to do most kinds of experiments. Monod and his team excelled at developing the techniques to sort out different possibilities. The key observation was that the presence of the sugar caused the appearance of the enzyme. One possible explanation for this was that the sugar somehow activated

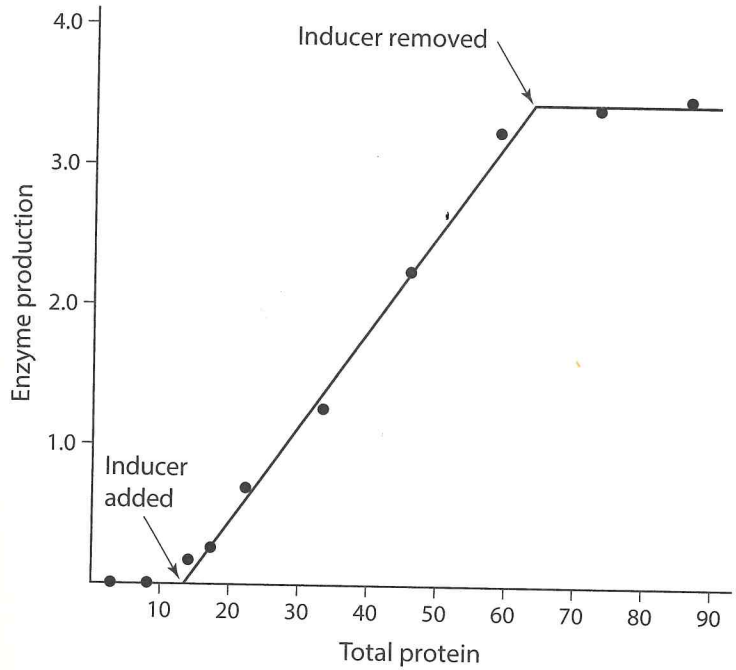

FIGURE 3.5 The induction of enzyme production. When an inducer such as lactose is added to a population of growing *E. coli* bacteria, the β-galactosidase enzyme is produced; when the inducer is removed, synthesis of the enzyme stops.

From Monod and Jacob (1961), redrawn by Leanne Olds.

the enzyme by binding directly to a preexisting, inactive form of the enzyme in the bacteria and converting it into an active form. In a series of clever and technically challenging experiments, Monod and his team ended up blasting this idea apart.

Monod's experiments showed instead that lactose tightly regulated the *production* of the enzyme. When the bacterium *Escherichia coli* was grown in the absence of lactose, there were just a few β-galactosidase enzyme molecules present in a cell. When lactose was added, this rose to several thousand molecules per cell in just a few minutes. When the sugar was removed, the synthesis of the enzyme stopped. [Figure 3.5] This turning on and off of enzyme production was regulated somehow by the presence/absence of the sugar. The sugar was said to be an *inducer* of enzyme production.

This was all very logical on the part of bacteria—it only made the enzyme when lactose (a food source) was present, and it did not waste energy making the enzyme when there was no lactose around. But how did that logic work?

The rules of the regulation of enzyme production would elude Monod for several years. And the main reasons for that were twofold: first, he did not yet know all the players in the game; and second, he had a mental block about how the logic of regulation might operate. The simple observation was that in the presence of the inducing sugar, bacteria made the enzyme. Monod and his collaborators kept thinking of the inducer as something that positively controlled enzyme synthesis, (denoted schematically here and throughout the book with an arrow →):

Inducer (lactose)
↓
Enzyme (β-galactosidase)

To make a breakthrough, they would need to discover another key player and to flip their logic around.

I will explain how they eventually got it right, but the correct logic is so important to understanding regulation and to the entire book that I don't want to risk your getting mired in those experimental details and miss the bigger picture. So I will tell you straight off what Monod was missing and how lactose regulates enzyme synthesis. Then I will back up and tell you how he and his comrades figured it out.

The player that Monod needed to discover was another protein that acted in between lactose and the enzyme. This protein is called a *repressor*, because its job is to specifically repress β-galactosidase enzyme synthesis. The flip in logic comes from realizing that lactose does not positively control enzyme synthesis directly. Rather, lactose inhibits the repressor, so that it no longer represses enzyme production.

Logically speaking, two negatives make a positive.

The double-negative logic of enzyme regulation made great sense biologically: in the absence of lactose, the enzyme that breaks down the sugar is not needed, and a repressor prevents the synthesis of the enzyme (negative regulation denoted by the ⊥ symbol below and hereafter in the book); when lactose is present, it inhibits the repressor, which allows the enzyme gene to turn on and the enzyme breaks down the sugar, providing energy to the cell:

Double-negative logic

No lactose	Lactose
	⊥
Repressor	Repressor (inhibited)
⊥	⊥
Enzyme gene OFF	Enzyme gene ON

Such beautiful logic and economy for just a simple bacterium.

I will get to some of the details of how repression works shortly, but for my purposes here and in the rest of this book, the importance of enzyme regulation is not in the gritty details but in the logic. The breakthrough came from breaking free of a mental bias. When we observe some phenomenon, we are inclined to think of the most direct explanation, with the fewest links in the chain between cause and effect. When we see a car moving down the street, we think somebody is stepping on the gas, not that somebody released the brake.

When the presence of A (e.g., a sugar) leads to the appearance of B (an enzyme), we infer a positive relationship: A causes B. It requires a stretch of the imagination to conjure up the explanation that A inhibits something else (a repressor) that inhibits B.

But it turns out that life—from the molecular scale all the way up to the ecological scale—is usually governed by longer chains of interactions than we first imagine, with more links in between. We need to know about each of those links and the nature of the interactions between them to truly understand, and to intervene in, the rules of regulation on every scale.

To discover the repressor and figure out the logic of enzyme regulation, Monod needed a fresh approach.

DISCOVERING THE REPRESSOR

The fresh approach was to use genetics. Imagine, for instance, that you were interested in how some visible trait was made, say, the pink color of a flower. There are fundamentally two ways you could try to figure out all the players involved in making that pink color. You could take the biochemical approach, which would be to grind up

the flower and try to purify all the enzymes that work in making a pink pigment from some simpler chemicals. That turns out to be very difficult and time consuming.

Or, you could take a genetic approach. That would entail taking seeds from some pink plants; and growing thousands of seedlings; and looking for the rare ones that could not make pink flowers but, say, only white ones. Every white plant has some genetic defect, a mutation, in some gene that is involved in the making of the pink pigment. You would then study those genes.

The great advantages to the genetic approach are that it uses a simple visual test to find mutations in genes of interest and that it is unbiased—it makes no assumptions about the number of players or what they do. It can discover players that are not enzymes, for example. Many of the key breakthroughs in biology and medicine over the past half-century were catalyzed by a genetic approach (I will describe two medically important examples in the next two chapters).

Monod and his team went looking for mutations in bacteria that disrupted β-galactosidase production. They isolated two types of mutants. One type made a defective β-galactosidase enzyme: those were mutations in the gene encoding the enzyme itself. This type was expected. But a second type of mutant was especially interesting: these mutant bacteria did not require any lactose to make the enzyme. They made the enzyme all the time ("constitutively"), whether lactose was around or not. In this mutant, the normal on/off regulation of the enzyme was broken. The constitutive mutations were in a separate gene from the enzyme and somehow disrupted the regulation of the enzyme gene.

Understanding how this new player worked would be key to understanding the regulation of the enzyme. But Monod was stumped at first. He had interpreted the constitutive mutants through the logic of the inducer acting as a positive regulator of enzyme production. He reasoned that, if the mutant bacteria required no added inducer to produce the enzyme, then the mutants must make their own internal inducer of β-galactosidase. It would take a new partner to reveal that Monod's logic was faulty.

DISCOVERING DOUBLE-NEGATIVE LOGIC

That new partner was François Jacob. Originally planning on becoming a surgeon before the war, Jacob's career was derailed when he was severely wounded in Normandy while serving as a medic. He went into scientific research instead, and wound up by chance in Lwoff's lab just down the hall from Monod. He was studying a different phenomenon in which bacterial viruses hide out quietly inside bacterial cells until something triggers them to multiply and burst forth. In a short period of time, Jacob had developed important techniques for studying genes in bacteria. He teamed up with Monod in 1957, and one new method from his bag of genetic tricks finally cracked the logic of enzyme regulation.

Unlike humans and most animals, which have two copies of each chromosome (one from each parent) and two copies of most genes, *E. coli* has a single chromosome with one copy of each gene. One trick that Jacob had pioneered was a way to transfer genes between bacteria. This allowed him to construct bacteria that had extra copies of genes and to test how bacteria behaved when mutant and normal genes were mixed together. If Monod was right about the constitutive mutants, then when a normal copy and a mutant copy of the gene were put together in the same bacterial cell, the prediction was that the internal inducer would be produced and the enzyme would be made constitutively.

But when Jacob and visiting American scientist Arthur Pardee did the experiment, they got exactly the opposite result: the bacteria required the inducer (lactose) to make the enzyme. The researchers were stumped at first. Perhaps they had made some technical mistake? But that was not the case: the same result was obtained when they repeated the experiment.

If their technique was not faulty, than perhaps their logic was. Indeed, that is exactly what Leo Szilard, a physicist-turned-biologist and frequent visitor to the Pasteur, suggested to Monod and Jacob. Maybe they were thinking about the inducer in the wrong way? Maybe the inducer did not activate enzyme synthesis directly, as Monod thought, but rather it *inhibited* a *negative regulator* of enzyme synthesis?

Bingo. The double-negative logic made sense of all of their results.

The constitutive mutants were not making an internal inducer, they were mutants that lacked a player in enzyme regulation—a *repressor* of enzyme synthesis. The absence of the repressor in the mutant allowed enzyme synthesis to take place continuously without an inducer. And when a bacterium had one good copy of the repressor gene and one mutant copy of the repressor gene, the good copy prevailed and repressed enzyme production, unless an inducer was added.

Once Monod and Jacob overcame that bias of simple positive cause and effect relationships, they started thinking in new ways and seeing connections that had not and would not have occurred to them (or anyone else) before.

One Sunday afternoon, while sitting in a Paris movie theatre with his wife, Jacob's mind started to drift from the film to the puzzle he had been working on for years. Some bacteria harbored hidden viruses that could be activated when bombarded with ultraviolet light. How that worked had stumped Jacob, and no one thought there was any connection between what he was studying and what Monod was researching at the other end of the hallway. Until, in the darkened cinema, Jacob began to picture the virus with its many genes somehow being kept off inside the bacterium.

Then he had a flash of insight—the logic of virus activation was the same double-negative logic of enzyme induction. A repressor must also be keeping the viral genes repressed, until ultraviolet light destroyed or removed it, and the virus genes turned on.

What appeared to be positive activation was again the inhibition of repression.

Virus silent	*Virus activated*
	UV light
	⊥
Repressor	Repressor
⊥	⊥
Viral genes OFF	Viral genes ON

Convinced by what were once believed to be two entirely different phenomena, Monod and Jacob proposed that there were fundamentally two kinds of proteins inside cells; *structural* proteins, such as enzymes that carry out the chemical reactions in cells or build the parts of a virus; and *regulatory* proteins that controlled which structural proteins were made or were not made, depending on conditions. When it came to regulation then, not all proteins were equal. Some proteins were dedicated to controlling others.

Monod and Jacob began seeing negative regulation everywhere and finding it at work in other ways.

FEEDBACK

In addition to breaking down nutrients into useful compounds, bacteria and other organisms are also able to build important compounds out of simpler ingredients. The proteins that do all the work in living things are constructed from building blocks called amino acids. When bacteria are grown in a basic medium containing glucose and carbon dioxide as carbon sources, they can make all twenty kinds of amino acids.

However, when specific amino acids are provided to the bacteria, the biosynthesis of that particular amino acid stops quickly. That rapid response suggests that when an amino acid is plentiful, bacteria have some mechanism for specifically shutting off the enzymes that synthesize it.

In the 1950s, many biochemists were busy deciphering the ways that various amino acids were manufactured. They were finding that the synthesis of every amino acid usually involves a several-step-long "pathway" in which an initial chemical precursor (P) is modified by a series of enzymatic reactions into the amino acid. These pathways are drawn schematically as a chain of intermediate reaction products (I1, I2, etc.), each produced by a different enzyme:

$$P \rightarrow I1 \rightarrow I2 \ldots \rightarrow \text{amino acid}$$

It was discovered, for example, that when the amino acid tryptophan was provided to bacteria, the synthesis of an intermediate was halted. This suggested that tryptophan acted on an early-acting enzyme in the pathway. Similarly, it was found that providing the amino acid isoleucine also inhibited the activity of the first enzyme in its synthetic pathway.

These discoveries inspired the concept of *negative feedback*, whereby compounds feed back on their own synthesis as a way of controlling their levels in cells. The study of all sorts of biosynthetic pathways subsequently revealed that not only was negative feedback widespread, but that it also almost invariably operated by the end-product of the pathway directly inhibiting the first enzyme in its pathway.

Like the double-negative logic of enzyme induction, the logic of negative feedback in biosynthetic pathways also made great biological sense: when the end-product of a pathway is abundant, cells do not waste energy making it or any intermediates; but when the concentration is low, the synthetic machinery is not inhibited, and the needed product is synthesized.

These pioneering studies of bacteria revealed four basic ways that one molecule can affect the abundance of another molecule. They constitute a set of general rules and a logic of regulation that, as we shall see, govern all sorts of processes in other species. (You may want to bookmark this page.)

GENERAL RULES OF REGULATION AND THE LOGIC OF LIFE

Positive regulation

A → B	A positively regulates the abundance or activity of B

Negative regulation

A ⊣ B	A negatively regulates the abundance or activity of B

Double-negative logic

A ⊣ B ⊣ C	A negatively regulates B, which negatively regulates C; A increases the abundance of C through double-negative logic

Feedback regulation

A → B → C (C feeds back to A)	The accumulation of C feeds back to negatively regulate A and the production of B and C

THE SECOND SECRET OF LIFE

The discovery of repressors and feedback inhibition prompted intense interest in understanding exactly how these two kinds of regulation worked at the molecular level. What did a repressor do? How did inducers work? How did feedback occur?

Late one evening in the fall of 1961, Jacques Monod walked into the laboratory of his colleague Agnes Ullmann. Usually well-dressed and energetic, Monod's tie was loose, and he looked tired and worried. After a long silence, he told Ullman, "I think I have discovered the second secret of life."

Ullmann thought Monod did not look well, so she suggested that he sit down and have a scotch, their favorite drink. After another drink or two, Monod stood back up and started a long explanation. He was not ill. He was in top form. He recapped years of observations about repression and feedback inhibition, then offered a single unifying explanation for *both* phenomena.

Monod's breakthrough came from picturing the shapes and sizes of molecules. He was thinking about an enzyme his lab was then studying. Enzymes are large proteins, more than one hundred times larger than the substances they work on (called *substrates*), such as sugars or amino acids. Like a key fitting into a lock, substrates fit snugly into a cavity in the enzyme called the active site, where they get cleaved or modified.

The enzyme Monod was studying is the first enzyme in a pathway that makes the amino acid isoleucine. It works on a substrate called threonine and is inhibited by isoleucine, the final product of the pathway. Monod was trying to picture how the small isoleucine molecule might fit into the active pocket in the enzyme and stop it from working. But then it struck him, isoleucine is not the same shape as threonine. Maybe it can't fit into the cavity?

Then he thought about other feedback-inhibited enzymes and realized the same was true for them: they were inhibited by molecules that looked very different from their substrates. What could that mean? Monod figured the place where the feedback inhibitor bound must different from the active site. The enzyme—the "lock"—must have two keyholes: one for the substrate and one for the inhibitor.

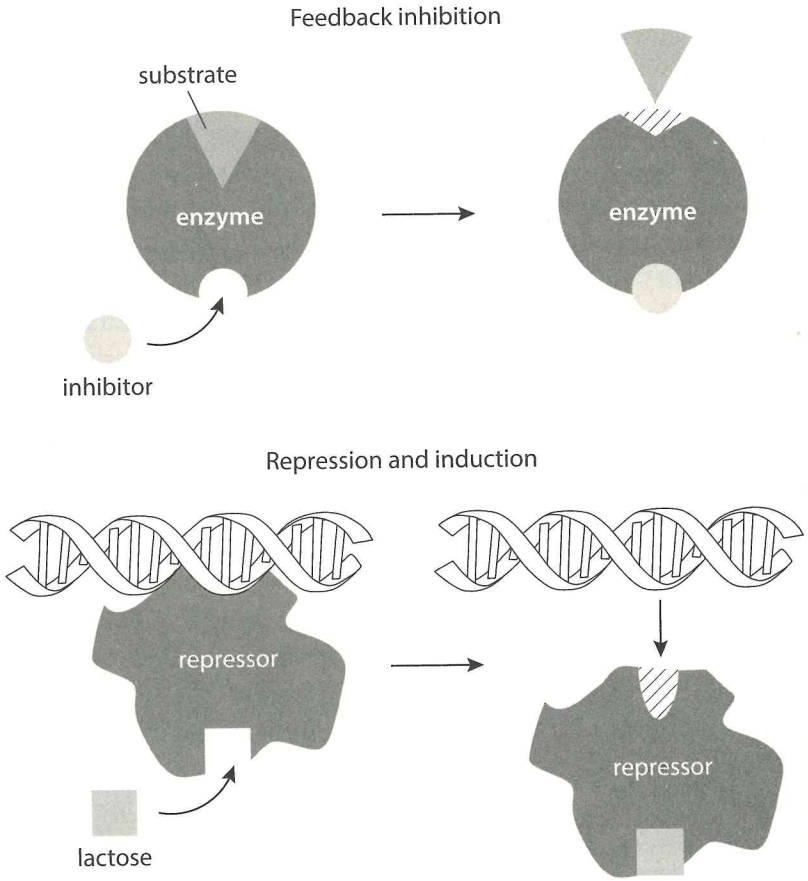

FIGURE 3.6 Allostery is the basis for feedback inhibition and enzyme induction. (Top) The substrate fits into the active site of the enzyme; the inhibitor into a different pocket. When the inhibitor is bound, the shape of the active site changes such that the substrate no longer fits. (Bottom) One site on the repressor binds DNA, another site binds lactose. When lactose is bound, the shape of the repressor changes, and it no longer binds DNA, which allows the enzyme gene to turn on.

Illustration by Leanne Olds.

Somehow, the binding of the inhibitor altered the shape of the enzyme in a way that it could no longer bind its substrate (that keyhole was closed). Monod dubbed this phenomenon *allostery* (from the Greek *allos*, meaning "other," and *stereos*, meaning "solid or object.") He thought allostery could be an important way to regulate the activity of proteins (see Figure 3.6, top).

Then, that one evening, all the pieces fell into place. The inducer and repressor worked in exactly the same way as feedback inhibition, by allostery. The repressor must also have two sites: one site for binding DNA, one for the inducer. When no inducer is present, the repressor binds to DNA, keeping the gene off; when the inducer is present and binds to the repressor, it would induce a change in the physical shape of the repressor, causing it to fall off the DNA and allowing the gene to turn on (see Figure 3.6, bottom).

Monod had two lines of evidence for one simple but big unifying idea: small molecules (amino acids, inducers) regulate the shape and activity of big molecules (proteins). Having connected the seemingly unrelated phenomena of enzyme repression and feedback inhibition, Monod imagined the potential generalities. Allostery could explain, for example, how small molecules like hormones and neurotransmitters regulate the endocrine and nervous systems. Staggered by the potential scope of his idea, he wandered in to test it on Ullmann.

Since DNA was the first secret of life, then perhaps allostery—with all its implications for understanding how genes and proteins were regulated—was the second. At the very least, the Nobel Committee thought that it and all of Monod's and Jacob's discoveries merited the 1965 Nobel Prize in Physiology or Medicine.

E. COLI AND ELEPHANTS

The importance of Monod's and Jacob's research was not a matter of the specifics of solving the mystery of β-galactosidase enzyme regulation in *E. coli*. Like Elton and Cannon, the power of their ideas stemmed from their originality and generality concerning the rules of regulation they uncovered.

Just as Elton conceived of ecosystems as a society of organisms interacting with one another through food chains, and Cannon saw the body composed of a collection of organs communicating with one another through the nervous and endocrine systems, Monod and Jacob pictured life in cells as a "society of macromolecules bound together by a complex system of communications regulating both their synthesis and activity."

Monod and Jacob eloquently explained how their insights, derived entirely from the study of single-celled bacteria, had implications for

understanding complex phenomena in much more complex organisms. In a masterful synthesis of the state of knowledge in 1961, they quipped that it was a "well-known axiom that anything found to be true of *E. coli* must be true of Elephants."

That was more of a bold wish than a proven or accepted fact, but that did not restrain their speculations. While admitting that regulation in higher organisms might be "immeasurably" more complex, they suggested:

> On the other hand, it seems very unlikely that the main mechanisms recognized in lower forms: allosteric inhibition, induction and repression, should not also be used in differentiated organisms. But it is clear that these mechanisms, by their very nature, can be adapted to widely different situations, and would serve entirely different purposes in *E. coli* and Man, respectively.

Not only these mechanisms, but the logic of negative regulation also appeared to Monod and Jacob to be of utmost importance in higher organisms. Recognizing that cancer cells have lost their sensitivity "to the conditions which control multiplication in normal tissues," they suggested that cancers may result from genetic mutations or other agents that inactivate a repressor involved in the control of cell multiplication.

As I will show in Chapters 4 and 5, their speculations turned out to be highly influential and remarkably prescient.

CHAPTER 4

FAT, FEEDBACK, AND A MIRACLE FUNGUS

Rather than replacing genes, we can exploit regulatory principles to make good genes work harder.

–DR. JOSEPH GOLDSTEIN, TO DR. ROY VAGELOS, CEO OF MERCK & CO.

On June 29, 1935, American Ancel Keys and Englishman Bryan Matthews made camp near the summit of Mount Aucanquilcha, over 20,000 feet above sea level in northern Chile. They built a simple snow shelter by putting up a few poles and draping blankets over them, then crawled inside to escape the wind and the temperatures that plunged to fifty degrees below zero overnight. They remained above 20,000 feet for fifteen straight days, during which they ascended the summit several times. At the time, their achievement was one of the highest conquests of the Andes. But these intrepid mountaineers were not professional climbers—they were academic physiologists.

Keys was from Harvard, and Matthews from Cambridge University. The two were part of the ten-member International High Altitude Expedition (IHAE) that had traveled to Chile to study how the human body adjusted to very high altitude. Aucanquilcha was home to the world's highest permanent population at 17,500 feet, and the world's highest mine at 19,000 feet. The expedition was the largest,