**What Is the Weight of a Single Amoeba and Why Does It Matter?**

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**Abstract**

Cell size is an important variable in the study of cellular growth, metabolism, and the cell cycle. The large size of Amoeba proteus and the ease with which it can be collected and cultured have made it a star in biology education—and it was a model for research on cell biology before the introduction of molecular genetic methods. Measuring the cytoplasmic density of a single amoeba without modern instrumentation seems like a difficult task, but this was done with supreme accuracy in the 1940s. The solution was based on the familiar Cartesian diver that is used to demonstrate Archimedes’s principle. It required the fabrication of a tiny diver that would respond to the additional mass of a cell. Experiments using this method allowed investigators to study changes in size and density associated with feeding, starvation, and cell division. This research is an illustration of the ingenuity of cell biologists in the pre-molecular genetic era of their field, which is often overlooked by contemporary scientists. The consideration of the mass, density, and buoyancy of free-living amoebas encourages a new hypothesis about the evolution of testate amoebas.

**Key Words:** biomechanics; Cartesian diver; cell size; cell mass; cell density; testate amoebas.

The size of cells, measured as volume or mass, is a critical variable in the study of cellular growth, metabolism, and the cell cycle. Amoebas are jumbo microbes. Amoeba proteus, the iconic free-living cell studied in biology classrooms, often reaches beyond half a millimeter in length (Figure 1). Water fleas of similar size have dark gut contents and an opaque eye that make them noticeable as mobile dots in pond water without any magnification. Amoeba remains invisible until we use a microscope to increase the contrast between the colorless cells and the water. This is done by closing the ring of thin interlacing metal blades below the microscope stage called the iris diaphragm, which sharpens the incoming cone of light. With practice, this enables us to watch amoebas with a low-power 10x lens, which provides a 100-fold magnification when combined with a 10x eyepiece. The more numerous bacteria remain invisible until we turn the microscope turret to the 40x lens, and the gray mist surrounding the amoebas resolves into jostling dots and wriggling rods.

Amoeba proteus is millions of times larger than the commonest bacteria. It is as big as a whale compared with a mouse. This size comparison is made by treating the amoeba as a thin disk and the bacterium as a rod. If the discoid amoeba has a diameter of 0.5 mm and an average thickness of 20 µm, its volume is $4 \times 10^6 \mu m^3$; a 2 µm-long bacterium with a diameter of 1 µm has a volume of 1.6 µm$^3$. This is a 2.5-million-fold difference in volume. Using mass as a rough proxy for volume, this is equivalent to the difference in size between a 30 g mouse and a 75 metric tonne bowhead whale. The largest bacteria match the size of amoebas; the tiniest are 200 million times smaller than amoebas, which is the same as the size ratio between a dragonfly and a bowhead whale. These teeny bacteria cannot be seen with a light microscope, even at the highest magnification, because they are smaller than the wavelength.
of light. This means that light waves pass around them without interruption.

If an amoeba had the same density as pure water, we could calculate its mass from its volume: $4 \times 10^5$ $\mu$m$^3$ of water has a mass of 4 $\mu$g. But the inside of an amoeba contains dissolved ions, including potassium, sodium, and chloride, and its internal structures are built from proteins, fats, carbohydrates, and the nucleic acids—DNA and RNA. Billions of protein molecules are organized within this single cell. Many of these are the enzymes that drive the chemical reactions of digestion to release energy from the microbes consumed by the amoeba. Other proteins are structural, forming the cables of the cytoskeleton, and act as spools for winding the DNA into chromosomes. The combination of these biological molecules and the intermingling minerals increases the density of the cell so that an amoeba is heavier than a drop of water of the same size. We know this because amoebas sink in water. They must crawl over the surfaces of submerged plants to move upwards in a pond. The pseudopodial movements of an amoeba that loses contact with a surface do no more than the flailing limbs of a drowning sailor as he drops into the dark.

We can determine the density of the amoeba from the syrupiness of a fluid needed to keep it afloat, and with this scrap of information, we can calculate its mass from its volume. Density gradient centrifugation uses a substance called Percoll that is layered in a tube; made from silica particles, it is concentrated and densest at the bottom, more dilute and less dense toward the top. When cells are added to the top of the tube and centrifuged at high speeds, they accumulate in a distinct band within the Percoll layer that matches their own density. The density of the amoebas of the slime mold *Dictyostelium discoideum* was measured in this way by Fukui et al. (2000), who found that the wild-type cells (meaning non-mutant) in their study had a density of $1.07 \times 10^{-3}$ $\mu$g $\mu$m$^{-3}$. Applying this figure to estimate the mass of *Amoeba proteus* cells with a volume of $4 \times 10^5$ $\mu$m$^3$, we multiply by $1.07 \times 10^{-3} \mu$g $\mu$m$^{-3}$, which equals 4.28 $\mu$g. The dry matter content of the cell adds 7% to the mass of the amoeba.

To delve deeper into the changes in the size and density of cells under different conditions, a very tiny and very sensitive scale is needed that responds to the mass of a single amoeba. This seems like an immense technological challenge, but, astonishingly, Danish zoologist Erik Zeuthen solved this problem in the 1940s by fabricating a miniature Cartesian diver with a microscopic cradle tailored for an amoeba (Zeuthen, 1947). A Cartesian diver provides a familiar demonstration of Archimedes’s principle (a refresher is provided in the Appendix). The miniature diver was made from a glass tube topped with a polystyrene cradle. To weigh the amoeba, the cell is delivered to the cradle at the top of the diver using a pipet (Figure 2). The amoeba adds mass to the diver, which descends in the water. The added weight of the amoeba is calculated from the reduction in pressure needed to restore buoyancy to the diver. The diver technique was used to study the change in the mass and volume of amoebas that were starved by separating them from the freshwater ciliates that serve as their live prey (Holter & Zeuthen, 1948). The mass and volume of the starving amoebas fell rapidly—the volume fell by as much as 75% before death—as the cells consumed their energy reserves.

The diver was also used to determine the permeability of the amoeba cell membrane (Pigon & Zeuthen, 1951; Prescott & Mazia, 1954). Permeability is a measure of the resistance of the membrane to the diffusion of different substances. The rate at which water and dissolved substances flow through the membrane is an important feature of cellular physiology. It is one of the factors that determines the responsiveness of the cell to changes in the chemistry of its environment. Heavy water was used to measure the permeability of the amoeba membrane. Heavy water is water in which the hydrogen atoms in each molecule of H$_2$O are replaced with deuterium atoms to form D$_2$O. Deuterium is one of the isotopes of hydrogen. The nucleus of the hydrogen atom contains one proton; the nucleus of the deuterium atom contains one proton and one neutron. Deuterium has twice the atomic mass of hydrogen, and heavy water is 11% heavier than normal water. Cells cannot tell the difference between H$_2$O and D$_2$O, and so D$_2$O replaces the H$_2$O in an amoeba when it is grown in heavy water; the result is an increase in cell density (mass). This was tracked using the Cartesian diver method, providing a measure of how fast water was exchanged across the amoeba membrane.

These experiments with the tiny divers are one illustration of the ingenuity of biologists who studied amoebas in the last century. It is easy to dismiss the work of previous generations of scientists as the technology of research evolves to address new questions, but these early cell biologists were quite brilliant. Shinya Inoué (1921–2019) was a model of this kind of brilliance within the field of cell biology. He developed the polarized microscope that was used to study the dynamic behavior of the cytoskeleton inside living cells. Inoué assembled the first version of his microscope from an assortment of unlikely components—including a discarded machine-gun base and a tin tea can—at the Misaki Marine Biological Station, south of Tokyo, in the aftermath of World War II (Dell & Vale, 2004). He was a co-author of the study on amoeba cell density mentioned above.

Methods for directly weighing cells have been perfected in our time, with the development of a balance that is sensitive to picogram ($10^{-12}$ g) fluctuations in mass (Martinez-Martín et al., 2017). This is one thousand times more precise than the Cartesian diver.

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**Figure 2.** Diagram of the Cartesian diver method for measuring the “reduced weight” of a single cell. The reduced weight is the weight of the cell less the buoyant force of the surrounding fluid. (a) The diver with mass $M$ is neutrally buoyant with an air bubble in the bulb with volume $V_o$. (b) The addition of the amoeba with mass $m_a$ causes the diver to sink. (c) Reducing the pressure in the fluid causes the air bubble to grow ($V_o + V'$), and neutral buoyancy is restored.
method and gives a direct measurement of mass uncomplicated by buoyancy effects. The picogram balance is used to measure the mass of a cell that attaches itself to the end of a silicon beam. This beam vibrates when its base is energized by a blue laser. As cells stuck to the beam increase in mass, the beam waggles more slowly, and this change in the frequency of oscillations is detected with a second laser. This physical principle takes us back to our drowning mariner. Moments before he plunged into the sea, he had stood on the end of a wooden plank. He had been caught stealing the grog, whose diminishing supply had been a longstanding concern on this terrible voyage. Indeed, the same plank had been rolled out in the previous week when the slender cabin boy was executed for refusing to explain where all the grog had been going. In his logbook, the pirate captain recorded that the plank had waggled more slowly the second time when his older and considerably heavier shipmate stood on the end, flexed up and down on his toes, and said he was sorry. There is also, no doubt, a way of explaining Cartesian divers with piratical metaphors, but I will leave this to the imagination of readers.

This inquiry on the mass of amoebas can be extended to species that fashion their own shells or “tests” from debris in their aquatic habitats and from sculpted plates of silica. These are the testate amoebae and include species of *Difflugia* and *Quadrulella* that live in freshwater habitats (Figure 3). *Difflugia* assembles its test from tiny sand particles and diatom shells that it cements on its surface. Some *Difflugia* shells look messy, but this amoeba is discriminating in the assembly process, reserving smaller particles for the region surrounding the opening, or pseudostome, through which it extends its pseudopodia. This architectural finesse is clearer in *Quadrulella*, which manufactures square scales in vesicles within the cytoplasm, and releases them onto the cell surface by exocytosis. The test is assembled with bigger scales covering the larger curved base of the structure and smaller ones decorating the neck. Bian et al. (2019) reported that testate amoebas will assemble shells from microspheres made from silica and polystyrene if they are cultured without access to their natural sources of construction materials. The precision of test assembly is remarkable when we highlight the fact that this operation is performed by a single cell with no nervous system. The molecular processes that underlie this expression of cellular intelligence are a complete mystery.

The function of test formation is similarly obscure. The prevailing view was expressed in an article by Hansell (2011): “What are these cases for? We have almost no information on this. They are very reminiscent of the protective cases of some insect larvae, so they might similarly provide physical protection, in this instance against predators or possibly pathogens.” In this article on weighing amoebas, we propose that an additional function of the test may be to weight the cell and function as a natural diving bell (Figure 4). By increasing the density of the cell, the formation of a test will allow the amoeba to remain in contact with surfaces when the slightest turbulence in the surrounding water would dislodge a naked amoeba.

We can estimate the density of a testate amoeba from published data on the density of diatom shells and silica. Diatom shells, or frustules, are made from amorphous silica and organic compounds. The silica fraction varies from 10–70% of the total dry mass and has a density of 2600 kg m\(^{-3}\), and the carbohydrates and proteins have a density of 1300 kg m\(^{-3}\) (Miklasz & Denny, 2010). This means that the overall density of a diatom frustule ranges from 1400 to 2200 kg m\(^{-3}\) (for 10% to 70% silica). Test composition in amoebas varies a great deal between species (e.g., *Difflugia* versus *Quadrulella* in Figure 3) and between individuals within some single species (e.g., *Difflugia*). The mixtures of silica and organic compounds in the diatom test offer a rough guide to the density of a broad range of amoeboid tests. Using the estimated density range of 1400 to 2200 kg m\(^{-3}\), and assuming that the amoeba test occupies 50% of the volume of the shelled cell, we derive a very rough prediction of the overall particle density of a testate amoeba of 1200 to 1600 kg m\(^{-3}\). The maximum estimated particle density is 50% higher than the density of a naked amoeba, boosting the mass of the 0.5 mm-diameter cell from 4.3 to 6.4 μg. This increase in mass will make the cell sink ten times faster than a naked amoeba and increase the resistance of the amoeba to being displaced from a surface. The mass of amoebas that build their tests from quartz grains will be somewhat higher, and these cells will sink even faster (Armynot du Châtelet et al., 2013). Either way, the presence of a rigid test is likely to help an amoeba remain clamped to the leaves of *Sphagnum* moss in a peat bog or silt at the bottom of a pond. These

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**Figure 3.** Scanning electron micrographs of testate amoebas. (a) *Difflugia* with a test assembled from diatom frustules and sand particles embedded in an organic matrix. The length of this cell is 160 μm. (b) *Quadrulella* with a test made from quadrangular plates of biosilica. The length of this cell is 85 μm. Image credit: http://penard.de/.

**Figure 4.** Early diving bell designed by Franz Kessler in 1616 and the structure of the testate amoeba *Difflugia*. The diver in Kessler’s bell sat on an internal frame and looked through the bull’s-eye windows around the top. The ball hanging below the rim of the bell is the ballast weight that allowed the diver to walk on the seafloor. In English, *Wasserharnisch* means water-harness or water-armor. *Difflugia* image credit: https://arcella.net.
biomechanical considerations may help explain some features of the distribution of testate and naked amoebas in different habitats. From this simple inquiry on the size of amoebas, we have revisited some obscure but very clever experiments from the 1940s, considered how some foundational principles in physics apply to biology, looked at modern instrumentation for measuring the mass and density of cells, and proposed new ideas about the adaptive function of test formation by single cells.

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Appendix: The Cartesian Diver Experiment

Water is sucked into a plastic eye dropper, leaving an air bubble in the bulb. This is weighted at the open end with a threaded nut or chain of paperclips and placed, bulb uppermost, in a flexible plastic bottle filled with water. The bottle is closed tightly with a screw cap. The eye dropper, which is the diver, will remain stationary, floating in the water if the air bubble is the right size to make it neutrally buoyant. When the sides of the bottle are squeezed, the hydrostatic pressure of the water increases, the air bubble in the diver is compressed, and the diver descends. Releasing one’s grip on the bottle causes the hydrostatic pressure to decrease, which allows the air bubble to expand and the diver to ascend. This illustrates Archimedes’s principle, which states that the buoyant force acting on an object immersed in water is equal to the weight of the water that the object displaces. If the weight of the displaced water is less than the weight of the object, it will sink. When the bottle is squeezed, the air bubble shrinks as water is forced into the open end of the diver, and the decrease in weight of the displaced water causes the diver to sink. The Cartesian diver can also be used to demonstrate Boyle’s law of 1662 (the relationship between pressure and volume), Pascal’s law of 1663 (pressure transmission through fluid), and the ideal gas law of 1834 (the state of a gas is determined by its pressure, volume, and temperature, or PV = nRT). The dates refer to the years of publication. The uncredited gas law was derived by Benoît Clapeyron, who combined Boyle’s law with other physical principles.