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The Roles of the Sense of Taste and Clean Teeth in the Discovery of Bacteria by Antoni van Leeuwenhoek

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INTRODUCTION

The discovery of protozoa, unicellular algae, unicellular fungi, and bacteria by Antoni van Leeuwenhoek is well recorded in standard books on the history of microbiology (1, 4), the history of biology (5, 6), and the history of medicine (3). The discovery of such a variety of microorganisms is the reason for books devoted entirely to van Leeuwenhoek (2). Furthermore, many microbiology and biology books, for whatever purpose they were written, introductory textbooks or otherwise, give some attention to the discoveries.

It was a remarkable achievement to discover eucaryotic unicellular organisms, but it required even greater skill to discover bacteria. Of the many discoveries made with single-lens microscopes that he constructed himself, the discovery of bacteria by van Leeuwenhoek is generally considered by microbiologists to be his greatest discovery.

Although, as indicated above, there are numerous writings on the subject, from cursory mention to in-depth studies, there has been no consideration given to what led van Leeuwenhoek to the discovery of bacteria. No matter at what level one reads, whether superficial account or lengthy treatise, one receives the impression that van Leeuwenhoek applied the microscope to virtually anything or everything he could observe with the instrument, and in doing so he happened to observe bacteria and other kinds of microorganisms. There would have been nothing wrong in that, since whatever approach was used, the discovery of bacteria ranks high in the history of microbiology. However, although the discovery was fortuitous, it did not occur from random observations. It was fortuitous in the sense that while deliberately investigating a certain subject with a designed approach, van Leeuwenhoek observed bacteria in the course of the study.

It is true that van Leeuwenhoek's numerous microscopic observations covered a broad spectrum of subjects, but they were not made without definite aim. If one reads the letters van Leeuwenhoek sent to the Royal Society in London, and the extant letters the Royal Society and individual persons sent to him, one can see that he pursued investigations which he originated because the subject interested him and also that studies were made in response to requests by others to investigate a specified subject with the aid of a microscope. Furthermore, the published works of contemporary scientific investigators were the stimulus for studies by van Leeuwenhoek.

An example of a subject which interested van Leeuwenhoek for many years was the disease called gout. He sent reports on the study of gout to the Royal Society, considered himself more knowledgeable about the disease than physicians, disagreed with a report on gout published in the *Journal des Scavans*, and on one occasion asked the Royal Society to send him information concerning the treatment for gout in England (19).

A request from the Royal Society to examine hair with a microscope is an example of a study in response to the ideas of others (17). At that time it was not known whether hair grew in length by addition to the tip, as is the case in plants, or whether it grew from the bottom and pushed previous growth upward.

Robert Boyle (1627–1691) made major contributions to the study of chemistry and physics, among which was Boyle's Law concerning properties of gases. The publication of an investigation by Boyle in *Philosophical Transactions* was the cause for microscopic studies by van Leeuwenhoek on certain chemical crystals (15), an

example of the published work of a contemporary being the stimulus to study a new subject.

The studies of gout, hair, and chemical crystals did not lead to the discovery of any microorganisms. However, van Leeuwenhoek was also curious about the sense of taste, and while pursuing investigations to satisfy that curiosity he discovered bacteria.

INVESTIGATIONS ON THE SENSE OF TASTE AND THE DISCOVERY OF BACTERIA

Van Leeuwenhoek's first letter to the Royal Society was dated 28 April 1673 and dealt with microscopic observations on mold, parts of a bee, and parts of a louse. The original letter is lost, but its contents are known from the report published in *Philosophical Transactions* (7). His first letter dealing with the sense of taste was that of 19 October 1674 addressed to the Royal Society (9). The report of the discovery of bacteria was contained in a letter sent to the Royal Society and dated at Delft on 9 October 1676 (16).

The impetus for van Leeuwenhoek's interest in the sense of taste is revealed in his letter of 19 October 1674. A diminished sense of taste during the course of a mild illness was a good reason to study that particular sense (9): "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." ("Voorleden Winter sieckelijck sijnde, ende als bij na gansch geen smaeck hebbende, besichtichde ick verscheijde malen mijn tonge, die seer beslagen was, in een spiegel, ende oordeelde alsdoen, dat mijn gansch weijnige smaeck veroorsaeckt wiert, door de dicke huijt, die op de tonge lach.")

When speaking of "thick skin" (*dicke huijt*), van Leeuwenhoek was referring to the furry coating of his tongue, which he concluded was interfering with what he called the "little points on the tongue" (*punctgens op de tonge*) that have a role in the sense of taste. The little points were the papillae of the tongue, and the taste buds are located in the papillae.

van Leeuwenhoek then examined the little points of the tongue with a microscope, using the tongue of an ox for the investigation. Ox tongues would have been easy to obtain in van Leeuwenhoek's time, since before the discovery and widespread application of practical methods of refrigeration, all reasonable size communities had a slaughterhouse for domestic animals, since the time between killing an animal and consuming the meat before decomposition occurred was short. He may also have obtained the tongue from a butcher's shop, as the tongues of domestic animals were then, and still are, eaten as meat.

Microscopic examination of the little points on the surface of the intact tongue, and after removal of the upper layer of skin, showed that the little points had "very fine pointed projections" (*seer subtijle puntige uijsteeksels*) that were composed of "very small globules" (*cleijne clootgens*).

That van Leeuwenhoek observed very small globules in the papillae of the tongue should not be interpreted as meaning that the globules were cells and hence that he discovered the sensory receptor cells for taste. "Globules" ("clootgens" or "klootgens" or "globule" in the letters), is, if not the most frequently used word, at least one of the most frequently used words in van Leeuwenhoek's letters. He saw globules in nearly everything he looked at with a microscope. Unless he gave further information about the globules, as he sometimes did, it is difficult to understand what he was seeing. When he speaks of "the globules that make the blood red" (*de globule die het bloet root maken*), he is speaking of red blood cells (11). However, in the same letter he confounds the reader by stating that the clear fluid, separated from the red globules after clotting of the blood, was composed of globules. He further confounds the reader by allowing the serum to evaporate and then reports that the dry matter which remained was made up of globules. Needless to say, together with many other materials he looked at, particles of soil and chalk were reported to be composed of globules; it was reported that the globules of chalk were transparent, and it was the way the transparent globules were arranged that gave chalk its white color (8).

van Leeuwenhoek was criticized by scientists in France for reports of globules in the many different materials he observed microscopically. He knew about the criticism (11), and although he claimed he did not mind it, it may have disturbed him, since he brought the subject up in at least two letters to the Royal Society.

The surface of the tongue has a bacterial flora, and the bacteria residing there are not sparse. However, van Leeuwenhoek did not report the observation of any microorganisms when examining the tongue with a microscope.

After the investigation of the papillae of the tongue, van Leeuwenhoek turned his attention to the constituents of food involved in the sense of taste. As will be seen later, he appeared to be satisfied that he had discovered the essential component of the tongue with respect to taste, namely, the globules.

Herring is a very popular Dutch food and is frequently eaten raw. In a letter of 11 February 1675 are details of an inquiry into the taste of

herring (10). van Leeuwenhoek had been studying the formation of crystals after placing common salt in water and allowing the water to evaporate at either room temperature or after the application of heat to quicken the process. He noted that at room temperature "square crystals" (*viercantige stukje*) were formed, but at the higher temperature the crystals had the appearance of "thin sharp-pointed pipes" (*dunne spitse pijpjens*). He then applied this information to the taste of herring. He concluded that the reason people do not like the taste of cooked salted herring is because the heat of cooking causes the salt to form sharp-pointed crystals which irritate the globules of the tongue, whereas the preference for raw salted herring is because the salt crystals are square and less irritating to the globules.

Microorganisms were not discovered in the investigation on the taste of herring, but the move away from the study of the tongue to the study of the role of food in the sense of taste was a move in the right direction for the eventual discovery of bacteria. Although the surface of the tongue has a resident bacterial flora, it is doubtful that van Leeuwenhoek would have discovered bacteria if he had done further microscopic studies on the surface of that organ. It is easier to observe bacteria in saliva than bacteria on the tongue. van Leeuwenhoek made several investigations on saliva, but did not see microorganisms. Even after he had discovered eucaryotic unicellular organisms and bacteria, and as a result of these discoveries examined saliva from several different people for the purpose of determining whether or not microorganisms are present in saliva, he reported that microorganisms are absent from saliva (18).

After the study on salted herring, van Leeuwenhoek sent several more reports on the sense of taste to the Royal Society before his discovery of bacteria. The subjects of those reports were investigations into the reason for the difference in taste between salt and sugar and on the taste of arum, asparagus, and cinnamon (12–14). Generally, the conclusions were that microscopic crystals or particles of food affected the microscopic globules in the little points of the tongue and that dissimilarities in the shapes of the crystals or particles of unlike foods were the reason for differences in taste of different foods.

Starting in September 1675 and continuing until August 1676, van Leeuwenhoek did a series of microscopic observations on rain, canal, well, and seawater. The results were communicated to the Royal Society in his letter of 9 October 1676 and reported observations of several different eucaryotic unicellular organisms, namely, protozoans (16). The letter also announced the discovery of bacteria. They were not observed in the various natural waters studied, but rather in an unnatural water environment, pepper water.

Why was van Leeuwenhoek studying water in which peppers were being soaked or steeped? It is unusual to find a report of just one investigation in any of the many letters van Leeuwenhoek sent to the Royal Society. Usually a letter gave reports on several investigations. In addition to studies on water from natural sources, van Leeuwenhoek was continuing his studies on the sense of taste and at that time was trying to discovery why pepper had such a potent taste: "After several endeavors, now and then, to discover if possible the cause of the power, or heat, of pepper on our tongue . . . I put about 1/3 of an ounce of whole pepper in water, and placed it in my office, for no other purpose than that the pepper should become soft, so that I could better observe it." ("Na dat verscheijde devoiren soo nu en dan, heb aengewent, omme waer het mogelijk, te ontdecken, de kragt, of hitte, die de peper op onse tonge aenbrengt . . . ik heb dan op nieu ontrent 1/3 once heele peper in water geleijt, en op mijn comptoir gestelt, uijt geen ander insigte, dan om dat de Peper soude sagt worden, omme deselve des to beter to connen observeren.").

van Leeuwenhoek allowed the pepper to remain in the water for about 3 weeks. He did not describe the vessel in which the pepper was steeped or give the quantity of water used. It must have been an open vessel, with probably just enough water to cover the pepper, since he reported that he twice added some water because of evaporation. On 24 April 1676 he examined his steeped-pepper preparation with a microscope and with "great wonder" (*groote verwondering*) observed several kinds of microorganisms in the water, including what are now called bacteria.

Conclusions that bacteria were observed for the first time are based on expressions van Leeuwenhoek used with respect to the sizes of the microorganisms observed.

In some of his previous studies van Leeuwenhoek had discovered protozoa. His written descriptions of one of the organisms allows it to be recognized as a species of vorticella, bell-shaped aquatic protozoans with a stalk by which they attach to objects. Protozoans were reported as being "little animals" (*kleijne diertgens*). Little animals were observed in the pepper water, but van Leeuwenhoek also reported that he saw other organisms that were "incredibly small" (*ongelooflijk kleijn*). An organism that is incredibly small compared with a protozoan is the reason for the belief that van Leeuwenhoek discovered bacteria on 24 April 1676. He also reported that by his judgment 100 of them ar-

ranged lengthwise would not equal the length of a grain of coarse sand and estimated that 10^6 of them would not equal the dimensions of a grain of coarse sand.

Later in the letter van Leeuwenhoek gave his report on the original purpose of the study, the taste of pepper. He explained that sharp-pointed microscopic particles in pepper greatly irritated the microscopic globules of the little points of the tongue and that was the reason for the hot taste of pepper.

van Leeuwenhoek's interest in the sense of taste did not wane after his discovery of incredibly small organisms. He continued his reasearch on that sense and transmitted his findings to the Royal Society for many more years.

VAN LEEUWENHOEK'S PRIDE IN HIS CLEAN TEETH AND THE DEFINITIVE EVIDENCE FOR THE DISCOVERY OF BACTERIA

The report of the discovery of bacteria consists of 72 words in a very long letter, which if printed in *Microbiological Reviews* would be 20 or more pages in length. Except for noting the observation of incredibly small organisms and comparing their size with a grain of coarse sand, no other information about them was given. Therefore, the shape of the first bacteria to be seen is unknown. However, when estimating their size van Leeuwenhoek spoke of them as being arranged lengthwise; thus, they may have been rod shaped.

There have been speculations that some of the other microorganisms reported in van Leeuwenhoek's letter of 9 October 1676 were bacteria, but there are no meaningful foundations for such speculations.

It is a pity that van Leeuwenhoek did not provide illustrations of the microorganisms reported in the letter of 9 October 1676. He certainly knew the value of drawings in scientific reports. Although he could not read the text of a book written by Francesco Redi (1626–1679), he could understand the illustrations of microscopic observations included in the book (10).

The definitive evidence for the discovery of bacteria was sent to the Royal Society in a letter dated 17 September 1683 (20).

van Leeuwenhoek was proud of his clean teeth and described his regimen for keeping them in that condition in his letter of 17 September 1683. Each morning he rubbed his teeth, using salt as an abrasive, and then rinsed his mouth with water. After eating he picked his teeth with a toothpick and then rubbed them well with a piece of cloth. He was well satisfied with his regimen and commented that few people of his age had such clean and white teeth. He was then just 5 weeks short of his 51st birthday. Despite his thorough regimen, on examining his teeth with a magnifying mirror he found that they were not as clean as he thought: "Yet by doing so my teeth are not clean, for when I look at them with a magnifying mirror there remains or grows between some of the molars and teeth a little white matter, about as thick as batter." ("Soo en sign mijn tanden daar door soo suijver niet, of wanneer ik deselve met een vergrootspiegel besag daar blijft ofte groeijt, tusschen eenige van de kiesen, en tanden, een weijnig witte materie, die soo dik is, als of het beslagen meel.")

Although the consistency of the batter-like matter prevented good microscopic observations, van Leeuwenhoek's suspicion was aroused as to the possibility that living organisms were present in the white matter. He then mixed some white matter with clean rainwater, after determining that the water was free of little animals. He also mixed some white matter with saliva from his own mouth, after eliminating air bubbles in the saliva so that they would not interfere with microscopic observations on the mixture. Again, this is a case of van Leeuwenhoek's belief, based on microscopic observations, that saliva is free of microorganisms.

Microscopic examination revealed that the preparations contained "many very small living animals, which moved very prettily" ("veele seer kleijne levende dierkens, dier haar seer aerdig beweegden.")

On this occasion van Leeuwenhoek made drawings of the organisms, which are now reproduced in virtually all introductory microbiology books and also in many higher-level books.

Rod-shaped bacteria of different sizes were observed and appropriate drawings were made. However, to look at reproductions of van Leeuwenhoek's drawings can be misleading with respect to some bacteria he saw, since the text of his letter is not usually given with the reproductions, and what is said in the text and what some drawings show are quite different. The drawings of bacteria which are probably misinterpreted are those shown in his illustration as having a spherical shape, and consequently it is concluded that van Leeuwenhoek observed coccus-shaped bacteria. According to the letter these little animals "moved quickly" (*vaardige voortgang*). Therefore, there is a question of what fast-moving cocci can be found in matter taken from between the teeth? Furthermore, other information in the letter casts doubt regarding the observation of cocci. The letter states that the organisms appeared at one time to be "long and round" (*lang-ront*) and at another time to be just "round" (*ront*). van Leeuwenhoek clearly states that he could not determine the shape of these fast-moving organisms, i.e., whether they were elongated or round, but pre-

sented them in his illustration only as they appeared when they seemed to have a spherical shape and without a drawing of how they appeared when they seemed to him to be rod shaped.

Dobell translated the description of these microorganisms at one time as being "oblong" and at another time as being "round" (2). Therefore, he is incorrect in stating that the microorganisms were coccus shaped, and he is even more remiss when he concludes that the organisms were micrococci, since micrococci are nonmotile bacteria. Dobell appears to have been led astray by giving more attention to the illustrations than to van Leeuwenhoek's written descriptions. Indeed, he more or less admits to that by making the following statement about van Leeuwenhoek's illustrations (2): "To anybody familiar with these organisms his figures speak so clearly that his words are almost superfluous."

van Leeuwenhoek immediately extended his study to see if he could find little animals in white matter from between the teeth of other people. He collected samples from people of both sexes, and of different age groups, and also from people with and without clean teeth. He took saliva from each person and, after ascertaining that it was free of little animals, mixed white matter from between their teeth with their saliva. He also made preparations of white matter in water. The mixtures consisted of one part of white matter and nine parts of saliva or water.

That he did not find little animals in any of the saliva specimens and also the fact, as previously reported herein, that he had not observed them in other studies to determine whether or not little animals occur in saliva casts more doubt on the observation of cocci in matter from between his teeth. Saliva has a relatively high concentration of bacteria, with cocci being by far the predominant form. If he could not observe cocci in any of the many specimens of saliva he examined, would he have seen cocci in his preparations of matter from between the teeth?

Included in the extended study were two women. Since he knew they cleaned their mouths each day, one of them was most likely his second wife and the other was probably his daughter from his first marriage. His first wife died in 1666, and four of the five children of that marriage did not survive infancy. An eight-year-old child was included in the study. A man who led a sober life and did not smoke tobacco was a member of the group. He also included an old man who smoked and frequently drank alcoholic beverages. van Leeuwenhoek was quite curious as to whether or not little animals could live in a mouth which was frequently used to take in alcoholic drinks. This old man had lost most of his teeth, having only a few of his front ones remaining, and they were very dirty. van Leeuwenhoek asked him if he ever cleaned his mouth. The old man replied in a manner indicating that he never did so with water, but he flushed his mouth every day with wine.

In all of the specimens van Leeuwenhoek observed rod-shaped little animals, some of which were larger than others, but the old man who was fond of alcohol did not have any of the larger kind of rod-shaped organisms. The fast-moving little animals that van Leeuwenhoek saw in white matter from between his own teeth and which he could not determine to be elongated or spherical were seen in all specimens of the extended study. In the matter from the man who lived a sober life van Leeuwenhoek saw, in addition to the other kinds of organisms, spiral-shaped little animals. His written description is not very good, but he provided a drawing showing the spiral form of the organisms. Unfortunately the drawing was not reproduced well in *Philosphical Transactions*, and the original drawing is now lost. However, a good illustration of the spiral-shaped organisms observed on this occasion was included in van Leeuwenhoek's *Ondervindingen en Beschouwingen* published in Delft in 1694.

The letter of 17 September 1683 to the Royal Society provides good evidence that van Leeuwenhoek saw motile rod- and spiral-shaped bacteria, but it is doubtful from the written descriptions of microorganisms reported therein that he observed spherical-shaped bacteria.

CONCLUSIONS

That van Leeuwenhoek discovered bacteria is well established, but there has been no previous consideration given to the activities van Leeuwenhoek was engaged in that led to the discovery.

It has most frequently been the practice of those interested in the history of microbiology to concentrate specifically on the relatively few letters written by van Leeuwenhoek that report observations of microorganisms and to neglect the numerous other letters: hence, the reason it is well known that van Leeuwenhoek was the first person to see bacteria and also why there is a dearth of information on the events antecedent to the discovery. By reading some of the other letters, those from April 1673 to September 1683, one can see that he was engaged in a logical sequence of investigations on the sense of taste; it was his interest in that particular sense that caused him to place peppers in water, and it was in that experimental setup that bacteria were observed for the first time. Proof beyond doubt for the discovery of bacteria came 7 years later when van Leeuwenhoek gave written

descriptions and made illustrations of bacteria found in matter from between his own teeth and the teeth of others. The illustrations should not, as is usually the case, be interpreted without the information provided by the written descriptions.

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