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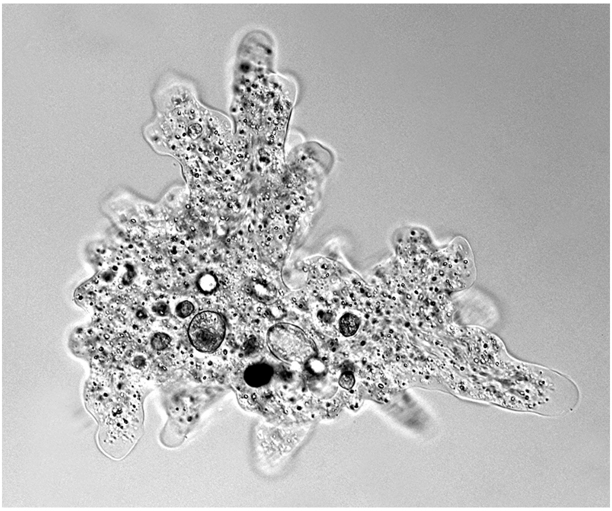
Animations



We are amoebas, Part I: Watching an amoeba glissade over the polished glass of a microscope slide can be an unsettling experience if we meditate on the creature in the spotlight. As we pay attention to the movements of this speck of life, the alleged simplicity of the amoeba gives way gradually to the unequivocal presence of a deeply sensitive being. Microscope becomes mirror, and in the beauty of the amoeba we see something of ourselves.

A circle of pond water lit and magnified with the microscope is painted with brown islands of fine organic silt, sparkling nuggets of sand, and crisscrossing filaments of green algae. The largest of the algae is distinguished by its bright green ribbons, wound as double helices inside each tubular cell. The surface of the algal strands is planted with smaller trumpet-shaped organisms, whose mouths bristle with motorized hairs that suck whirlpools of water into their open bells. Freewheeling microbes buzz along the waterways, colliding with the algae and careening across the entire field of view, seeking relief from the squash of the coverslip. The carnival is in full swing, but the real star is still to appear.[1]

Patience is essential here, and the appearance of the amoeba, when it moves into the spotlight, is at once familiar and surprising. It is so different from everything else, larger than the other single-celled organisms and almost transparent—"body clear as water," judged a nineteenth-century naturalist.[2] The commonest species, called *Amoeba proteus*, appears as a faint shadow in the water, a wavering boundary between the vibrancy of life and the surrounding fluid. This cell is thinnest and most transparent toward its edges. Its presence is revealed by granules that surge through the center of each limb-like extension, looking like gravel scrambling on the bed of a clear stream. My god, it is a beautiful thing.

Besides its notable glassiness, *Amoeba* draws our attention with its curious movement. This seems such an alien way of getting around, and there is no substitute for the eponymous "amoeboid" to describe the mannerism. *Amoeba* has mastered mobility without urgency, unlike the faster microbes called flagellates and ciliates thrashing around the same neighborhood. It pours its substance from one direction to the next, simultaneously flowing ahead and retracting behind. When the cell keeps going along the same track for a minute or so it becomes elongated, with smooth pseudopodia at the front end and the membrane puckering over its trailing backside. Exploring other options, the cell stretches in two or more directions at the same time and can assume the shape of a starfish.

Amoeboid movements seem strange because they are so different from the way that big animals like us move. Animal motion is powered by the contraction and relaxation of muscles. When a cheetah sprints through the Namibian grassland, its limbs fly out and pull together four times per second, power-assisted by the spine that operates as a spring and steered by its meaty tail. It is a phenomenal muscular machine. Muscle contractions are apparent in simpler anatomies too, in the flapping of insect wings, wrigglings of worms, and hypnotic pulsations of jellyfish bells. But among single-celled organisms, where we find a great range of movements, there are no muscles, and these microbes face physical challenges that are ignored by larger species.

Water is experienced as a viscous fluid by a microscopic particle. A bacterium will stay suspended in it rather than sinking. To move, it must drag or push itself through its treacly world. Many bacteria and other microorganisms use tails or flagella to stir the water. Some flagellate species swim at comparatively high speeds, traveling many times their own body length per second, seeming swift as dolphins as they cross their magnified puddles. Unlike a marine mammal or a fish, however, the microbe stops dead the moment it stops lashing its tail. This is the influence of viscosity, which robs the cell of any inertia—the Newtonian tendency to keep moving. Microbes have contrived many other methods of locomotion besides swimming with flagella, including the use of grappling hooks to yank themselves along surfaces, and rotating the whole cell like a corkscrew, but the controlled flow of fluid inside the amoeba is the most graceful by far.

If *Amoeba* were not so mobile, it seems possible that this colorless creature would never have been discovered. This thought experiment raises the possibility that many microorganisms remain unknown, sitting quietly in their ponds, blending in with the surrounding silt. They might collect nourishing particles on their surfaces and munch away in obscurity forever. Even in this age of genetic fingerprinting, it seems doubtful that these ghosts would be found. We may be fishing their genes from water samples all the time and labeling them as "unknown" in genetic databases because their sequences of As, Ts, Gs, and Cs do not match those of known species with a Latin name, or presumptive species tagged with a code number. Unless we isolate whole cells of these cryptic organisms, there is no way to link one of these unique genomes to a particular microbe. Perhaps we have missed more than we have found.

But *Amoeba* does move, and it caught the attention of the German naturalist August Johann Rösel von Rosenhof, who described "the little Proteus" in 1755. Proteus was the shape-changing sea god of Homer's *Odyssey*, and von Rosenhof provided drawings of the ambulating cell in a book whose principal concern was with insects. Watching these cells for two or three hours at a stretch, he waited to see if they would assume a particular shape and show "something like [a] head, feet, or even a tail." Hoping to learn more about its structure, von Rosenhof used the tip of a sharpened quill to hold the cell in place and concluded that "his inanimate substance was held together by a delicate layer of skin, and when this was torn, he would crumble before my eyes."[3] It was a most peculiar kind of insect.

The name *Amoeba* came in the next century, and its Greek root ἀμοιβή (amoibē) refers to "change," so that the combination, *Amoeba proteus*, doubles down on the extraordinary malleability of this cell. Other pond microbes have firmer bodies. *Paramecium* cells, for example, are framed like slippers, with a mouth on the side into which one can imagine a miniaturized Cinderella sliding a dainty foot. Its surface, called the pellicle, is flexible, although much less so than the membrane of the amoeba so that the cell bends without losing this unmistakable slipper shape. Diatoms have brittle cell walls called frustules, dinoflagellates are fitted with overlapping panels, and so on. *Amoeba* is exceptionally plastic. And it seems so vulnerable, see-through from top to bottom, spreading its naked membrane into a world of vicious armor-plated competitors. It must have its reasons.

Some relatives of the bare-membraned amoebas lack this raw confidence. These “testate amoebas” fabricate protective shells or tests from mineral grains, silica plates, and the glassy frustules of dead diatoms, and hide within these elaborate constructions like tiny turtles (the testate amoebas are featured in chapter 6). The sight of the diaphanous arms of these cells elongating from their sparkling pots is mesmerizing. Like an octopus unfurling its tentacles from a jar, the amoeba reaches so far from its test that it seems improbable that it could have hidden all of its substance inside. This magical performance is explained by the pneumatic nature of the pseudopodia, which fill with fluid as they draw themselves out. Buy yourself a microscope before it’s too late and dip a jar into a pond.

A note is necessary here to explain the slippage from *Amoeba* to amoeba and amoebas in this book. The italicized name, *Amoeba,* is the Latin name for the genus, or larger grouping, of dozens of species of microorganisms believed to be the closest relatives of *Amoeba proteus*. It was fashionable to use the *o* and *e* ligature for *Amœba* in the early twentieth century, but this disappeared decades ago. *Amoeba proteus* is the species that defines the genus *Amoeba*.[4] Biologists call this the type species. This organism is an "amoeba," its relatives are "amoebas" or "amoebae," and these nouns also apply to 15,000 other microbes that move in an amoeboid style. These include naked species of amoebas, the amoebas with shells that just got a mention, slime molds with amoeboid phases in their life cycles, and all manner of amoeboid species that have been fished from every wet ecosystem on Earth. All of the amoebas and other single-celled pond creatures larger than bacteria are called protists, which is the modern name for the protozoa.[5] Biologists that specialize in their study used to be called protozoologists, but they are protistologists today.

One would think that in this third century after the discovery of amoeboid cells, we would have a pretty clear picture of their mechanism of movement. It cannot be that complicated, can it? Well, yes it can. It is. Here is the conclusion reached in a 2013 paper titled "How Do Amoebae Swim and Crawl?" by a trio of University of Cambridge biologists: "Simply put, we do not understand how these cells swim, and therefore how they move."[6] One of the authors was a Fellow of the Royal Society of London, not that this distinction, in itself, is a guarantee of sagacity. More convincingly, nobody else has answered the question satisfactorily despite measuring their internal pressure and the voltage across their membranes, dissecting them to see what happens when they are deprived of their nuclei, spinning them in a centrifuge to see how they cope, looking at the cells with all manner of microscopes, modifying their genes, simulating their movements with supercomputers, and thinking really hard about the problem. If we cannot understand exactly how the amoeba crawls, what chance do we have of solving the big questions in biology? How, for example, does the human body of trillions of cells develop from a single fertilized egg? An explanation of amoeboid motility should be child's play compared to solving the mysteries of the womb.

This is what we do know about amoeboid movements. Inside cells there is a web of filaments made from a protein called actin. This is one of the components of the cytoskeleton. Metal scaffolding draped in plastic sheeting offers a useful model for the cell and its internal skeleton if we imagine that the bars can change in length and slide through their joints to produce an ever-changing shape.[7] In this representation, the plastic represents the cell membrane, which is pushed outward and pulled inward as the bars elongate in some places, shorten in others, and slide past one another. The presence of fluid around all of the protein filaments in the cell is a significant addition to the scaffold model. This is the liquid portion of the cytoplasm, which is squeezed when the membrane retracts and relaxes when the membrane flows outward. This push and pull is one of the ways in which the amoeba moves in a particular direction, with the filaments extending within a pseudopodium here and withdrawing from another one there.

In the 1990s, researchers monitored pressure changes inside amoebas by piercing the cell membrane with a glass needle or micropipet.[8] The cell reacted to being pricked by recoiling from the pipet, which made things difficult for the scientists, but the experiments showed that the pressure increased before a pseudopodium began to stretch outward, and then fell as the membrane bulged. The order of cause and effect is complicated here, because the changes in membrane tension and the pressure in the fluid are so intimately connected. It was unclear whether the pressure changes provoked the alterations in membrane tension, or vice versa. To confuse things further, the pressure rose on some occasions without any resulting expansion of the membrane. The amoeba seemed to be acting in a willful fashion, not like a simple machine at all.

Another complication arises from the observation that the fluid cytoplasm thickens from the free-flowing stream toward the middle of the amoeba to a firmer jelly close to the inner surface of its membrane. The thickening of the cytoplasm is called the "sol-gel transition" and is controlled by crosslinks that tie the actin filaments together: crosslinking stiffens the filaments to produce the gel that extrudes into pseudopodia.[9] The idea that reversible changes in consistency, from sol to gel and back again, were responsible for movement became popular in the 1920s, and they do account for a lot of the behavior of the cell. But they are not enough on their own. Whenever a biologist announces a newfangled theory of amoeboid movement, the excitement fades when we recall some incongruous feature of the behavior of these cells as they glide over surfaces, reach into the water, and feed on bacteria. No single explanation has ever worked satisfactorily. The true picture lies somewhere in the ensemble of filament elongation and sliding, changes in pressure and internal fluidity, and, no doubt, some biomechanical contrivance that awaits discovery.

Regardless of how it moves, the amoeba is a highly mobile and virtuoso predator, constantly probing the water with its pseudopodia, streaming more of its substance into arms that make contact with potential prey by redirecting cytoplasm from other parts of the cell, and chasing mobile targets at a top speed of 20 millimeters per hour.[10] This pace is equivalent to two cell lengths per minute, which is unhurried by any reckoning. *Paramecium,* the pond microbe shaped like a slipper, can swim two hundred times faster than an amoeba, covering more than 1 millimeter per second. *Paramecium* is a type of ciliate that is powered by thousands of beating hairs called cilia organized in rows along its surface. (These hairs have the same structure as flagella, but they are called cilia when they are arranged on cells in large numbers, and cells that bear them are classed as ciliates rather than flagellates.) Even with this impressive speed, these athletic *Paramecium* cells are caught and eaten by *Amoeba*, and can be seen jiggling around inside the predator until they succumb to its digestive juices. Bloated with one of these energetic ciliates, an *Amoeba* resembles a snake that has swallowed a live rodent.

Predators that move more slowly than their prey depend on a combination of good luck (being in the right place at the right time) and the application of the strategy of the tortoise in its famous race with the hare. *Amoeba* is left hungry in a swirl of silt when *Paramecium* has a clear run through the water. The odds shift in favor of the predator when *Paramecium* encounters the tangled threads of algae or other obstructions that compel it to back away, which it does by reversing the direction of its beating cilia, selecting an escape route, and rocketing off as fast as possible as the amoeba bears down. Death comes when it finds itself cornered and the shadow of the *Amoeba* grows. *Paramecium* also loses when it swims in the wrong direction, straight into the arms of a waiting amoeba.

Diagrams of amoebas in textbooks show them hugging their prey with a pair of pseudopodia that fuse at their tips to encircle the victim in an internal pool labeled as the food vacuole. This is a two-dimensional illustration of the feeding mechanism that is called phagocytosis. It is difficult to see what happens in three dimensions, because the *Amoeba* is so thin and transparent. But if we watch the feeding cell under the microscope and keep focusing the lens up and down very carefully, peeping between its upper and lower surfaces, we begin to see that the amoeba flows all around its prey, extending a fine veil above and below the food so that the meal is completely surrounded. This observation requires something of the skill with the microscope that a devoted bird watcher develops through familiarity with binoculars. *Amoeba* loses an entire physical dimension without this patient observation, leaving us with a silhouette rather than an appreciation of its essence as a fleshier manifestation of life. I have a plastic classroom model of an *Amoeba* mounted to the side of a bookcase in my home office. It is as big as a newborn baby, and half the upper surface is cut away to show the structures inside the cell. Even with its fat pseudopodia, this painted figurine is a mediocre representation of the reality of the restless organism that continually adjusts its shape and speed. And even when we watch an amoeba with a microscope, imagination is needed to begin to appreciate the splendor of the dynamic cell. There is so much that is going on in this slip of a thing.

The significance of the three-dimensional shape of the amoeba and its flexibility were recognized by Oris Park Dellinger from Clark University in Massachusetts before the First World War.[11] Dellinger developed a method for viewing amoebas from the side by making a shallow trough on the edge of a glass slide and rotating the microscope into a horizontal position so that the slide was oriented vertically on its stage. This revealed that the cell could stand on end and assume the form of a baseball mitt to catch a speeding *Paramecium* like a slugged ball. Fascinated by the behavior of the cell, he convinced his colleague, David Gibbs, to take turns with him, watching amoebas over six days and five nights in the winter of 1905–1906. For these experiments they used conventional horizontal slides but propped the coverslips on pieces of glass to increase the depth of the pool more than tenfold. They also added water periodically to compensate for evaporation, keeping the cells as comfortable as possible. Using this setup, they tracked several individual cells as they moved around, fed and rested, and bumped into one another (the cells—not Dellinger and Gibbs), and continued to watch them daily for weeks following the stint of constant surveillance.

Through the days and nights that winter, Gibbs and Dellinger witnessed the richness of individual experience in the lives of the amoebas. They plotted the position of the cells as the hours passed, noting "traveling with many pseudopods" when the line on the graph turned upward indicating an increase in velocity, followed by "feeding" and "taking in masses of algae." Then, as the same cell slowed down, they wrote "sluggish" as the plot bottomed out, followed by "full of food" and "inactive." This siesta preceded the division of the cell, and a renewal of intense activity as the resulting pair of daughter cells began feeding. The plots showed a rhythm of work and rest that corresponded to the changing energy needs of the cell. Gibbs and Dellinger also recorded the position of amoebas as they pursued *Paramecium* cells and found that some kept up the chase for more than 20 minutes before catching or abandoning their quarry. They concluded, "amoeba can no longer be considered as a bit of but slightly differentiated protoplasm, but must take its place in the true animal series with the rudiments of true animal behavior."

This heroic study was described in a classic paper, titled "The Daily Life of *Amœba proteus*," which was published in *The American Journal of Psychology* in 1908.[12] The choice of journal was unconventional for a biological investigation. Gibbs and Dellinger had strayed across the boundaries between disciplines, and the paper is not cited in an otherwise encyclopedic book dedicated to this organism, *The Biology of Amoeba*, a 628-page doorstopper published in 1973.[13] Imagine an academic thesis titled *The Biology of Hyenas* that failed to describe the hunting behavior of these carnivores. I think that would rank as an equivalent omission. The reason that the paper by Gibbs and Dellinger was ignored by most amoeba experts lies in the differing languages of animal behavioral studies and those of cell biology. In the decades following their highly original observations, interest in the foundations of cognition, memory, and consciousness in single-celled organisms waned as biochemists and geneticists transformed the study of cell structure and function. These scientists were too busy to pay attention to the behavior of the cells that they deconstructed. But this situation has changed in recent years, with a revival of experiments on cellular consciousness (as we shall see when we return to the subject in chapter 5). Psychologists and philosophers have also been influential in this field of interdisciplinary research.

And now we come to the bowel and bladder of the amoeba. (Of course we do.) The food vacuoles that hold ciliates and other prey that have been absorbed into the amoeba function as its digestive system. These spherical sacs extract all of the useful nutrients from the food morsels before the amoeba expels the undigested waste from its wrinkled rear end. A second kind of larger compartment inside the amoeba called the contractile vacuole is charged with removing water from the cell. Water enters the cytoplasm by the process of osmosis because the mixture of chemicals inside the amoeba is more concentrated than the surrounding water. The purer the water outside the cell, the faster it diffuses into the cell and the faster the vacuole has to collect and bail water. The contractile vacuole can be likened to a heart, which cycles between a slow filling or diastole for two or three minutes, before each contraction or systole, when it fuses with the exterior cell membrane and flushes as vigorously as a toilet.[14]

The contractile vacuole is a vital "organelle," which is the name for mini-organs like this and the mitochondrion and nucleus within cells. If this vacuole did not flush the cell, it would inflate like a water balloon and explode. Pressure changes can be important in the extension of pseudopodia, as we have seen, but there is a limit to how far this hydrostatic force can increase before the swelling becomes unsustainable. If the contractile vacuole stops working, the hypertensive cell bursts and its granular cytoplasm is discharged as a slick beneath the glass coverslip. The dying amoeba resembles a leaking oil tanker.

The membrane surrounding an amoeba is too thin to be seen using a light microscope, but it is always there. No membrane, no cell, because the chemical order of the interior cytoplasm has to be guarded, or has to guard itself, from the chemical disorder of the outside world. Membranes provide life with a temporary defense against entropy. They are made from two layers of greasy molecules, or lipids, and are peppered with proteins. The integrity of the membrane is everything to the health of the cell. It serves as the lung of the amoeba, allowing oxygen dissolved in the water to diffuse into the cell and for carbon dioxide to escape. Oxygen is absorbed by the hundreds of mitochondria in every amoeba and used to break down (oxidize) the sugars and other small molecules that are released from the food that the cell digests in its vacuoles.

Proteins that function as pumps shove charged atoms, or ions, from one side of the membrane to the other. This process charges the membrane and provides the cell with a source of energy for controlling the balance of chemicals within its interior fluid.[15] The membranes of all organisms are energized in this fashion. Our nerve cells use changes in membrane voltage to communicate information, and the summing of trillions of these "on" and "off" signals into meaningful patterns in the brain allows us to ponder the lives of amoebas and contemplate other less interesting subjects.

When the amoeba has gobbled enough ciliates to sustain a pair of cells, it displays its famous androgynous solution to reproduction by dividing in half. It prepares for duplication by making an identical copy of its chromosomes so that each of the pair of newborn cells will have all of the instructions for its solitary life. Division begins with the withdrawal of the pseudopodia and reshaping of the cell as a sphere.[16] If the amoeba was attached to a surface beforehand, it detaches as it rounds up in this way. Next, the membrane ruffles itself into little bulbs so that the amoeba assumes the appearance of a warty pumpkin. Beginning as smooth bulges, the swellings go on to grow spikes, and these begin to reattach the cell to the surface. As this is happening, the amoeba flattens into a disc. This disc stretches and develops a waist, and the dumbbell-shaped cell draws itself into two halves. The nucleus, which contains the DNA, is bisected as this takes place so that both daughter cells are endowed with a complete set of chromosomes. The whole process is completed in 10 to 20 minutes, but if the cell is floating in water before it divides this can take an hour. It appears that the division is assisted by the adhesion of the ends of the dividing amoeba to a surface, which provides the traction needed to expedite the separation of the two halves of the cell.

Experiments on live amoebas and other cells are subject to a limitation called the observer effect.[17] This phenomenon is part of the ethos of physics, wherein it is acknowledged as an unavoidable feature of research, ranging from the simplest task of measuring temperatures to the arcane explorations of particle physics. Thermometers absorb a little of the thermal energy of an object when they measure its temperature, and atoms are disturbed when lasers are used to study their behavior. In both examples, the experiment alters the state of the subject under investigation. The observer effect deserves more attention from biologists. Remote wildlife cameras capture different interactions between African fauna than the sunburned tourists on safari who startle the animals by arriving in a bus, swigging from water bottles, and changing camera lenses. The problem is just as pressing when the solitary investigator watches an amoeba. A coverslip placed directly on a drop of pond water on a slide squishes the residents into a sliver of fluid that is no more than a few tenths of a millimeter in depth. Even quite small cells of *Amoeba proteus* can extend over twice this span, so the coverslip limits their motion in the third dimension. There is little in the experience of the amoeba that compares with the oppression of a caged or concrete-tanked mammal, but we cannot expect any living thing to behave naturally in captivity. The situation worsens for the amoeba as time passes and water evaporates from its little pool, which becomes shallower and shallower, narrower and narrower, until the shrinking drop reaches the cell membrane, exposing this watery mote of life to the air, which shrivels it to a crisp.

These problems were addressed by Gibbs and Dellinger in their classic behavioral studies by providing their amoebas with plenty of water to maximize their mobility, but the bright light from the microscope was a confounding variable that they could not control. In addition to divorcing amoebas from the darkness of their ponds, the concentrated light beam from the microscope lamp heats the water. Most of the mobile pond microbes swim to evade the light, abhorring the brightness as well as the associated heat. When we move the slide to keep the cells in sight, they try to escape again, which is one of the difficulties of studying pond samples. Larger species, like the crustacean water flea, *Daphnia*, whose shelled body fills the field of view, are trapped in place under a coverslip. Immobilized in this glassy vice, its little heart keeps beating until its feathery limbs lie still, oxygen runs out, and darkness closes its cyclopean eye.

Amoeba proteus loathes the sudden illumination of the microscope, pausing the stream of its internal cytoplasm, then moving out of the spotlight as swiftly as its pseudopodia can take it. It is particularly sensitive to the most energetic wavelengths of blue light that plants and many algae use for photosynthesis.[18] The preference shown by our subject for the shadowed recesses and unlit depths of the pond reflects this aversion to the sun, but nothing is known about the way that it detects light. To neutralize this disturbance, we could use cameras that are sensitive to very low light levels to watch amoebas in near darkness. It seems unlikely that this artifice would reveal an amoeba string quartet, but, even so, there is an occult to this organism that eludes us, something that is missing from our picture of its tentacled cells. Without going too far down this metaphysical rabbit hole, we will explore the marvels and mysteries of amoeba biology in the following pages. And may wonderment never cease.

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