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THE DISCOVERY OF MICROORGANISMS BY ROBERT HOOKE AND ANTONI VAN LEEUWENHOEK, FELLOWS OF THE ROYAL SOCIETY

by

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By the means of Telescopes, there is nothing so far distant but may be represented to our view; and by the help of Microscopes, there is nothing so small as to escape our inquiry; hence there is a new visible World discovered to the understanding. By this means the Heavens are open'd and a vast number of new Stars and new Motions, and new Productions appear in them, to which all the ancient Astronomers were utterly strangers. By this the Earth it self, which lyes so neer to us, under our feet, shews quite a new thing to us, and in every little particle of its matter, we now behold almost as great a variety of Creatures, as we were able before to reckon up in the whole Universe itself.

Robert Hooke, 1665 (in the Preface of *Micrographia*)

SUMMARY

The existence of microscopic organisms was discovered during the period 1665–83 by two Fellows of The Royal Society, Robert Hooke and Antoni van Leeuwenhoek. In *Micrographia* (1665), Hooke presented the first published depiction of a microrganism, the microfungus *Mucor*. Later, Leeuwenhoek observed and described microscopic protozoa and bacteria. These important revelations were made possible by the ingenuity of Hooke and Leeuwenhoek in fabricating and using simple microscopes that magnified objects from about 25-fold to 250-fold. After a lapse of more than 150 years, microscopy became the backbone of our understanding of the roles of microbes in the causation of infectious diseases and the recycling of chemical elements in the biosphere.

Keywords: Robert Hooke; Antoni van Leeuwenhoek; Fellows of The Royal Society; *Micrographia*; microscope

INTRODUCTION

Microorganisms were first discovered by two remarkable geniuses, Robert Hooke and Antoni van Leeuwenhoek, between 1665 and *ca*. 1678. They came from very different backgrounds and their life histories as scientists converged through a complex series of events. Serendipity was at work, and the possibility of their interactions could not have been predicted or imagined. The common element that led to their discoveries of microorganisms was the making and use of microscopes, which they approached in different ways. Historical accounts frequently describe Leeuwenhoek as the 'first of the microbe hunters', and often cite his famous letters of 9 October, 1676[1] as giving the first unmistakable observations of bacteria. Generally overlooked is the earlier discovery of microscopic fungi by Robert Hooke in 1665. Hooke also significantly advanced microscopy, the backbone of microbiology, and was the first to confirm observations of Leeuwenhoek that were considered to be dubious by many contemporaries. Reexamination of the records and publications of The Royal Society from 1665 to 1678 show that Hooke and Leeuwenhoek both were the major discoverers of the microbial universe.

Hooke's career

Robert Hooke (1635–1702) became an 'undergraduate member' of Oxford's Christ Church College in 1653 but apparently never took a bachelor's degree. He became associated with a brilliant group of scholars (including Christopher Wren and Robert Boyle) that met regularly to discuss scientific problems. In due course, this group became the original nucleus of The Royal Society. Many details of Hooke's unusual life and career are documented in Birch's *History of The Royal Society*[2] and other accounts.[3]

Hooke was recognized for his outstanding abilities in 'mechanics', which had been evident at a very young age. His many talents resulted in appointment as Curator of Experiments of The Royal Society (1662–77). Hooke's duties as Curator were to conduct 'considerable' experiments at the Society's weekly meetings, and to do research officially recommended to him. Hooke became a commanding intellectual presence in the Society, and as Curator provided the main substance of many meetings.

The range of problems addressed by Hooke was formidable, and his achievements were very remarkable. Westfall[4] summarized Hooke's contributions to the field of instrumentation as follows:

> He added something to every important instrument developed in the seventeenth century. He invented the air pump in its enduring form. He advanced horology and microscopy. He developed the cross-hair sight for the telescope, the iris diaphragm, and a screw adjustment from which the setting could be read directly. He has been called the founder of scientific meteorology. He invented the wheel barometer, on which the pivoted needle registers the pressure. He suggested the freezing temperature of water as the zero point on the thermometer and devised an instrument to calibrate thermometers. His weather clock recorded barometric pressure, temperature, rainfall, humidity, and wind velocity on a rotating drum. Although it was not a scientific instrument, the universal joint was also his invention. Writing in the eighteenth century, Lalande called Hooke 'the Newton of mechanics'.

Several of Hooke's clever instrumentation inventions are described by Hunter and Schaffer[3] and Bennett *et al.*[3] Hooke's great imagination was not restricted to practical matters such as instrumentation. He developed theories on the nature of light and on universal gravitation. The latter led him into a long-standing controversy with Isaac Newton (1642–1727). Hooke believed that his idea of an inverse square law of gravitational attraction in orbital motion was plagiarized by Newton, and they remained enemies throughout Hooke's later years. According to Westfall,[4,5] Hooke did not discover the law of universal gravitation, 'But he did set Newton on the correct approach to orbital dynamics and, in this way, contributed immensely to Newton's later triumph.' In the *Principia*, Newton did make an acknowledgement of his debt to Hooke.[6]

Renowned X-ray crystallographer J. D. Bernal[7] notes that Hooke managed to supplement his meagre and irregular salary from The Royal Society by being largely responsible for the plans of the new City of London after the Great Fire of 1666. Bernal's summary of the scientific contributions of the 'Leonardo of London' gives a comprehensive insight into Hooke's many-sided genius:

> He [Hooke] was the greatest experimental physicist before Faraday, and, like him, lacked the mathematical ability of Newton and Maxwell. His interests ranged over the whole of mechanics, physics, chemistry, and biology. He studied elasticity and discovered what is known as Hooke's Law, the shortest in physics: *ut tensio sic vis* (extension is proportional to force); he invented the balance wheel, the use of which made possible accurate watches and chronometers; he wrote *Micrographia*, the first systematic account of the microscopic world, including the discovery of cells; he introduced the telescope into astronomic measurement and invented the micrometer; and he shares with Papin the credit of preparing the way to the steam-engine.
>
> Probably his greatest contribution to science is only now beginning to be recognized: his claim to have originated the idea of the inverse square law and universal gravity. Here, as we shall see, he was outclassed by the superb mathematical achievement of Newton, but it now seems that the basic physical ideas were Hooke's and that he was quite unjustly robbed of the credit for them. Hooke's life illustrates both the opportunities and the difficulties that the gifted experimenter could find in the seventeenth century. It also brings out the enormous store of inventiveness and scientific insight that had lain concealed for thousands of years in the brains and hands of natural craftsmen.

Hooke's *Micrographia* (1665)

Weak lenses for reading purposes and spectacle glasses had been in use since the thirteenth century. However, microscopes useful for research purposes were not available until the seventeenth century. The first illustrated book on microscopy was *Micrographia*,[8] published by Robert Hooke in 1665. This masterpiece of the seventeenth century created a sensation. Samuel Pepys bought a copy immediately and wrote in his diary, 'Before I went to bed, I sat up till 2 a-clock in my chamber, reading of Mr. Hookes Microscopical Observations, the most ingenious book that I ever read in my life.'

Micrographia contained detailed accounts of 60 'observations', mostly of objects examined microscopically. For these studies, Hooke usually used a microscope, about six inches long, that had two convex lenses. In the main, the specimens were biological (including sponges, wood, seaweed, leaf surfaces, hair, peacock feathers, wings of flies, eggs of silkworms, mites, a flea and a louse) even though Hooke's primary interests were in mechanics and the physical sciences. The printed illustrations of microscopic views in *Micrographia* were prepared from engravings based on Hooke's superb drawings, which attest to his acute powers of observation and his skill as a draughtsman. This aspect of Hooke's work is elaborated in an essay on 'Rhetoric and graphics in *Micrographia*' by J. T. Harwood.[9] Harwood notes that '*Micrographia* ensured Hooke's place in the history of science, but his rhetorical achievement in creating and diffusing an image of The Royal Society deserves recognition.… Through skillfully integrating text and graphics, Hooke persuasively explained the methods and achievements of The Royal Society and, more broadly, the New Philosophy.'

Hooke's description of a microscopic fungus

At the Royal Society meeting of April 23, 1663, 'Mr. Hooke brought in two microscopical observations, one of leeches in vinegar, the other of a bluish mould upon a mouldy piece of leather'. In *Micrographia*, Hooke described the microscopic structure of a white 'mould spot' in great detail.

Part of Hooke's description of microfungi follows:

Observ. XX. Of blue Mould, and of the first Principles of Vegetation arising from Putrefaction.

The Blue and White and several kinds of hairy mouldy spots, which are observable upon divers kinds of putrify'd bodies, whether Animal substances, or Vegetable, such as the skin, raw or dress'd, flesh, bloud, humours, milk, green Cheese, etc. or rotten sappy Wood, or Herbs, Leaves, Barks, Roots, etc. of Plants, are all of them nothing else but several kinds of small and variously figur'd Mushroms, which, from convenient materials in those putrifying bodies, are, by the concurrent heat of the Air, excited to a certain kind of vegetation, which will not be unworthy of our more serious speculation and examination. As I shall by and by shew.

But, first, I must premise a short description of this Specimen, which I have added of this Tribe [see figure 1] which is nothing else but the appearance of a small white spot of hairy mould, multitudes of which I found to bespeck & whiten over the red covers of a small book, which, it seems, were of Sheeps-skin, that being more apt to gather mould, even in a dry and clean room, then other leathers. These spots appear'd, through a good Microscope, to be a very pretty shap'd Vegetative body, which, from almost the same part of the Leather, shot out multitudes of small long cylindrical and transparent stalks, not exactly streight, but a little bended with the weight of a round and white knob that grew on the top of each of them; many of these knobs I observ'd to be very round and of a smooth surface, such as A A, etc. others smooth likewise, but a little oblong, as B.; several of them a little broken, or cloven with chops at the top, as C; others flitter'd as 'twere, or flown all to pieces, as DD. The whole substance of these pretty bodies was of a very tender constitution, much like the substance of the softer kind of common white Mushroms, for by touching them with a Pin, I found them to be brused and torn; they seem'd each of them to have a distinct root of their own; for though they grew neer together in a cluster, yet I could perceive each stem to rise out of a distinct part or pore of the Leather; some of these were small and short, as seeming to have been but newly sprung up, of these the balls were for the most part round, others were bigger and taller, as being perhaps of a longer growth, and of these, for the most part, the heads were broken, and some much wasted, as E; what these heads contain'd I could not perceive; whether they were knobs and flowers, or seed cases, I am not able to say, but they seem'd most likely to be of the same nature with those that grow on Mushroms, which they did, some of them, not a little resemble.…

There are a multitude of other shapes, of which these Microscopical Mushroms are figur'd, which would have been a long Work to have described, and would not have suited so well with my design in this Treatise, onely, amongst the rest, I must not forget to take notice of one that was a little like to, or resembled a Spunge, consisting of a multitude of little Ramifications almost as that body does, which indeed seems to be a kind of Water-Mushrom, of a very pretty texture, as I else where manifest. As a second, which I must not omit, because often mingled, and neer adjoining to these I have describ'd, and this appear'd much like a Thicket of bushes, or brambles, very much branch'd and extended, some of them, to a great length, in proportion to their Diameter, like creeping brambles.

At this point, Hooke tries to understand how the moulds (and 'Mushroms') reproduce in view of the fact that he could not observe any 'seed'. Thus, he believed that they may 'be generated without seed'. Expressed in another way: '… they rather seem to depend merely upon a convenient constitution of the matter out of which they are made, and a concurrence of either natural or artificial heat'. This is another way of saying 'sponta-

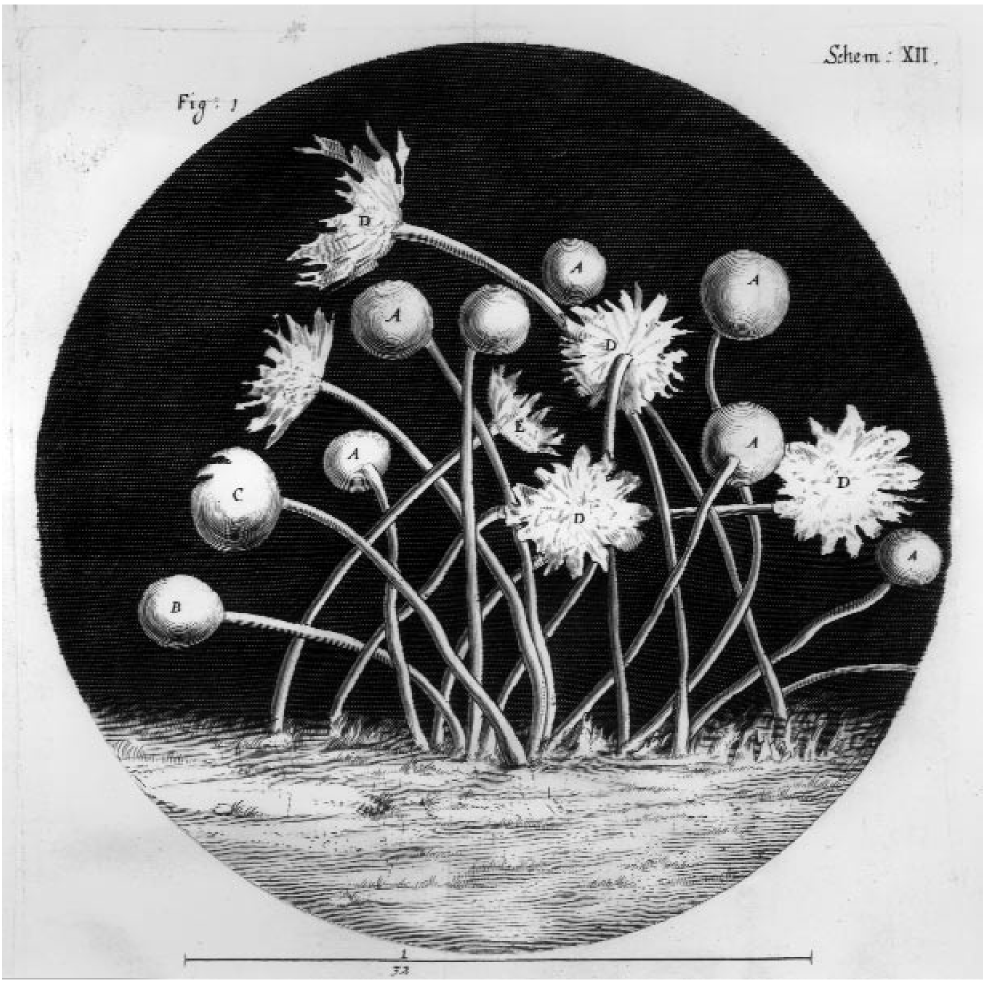

Figure 1. Microscopic view of a 'hairy mould' colony described by Robert Hooke in 1665 (in *Micrographia*). This image was the first published depiction of a microorganism. The reproductive structures (sporangia) are characteristic of the microfungus *Mucor*. Sporangia in different stages are identified by the letters A, B, C, and D. Hooke included a scale reference; the length of the bar under the diagram represents 1/32 inch. (Reproduced courtesy of The Lilly Library, Indiana University, Bloomington, Indiana.)

neous generation', which was a popular concept at the time (and not decisively disproven as a phenomenon until the mid-nineteenth century, by Pasteur).

Identity of Hooke's mould

In *Micrographia*, Hooke provided the first illustrations of microfungi (Scheme XII, Fig. 1). From the diagram and the detailed description, microbiologists[10] have concluded that his specimen can be identified as the widely distributed microfungus *Mucor*. There are many species of *Mucor*, frequently referred to as 'bread moulds', which occur commonly in soil, in manure, and on fruits and starchy foodstuffs. *Mucor* produces reproductive structures (sporangia) that appear as 'knobs' on the tops of slender transparent 'stalks'. When the sporangia burst to liberate sporangiospores, the knobs shatter giving rise to flower-like remains, as depicted by Hooke.

Hooke thought the 'knobs' might be 'seed cases', and attempted to release and observe the 'seeds' (sporangiospores), but failed because they were too small to be seen at the magnification he used. In any event, it was a sensible experiment on the part of someone primarily thinking as a physicist.

Antoni van Leeuwenhoek (1632–1723), self-educated scientific explorer

In 1654, at the age of 22 years, Leeuwenhoek set up a shop as a draper in Delft, Holland. He remained in Delft during his long life and at times held minor municipal offices (for example, Chamberlain of the Council Chamber, Official Wine Gauger). It has been suggested that the starting point of Leeuwenhoek's unique scientific career was probably the use of a low-power magnifying glass to inspect the quality of cloth. He developed the ability to make very small lenses—only about 1 mm in diameter—of excellent quality. The lens was embedded in a small metal sheet (usually about 1 inch × 2 inches) fitted with adjustable screws. The sample to be examined was ordinarily contained in a very thin glass tube positioned on a screw close to the lens. When held close to the eye, and focused by adjusting the screws, these simple microscopes revealed to Leeuwenhoek clear images of very small objects, magnified sometimes as much as 250 times or more.

Leeuwenhoek was a keen observer and had extraordinary curiosity about the living world, and with his simple single-lens microscopes he made many important discoveries. These were described in numerous letters (about 190) that were sent to The Royal Society of London. Eventually he was awarded the distinction of election as a Fellow. This remarkable shopkeeper from Delft, who had little formal education, described for the first time the sperm cells of animals, including humans, and he was also the first to recognize that in the fertilization process, the sperm enters the egg cell. He provided the first accurate description of red blood cells. At a time when it was widely thought that maggots, fleas and the like were formed by 'spontaneous generation', Leeuwenhoek showed that such creatures hatch from eggs.

Leeuwenhoek's first letter to The Royal Society (28 April 1673) was submitted by the Dutch anatomist Regnerus de Graff, a corresponding member of the Society ['A specimen of some Observations made by a Microscope, contrived by M. Leewenhoeck in Holland, lately communicated by Dr. Regnerus de Graaf']. The letter was very brief and reveals that Leeuwenhoek must have had access to *Micrographia*, published eight years earlier. This is indicated by his descriptions of 'mould' (presumably *Mucor*) and of a 'Lowse':

> The Mould upon skin, flesh, or other things, hath been *by some represented* [italics added] to be shott out in the form of the stalks of Vegetables, so that some of those stalks appeared with round knobs at the end, some with blossom-like leaves. But I do observe such Mould to shoot up first with a straight transparent stalk, in which the stalk is driven up by a globous substance, which for the most part places it self at the top of the stalk, and is follow'd by another globul, driving out

the first either side-ways, or at the top, and that is succeeded by a third and more such globuls; all of which make up at last one great knob on the stalk.

'*By some represented*': according to the Dutch scientists who edited *Leeuwenhoek's Letters 1673–96*,[11] this is 'in all probability' a reference to Hooke's description of *Mucor* in *Micrographia* (see footnote 1, vol. 1, p. 31).

In his description of a 'Lowse', Leeuwenhoek says: 'In a Lowse I observe indeed, *as others have done*, a short tapering Nose with an hole in it.… In the two Horns I find five Joints; *others* having marked but four.' In *Micrographia*, Hooke says: '… the head seems very round and tapering, ending in a very sharp nose which seems to have a small hole'. And Hooke specifies four joints on the Horns. '*As others have done*': In this regard, the Dutch scientists say: 'In all probability, there is a reference here to R. Hooke, who pictures the louse with antennae consisting of four articulations and also describes them (*Micrographia* 1665, Obs. 54). It is quite possible that Hooke saw an intermediate stage between the larva with three articulations and the full grown louse with five.' (see note 10, footnote 6, p. 33, vol. 1). An alternative explanation of the difference is that Hooke and Leeuwenhoek happened to examine different species.

J. Heniger[12] has provided a valuable precis of Leeuwenhoek's life and scientific career, especially in that he interprets his manifold observations and conclusions in the light of knowledge of the time:

> … he [Leeuwenhoek] was limited by his lack of skill in classical and foreign languages. He was, however, able to rely on such friends as de Graaf and Constantijn Huygens (as well as upon professional translators) to aid him. He derived much of his scientific knowledge from Dutch authors—for example, Cornelis Bontekoe on medicine and Swammerdam on insects—*and from Dutch translations of standard works, or, indeed from the illustrations of books that he was not otherwise able to read* (*Hooke on microscopy*, Grew on plant anatomy, and Redi on insects) [italics added]. He gained new information, too, from correspondence with The Royal Society (which had to be translated) and from conversations with visiting scientists.

Hooke initially needed help in translating Leeuwenhoek's letters, but he eventually learned Dutch so that he could read the letters of the 'ingenious Mr. Leeuwenhoeck.'

There is little doubt that Leeuwenhoek had access to information in *Micrographia* despite what he wrote to Secretary Oldenburg of The Royal Society on 22 January 1676:[13]

> Your letter of the 28th was received by me in good order, from which I learned that you do not doubt my knowledge of French, but I must tell you that I regret I do not understand any language but Dutch and when you write to me in French or Latin I can help myself all right, since I have enough friends who will translate it for me; but I cannot help myself with the English language since the death of a certain gentleman who was proficient in this language.

However, the Dutch editors of 'Leeuwenhoek's letters'[11] point out that although the English communities in Delft decreased in number as of *ca*. 1683, even by 1724 'the few remaining members understood Dutch so well that there was no further need of an English clergyman' (note 11, footnote 1, vol. 1, p. 343).

Discovery of bacteria

One of Leeuwenhoek's greatest contributions to biology, the discovery of bacteria, resulted from a serendipitous event that began with his interest in the sense of taste. In a letter of 19 October 1674[14] he wrote: 'Last winter while being sickly and nearly unable to taste, I examined the appearance of my tongue, which was very furred, in a mirror, and judged that my loss of taste was caused by the thick skin on the tongue.' This led him to examine 'little points' on an ox tongue with his microscope, and he saw that these had 'very fine pointed projections' which were composed of 'very small globules'. Obviously, he was observing the taste buds and he was particularly curious to know why pepper and other spices (ginger, nutmeg and cloves) had such potent tastes. This led him to microscopic study of 'infusions' made by soaking or pounding the spices in water.

On 24 April 1676 Leeuwenhoek scrutinized some 'pepper water' that had been sitting for three weeks and was astonished to see many very small organisms that he called 'animalcules' (or 'little eels'). They were actually bacteria, which typically are only about 1–2 μm in diameter. Leeuwenhoek immediately looked for the 'animalcules' in other places such as in the white matter that he found stuck to his teeth: 'I have mixed it with clean rain water, in which there were no 'animalcules', and I almost always saw with great wonder that there were many very little animalcules, very prettily amoving.'

Leeuwenhoek's often cited letters of 9 October 1676[1] are of special importance. In them, Leeuwenhoek refers to the potent taste of pepper as follows:

> Having several times endeavoured to discover the cause of the pungency of Pepper upon our tongue, and that the rather, because it hath been found, that though Pepper had lain a whole year in vinegar, yet it retained still its pungency; I did put about 1/3 of an ounce of whole pepper in water, placing it in my Study, with the design, that the pepper being thereby rendred soft, I might be enabled the better to observe what I proposed to my self. This pepper having lain about 3 weeks in the water, to which I had twice added some Snowwater, the other water being in part exhaled; I looked upon it the 24. of April 1676. and discern'd in it, to my great wonder, an incredible number of very little animals of divers kinds…

He goes on to describe several sorts of 'creatures' [probably mainly protozoa] and then: 'The 4th sort of creatures, which moved through the 3 former sorts, were incredibly small, and so small in my eye, that I judged, that if 100 of them lay [stretched out] one by another, they would not equal the length of a grain of course Sand; and according to this estimate, ten hundred thousand of them could not equal the dimensions of a grain of such course Sand.' Leeuwenhoek often made estimates of the sizes of 'animalcules' by comparison with grains of sand, hairs, or 'the diameter of a louse's eye'. Some microbiologists consider the April 1676 date to be the first unmistakable observation of bacteria (see footnote 61 of note 15) but this is not certain because of overlapping descriptions and observations in the numerous letters of Leeuwenhoek. Bulloch[16] notes that if there is any doubt about the 1676 observations, there can be none about the unequivocal description of bacteria that he [Leeuwenhoek] found on his own teeth in 1683.[17]

Confirmation of Leeuwenhoek's observations

Leeuwenhoek's observations were confirmed by Hooke.[18] in 'A Letter of the Ingenious and Inquisitive Mr. Leeuwenhoeck of Delft, sent to the Secretary of The Royal Society [namely, Hooke], 5 October 1677':

> In this Letter after the Relation of many curious Observations made with his Microscope, he [that is, Leeuwenhoek] adds, 'By some of my former Letters I have related what an innumerable company of little Animalcules, I have discovered in waters; of the truth of which affirmations that I might satisfie the Illustrious Philosophers of your Society, I have here sent the Testimonials of eight credible persons; some of which affirm they have seen 10000, others 30000, others 45000 little living creatures, in a quantity of water as big as a grain of Millet (92 of which go to the making up the bigness of a green Pea, or the quantity of a natural drop of water) in the desiring of which Testimonials I made it my request that they would only justifie (that they might be within compass) half the number that they believed each of them saw in the water, and even so the number of those little creatures that would thereby be proved to be in one drop of water would be so great, that it would exceed belief. Now whereas by my Letter of 9th of October, 1676. I affirmed that that there were more than 1000000 living Creatures contained in one drop of Pepper-water. I should not have varied from the truth of it, if I had asserted that there were 8000000; for if according to some of the included testimonials there might be found in a quantity of water as big as a millet seed, no less than 45000 animalcules. It would follow that in an ordinary drop of this water there would be no less than 4140000 living creatures, which number if doubled will make 8280000 living Creatures seen in the quantity of one drop of water, which quantity I can with truth affirm I have discerned.
>
> This exceeds belief. But I do affirm, that if a larger grain of sand were broken into 8000000 of equal parts, one of these would not exceed the bigness of one of those little creatures; which being understood, it will not seem so incredible to believe that there may be so great a number in the quantity of one drop of water.'
>
> [Hooke continues:] Upon the perusal of this Letter, being extremely desirous to examine this matter farther, and to be ascertained by ocular inspection as well as from testimonials, I put in order such remainders as I had of my former Microscopes (having by reason of a weakness in my sight omitted the use of them for many years) and steeped some black pepper in River water, but examining that water about two or three days after, I could not by any means discover any of those little creatures mentioned in the aforesaid Letter: though I had made use of small glass canes [that is, tubes] drawn hollow for that purpose, and of a microscope that I was certain would discover things much smaller than such as the aforesaid Mr. Leeuwenhoeck had affirmed these creatures to be; but whether it were that the light was not convenient (the reason of which I shall shew by and by) having looked only against the clear sky, or that they were not yet generated, which I rather suppose I could not discover any. I concluded therefore that either that my Microscope was not so good as that he made use of, or that the time of the year (which was in November) was not so fit for such generations, or else that there might be somewhat ascribed to the difference of places; as that Holland might be more proper for the production of such little creatures than England. I omitted therefore farther to look after them, for about five or six days, when finding it a warm day, I examined again the said water; *and then much to wonder I discovered vast multitudes of those exceeding small creatures, which Mr. Leeuwenhoeck had described* [italics added]; and upon making use of other lights and glasses, as I shall by and by shew, I not only magnified those I had thus discovered to a very great bigness, but I discovered many other sorts very much smaller than those I first saw, and some of these so exceeding small, that millions of millions might be contained in one drop of water. I was very much surprized at this so wonderful a spectacle, having never seen any living creature comparable to these for smallness: nor could I indeed imagine that nature had afforded instances of so exceedingly minute animal productions. But nature is not to be limited by our narrow apprehensions; future improvements of glasses may yet further enlighten our understanding, and ocular inspection may demonstrate that which as yet we may think too extravagant either to feign or suppose.

Of this, A later Discovery of Mr. Leeuwenhoeck does seem to give good probabilities; for by a Letter of his since sent (and which is hereunto annexed) it appears he hath discovered certain sort of Eels in Pepper-water, which are not in breadth above one thousandth part of the breadth of a hair; and not above a hundredth part of the length of a vinegar Eel.

Mr Leeuwenhoeck's second letter

Several pages of this letter deal with descriptions of 'Globules' in blood and 'Flegm' followed by more observations of the very small creatures in Pepper-water. Hooke then comments:

> The manner how the said Mr. Leeuwenhoeck doth make these discoveries, he doth as yet not think fit to impart, for reasons best known to himself; and therefore I am not able to acquaint you with what it is; but as to the ways I have made use of, I here freely discover that all such persons as have a desire to make any enqueries into Nature this way, may be the better inabled so to do.
>
> First, for the manner of holding the liquor, so as to examine it by the Microscope, I find that the way prescribed by Mr. Leeuwenhoeck is to include the same in a very fine pipe [that is, thin tube] of glass.…

Hooke says this is 'exceedingly ingenious', but then describes alternatives that he prefers. Using his own methods, he states: 'I examined water in which I had steeped the pepper I formerly mentioned; and as if I had been looking upon a Sea, I saw infinite of small living Creatures swimming and playing up and down in it, a thing indeed very wonderful to behold.'

The remainder of this 1678 communication[18] consists of a lengthy and very detailed account of procedures that Hooke devised for the microscopic examination of biological and other kinds of specimens. After Hooke's term as Curator ended, he was appointed Secretary of the Society (1677–82) and in this capacity he became one of the conduits for Leeuwenhoek's letters, including the important letter describing bacteria.

Microscopes used by Hooke and Leeuwenhoek

In the Preface to *Micrographia*, Hooke discussed the details of

> Microscopes and some other Glasses and Instruments that improve the sense … to promote the use of Mechanical helps for the Senses, both in the surveying the already visible World, and for the discovery of many others hitherto unknown, and to make us, with the great Conqueror, to be affected that we have not yet overcome one World when there are so many others to be discovered, every considerable improvement of Telescopes or Microscopes producing new Worlds and Terra-Incognita to our view.

Hooke produced various kinds of microscope but preferred using a relatively large instrument with two lenses, and he examined specimens under reflected light. It is particularly significant that in the Preface he also described 'simple microscopes' with only a single small lens for the examination of specimens with directly transmitted light. He noted that these simpler instruments have the advantage of giving a clearer image. To make such microscopes he first melted 'Venice Glass' and made threads that consisted of several small round globules ('or drops'). Then,

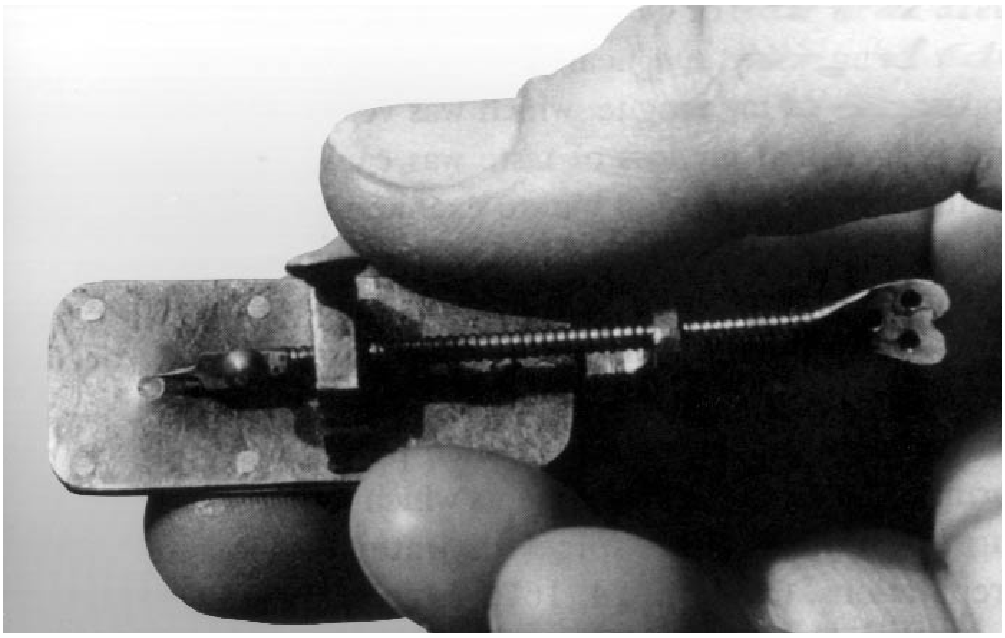

Figure 2. A replica of one of Leeuwenhoek's microscopes. The object to be viewed was placed on the pointed tip at the end of the screw. The single lens is in the small circle just to the left of the screw tip. (From H. Gest, *Microbes: an invisible universe*, American Society for Microbiology Press, Washington, DC (2003).)

… if further you stick several of these upon the end of a stick with a little sealing Wax, so that the threads stand upwards, and then on a Whetstone first grind off a good part of them, and afterward on a smooth Metal plate, with a little Tripoly, rub them till they come to be very smooth; if one of these be fixt with a little soft Wax against a small needle hole, prick'd through a thin Plate of Brass, Lead, Pewter, or any other Metal, and an Object, plac'd very near, be look'd at through it, it will both magnifie and make some Objects more distinct than any of the great Microscopes. But because these, though exceeding easily made, are yet very troublesome to be us'd, because of their smallness, and the nearness of the Object; therfore to prevent both these, and yet have only two Refractions, I provided me a Tube of Brass etc. Etc.

Hooke's concise description of the single-lens microscope is, in fact, an exact 'prescription' for making what was later commonly called a 'Leeuwenhoek microscope' (see figure 2). Leeuwenhoek was notoriously secretive about how he polished his lenses and never divulged his methods. Some of the details became known only indirectly in 1685 (see Appendix). In contrast, Hooke published detailed descriptions of his improvements in making and using microscopes in 1685 (*Micrographia*) and again in 1678 (*Microscopium*). His various innovations are noted in an interesting monograph on microscopy by Rooseboom.[19]

In 1678, Hooke[18] commented again on his aversion to the single-lens simple microscope:

I have found the use of them offensive to my eye, and to have much strained the sight, which was the reason why I omitted to make use of them, though in truth they make the object appear much more clear and distinct, and magnifie as much as the double Microscopes: nay to those whose eyes can well endure it, 'tis possible with a single Microscope to make discoveries much better than with a double one, because the colours which do much disturb the clear vision in double Microscopes is clearly avoided and prevented in the single.

This statement is followed by description of new methods that Hooke devised for producing single-lens microscopes that are 'exceeding easie to make'. The historian Daniel Boorstin[20] summarized Hooke's contributions to microscopy as follows:

> What Galileo's *Sidereus Nuncius* had done for the telescope and its heavenly vistas, Hooke's *Micrographia* now did for the microscope. Just as Galileo did not invent the telescope, neither did Hooke invent the microscope. But what he described seeing in his compound microscope awakened learned Europe to the wonderful world within. Fifty-seven amazing illustrations drawn by Hooke himself revealed for the first time the eye of a fly, the shape of a bee's stinging organ, the anatomy of a flea and a louse, the structure of feathers, and the plantlike form of molds. When he discovered the honeycomb structure of cork, he said it was made of 'cells.' Frequently reprinted, Hooke's illustrations remained in textbooks into the nineteenth century.

First observation of anaerobic bacteria

The conventional wisdom is that the first observation of microbial life in the absence of oxygen gas was made by Pasteur. In fact, Pasteur rediscovered the anaerobic lifestyle. The first person to observe anaerobic microorganisms was Leeuwenhoek, who did a remarkable experiment, detailed in his letter of 14 June 1680 to The Royal Society.[21] He filled two identical glass tubes about halfway with crushed pepper powder, to which some clean rain water was added. Using a flame, he sealed one of the tubes, whereas the other was left open. After several days, in the water of the open tube 'I discovered in it a great many very little animalcules, of divers sort having its own particular motion.' After five days, he opened the sealed tube in which some pressure had developed, forcing liquid out. He expected not to see 'living creatures in this water.' But, in fact, he observed 'a kind of living animalcules that were round and bigger than the biggest sort that I have said were in the other water.' Clearly, in the sealed tube, the conditions had become quite anaerobic owing to consumption of oxygen by aerobic microorganisms.

In 1913 the great Dutch microbiologist Martinus Beijerinck repeated Leeuwenhoek's experiment exactly and identified *Clostridium butyricum* as a prominent anaerobic bacterium in the sealed pepper infusion tube liquid. Beijerinck commented:[22]

> We thus come to the remarkable conclusion that, beyond doubt, Leeuwenhoek in his experiment with the fully closed tube had cultivated and seen genuine anaerobic bacteria, which would happen again only after 200 years, namely about 1862 by Pasteur. That Leeuwenhoek, one hundred years before the discovery of oxygen and the composition of air, was not aware of the meaning of his observations is understandable. But the fact that in the closed tube he observed an increased gas pressure caused by fermentative bacteria and in addition saw the bacteria, prove in any case that he not only was a good observer, but also was able to design an experiment from which a conclusion could be drawn.

Summing up

The most well-known source book on Leeuwenhoek's life and career is *Antony van Leeuwenhoek and his 'little animals'*[23] by Clifford Dobell FRS (1886–1949). This scholarly work, on which protozoologist Dobell spent many years of research, stresses Leeuwenhoek's observations of protozoa and bacteria, but takes a limited view of the scope of microbial life. Dobell omitted any mention of Hooke's discovery of microfungi and inexplicably dismissed Hooke's great contributions with the following footnote:

> Dr. Robert Hooke (1635–1703), an original Fellow of the Royal Society, was also an original and eccentric genius and inventor. His contributions to science are too well-known and numerous to mention; though his influence on his contemporaries and the part he played in the early days of the Society, are only just beginning to receive their due recognition …. *It is impossible and unnecessary to discuss this remarkable man and his work here* [italics added].

In Dobell's book there are 34 citations to Hooke in the extensive index; they are all trivial, mainly noting dates of various letters sent to Hooke. The Index does not cite *Micrographia*, which is simply listed among 25 pages of 'Other references and sources'.

The rarely cited important books of microscopist Brian Ford[24,25] reviewed Leeuwenhoek's career and discoveries in the context of the history of the microscope. On the basis of his careful study of the literature, Ford criticized several of Dobell's conclusions and believes, as I do, that Hooke's *Micrographia* was a major 'trigger for his [Leeuwenhoek's] own life-time of study and experiment'.

Milton Wainwright[26] recently pointed out that standard accounts of the early history of microbiology usually begin with the 'first sighting' of microorganisms by Leeuwenhoek. He goes on to say: 'Unfortunately, much of what is taught about the history of microbiology has been oversimplified to the point where plain untruths are being told; at best a fascinating and convoluted story has been reduced to the minimum, for easy, uncritical consumption.' On 2 October 2003 the author attended the Robert Hooke Tercentenary Commemoration Symposium at the University of Oxford. Notable speakers reviewed Hooke's numerous diverse achievements, and the programme announcement displayed Hooke's first image of a microorganism (*Mucor*) recorded in the scientific literature (figure 1, from *Micrographia*, 1665). All things considered, the major 'fathers' of microbiology in the scientific Renaissance of the seventeenth century are clearly identifiable as Robert Hooke and Antony van Leeuwenhoek.

Appendix: A description of Leeuwenhoek's microscope in 1685

A young Irish doctor, Thomas Molyneux (1661–1733), visited Leeuwenhoek on behalf of The Royal Society in 1685 and sent the following letter (to Secretary Mr Aston), which was read at a Society meeting.[27]

> I have hitherto delayed answering your last, because I could not give you an account of Mynheer Leeuwenhoeck; but last week I was to wait upon him in your name; he shewed me several things through his microscopes, which 'tis in vain to mention here, since he himself has sent you all their descriptions at large. As to his microscopes themselves, those, which he shewed me , in number at least a dozen, were all of one sort, consisting only of one small glass, ground, (this I mention

because ’tis generally thought his microscopes are blown at a lamp, those I saw, I am sure, are not) placed between two thin flat plates of brass, about an inch broad, and an inch an a half long. In these two plates there were two apertures, one before, the other behind the glass, which were larger or smaller, as the glass was more or less convex, or as it magnified. Just opposite to these apertures on one side was placed sometimes a needle, sometimes a slender flat body of glass or opaque matter, as the occasion required, upon which, or to its apex, he fixes whatever object he has to look upon; then holding it up against the light, by help of two small screws, he places it just in the focus of his glass, and then makes his observations. Such were the microscopes, which I saw, and these are they that he shews to the curious that come and visit him: but besides these, he told me that he had another sort, which no man living had looked through setting aside himself; these he reserves for his own private observations wholly, and he assured me they performed far beyond any, that he had shewed me yet, but would not allow me a sight of them, so all I can do is barely to believe, for I can plead no experience in the matter. As for the microscopes I looked through, they do not magnify much, if any thing, more than several glasses I have seen, both in England, and Ireland: but in one particular, I must needs say, they far surpass them all, that is in their extreme clearness, and their representing all objects so extraordinary distinctly. For I remember we were in a dark room with only one window, and the sun too was then off of that, yet the objects appeared more fair and clear, than any I have seen through microscopes, though the sun shone full upon them, or though they received more than ordinary light by help of reflective specula or otherwise: So that I imagine ’tis chiefly, if not alone in this particular, that his glasses exceeds all others, which generally the more they magnify, the more obscure they represent the object; and his only secret, I believe, is making clearer glasses, and giving them a better polish than others can do. I found him a very civil complaisant man, and doubtless of great natural abilities; but, contrary to my expectations, quite a stranger to letters, master neither of Latin, French or English, or any other of the modern tongues besides his own, which is a great hinderance to him in his reasonings upon his observations; for being ignorant of all other mens thoughts, he is wholly trusting to his own, which, I observe, now and then lead him to extravagancies, and suggest very odd accounts of things, nay, sometimes such, as are wholly irreconcilable with all truth. You see, Sir, how freely I give you my thoughts of him, because you desire it.

Notes

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4 R. S. Westfall, ‘Robert Hooke’, in *Dictionary of scientific biography*, vol. VI (ed. J. Hachette and J. Hyrtl), pp. 481–488 (Scribner’s Sons, New York, 1972).

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7 J. D. Bernal, *Science in history* (Hawthorn Books, New York, 1965).

8 R. Hooke, *Micrographia: or some Physiological Descriptions of Minute Bodies made by Magnifying Glasses with Observations and Inquiries thereupon* (Jo. Martyn and Ja. Allestry, Printers to the Royal Society, London, 1665).

9 J. T. Harwood, in Hunter and Schaffer, *op. cit.* (note 3), pp. 119–147.

10 G. C. Ainsworth, *Introduction to the history of mycology* (Cambridge University Press, 1976).

11 A. v. Leeuwenhoek, *Alle de Briefen van Antoni van Leeuwenhoek/The Collected Letters of Antoni van Leeuwenhoek 1673–1696* (eleven volumes), edited, illustrated, and annotated by committees of Dutch scientists (Swets and Zeitlinger, Amsterdam, published intermittently between 1939 and 1983).
12 J. Heniger, 'Antoni van Leeuwenhoek', in *Dictionary of scientific biography*, vol. VIII (ed. J. H. Lane and P. J. Macquer), pp. 126–130 (Scribner's Sons, New York, 1973).
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14 A. v. Leeuwenhoek, RS Manuscript letter no. 1836.
15 A. v. Leeuwenhoek, *1674, 1676, The discovery of unicellular life.* This is a 'keepsake' published by the editors of Chronica Botanica (Waltham, Massachusetts) in 1954, and consists of excerpts from letters dated 7 September 1674 and 9 October 1676 together with a forward by A. J. Kluyver and 88 interpretive footnotes
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17 A. v. Leeuwenhoek, RS Manuscript letter no. 1898.
18 R. Hooke, *Lectures and Collections; Microscopium* (J. Martyn, Printer to the Royal Society, London, 1678).
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21 A. v. Leeuwenhoek, RS Manuscript letter no. 1886.
22 H. Gest, 'Anaerobes in the recycling of elements in the biosphere', in *Biochemistry and physiology of anaerobic bacteria* (ed. L. G. Ljungdahl *et al.*), pp. 1–10 (Springer, New York, 2003). [Includes translation of comments, in Dutch, of M. Beijerinck.]
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