THE ORIGIN OF LIFE

A colorful account of the studies man has made in his attempt to discover the fundamental characteristics of living matter

by NORMAN H. HOROWITZ

FROM THE EARLIEST times it has been believed that living things can originate spontaneously from non-living material. For centuries it was thought, for example, that worms, frogs, insects and scorpions could originate in mud, or from dew or decaying meat, without parents. Known as the doctrine of spontaneous generation, this was the view of the classical Greek authors — Aristotle, Lucretius and others who influenced Western thinking for 2,000 years. It was the generally accepted view all through the Middle Ages and well into the 17th century.

The following quotation from the works of a well-known physician and chemist named Van Helmont, who lived from 1577 to 1644, is typical:

> "Furthermore, if a dirty undergarment is squeezed into the mouth of a vessel containing wheat, within a few (say 21) days the ferment drained from the garments and transformed by the smell of the grain, encrusts the wheat itself with its own skin and turns it into mice. . . . And what is more remarkable, the mice are neither weanlings, nor sucklings, nor premature; but they jump out fully formed."

It is important to note that Van Helmont was not making this up; he actually carried out the experiment and this is the way he says it worked.

Two hundred years later Pasteur commented on this quotation from Van Helmont. "What this proves," he said, "is that to do experiments is easy; but to do them well is not easy." One can see now how careless Van Helmont must have been when he set up this experiment. But the result came out just as he had expected; it was in keeping with the view of the times, and he didn't feel like being very critical about it. It is still true today, as it was then, that the easiest thing to do in a laboratory is to find the result you expect to find.

In 1668 an important discovery was made. An Italian physician named Redi decided to test the idea that worms were generated spontaneously in rotting meat. He put some rotting meat and fish in open jars, and he watched them. In time, he noticed that flies came and laid their eggs in the meat and that maggots hatched from the eggs. When he covered the jars with muslin he found that flies came and laid their eggs on the muslin, but as long as the eggs didn't get to the meat, no "worms" ever developed in it—and that was the beginning of the end of the theory of spontaneous generation of higher plants and animals. From this point on, this belief gradually died out among educated people.

A few years later, however, in 1675, another discovery was made which was to reopen the whole question at a different level. This discovery was made by Leeuwenhoek, a Dutch microscopist. Leeuwenhoek was the greatest microscopist of all time; he discovered a whole new world—the world of bacteria and protozoa. Nearly one hundred years later they were to form the subject of another controversy on the theory of spontaneous generation.

"The Origin of Life" has been adapted from a Friday Evening Demonstration Lecture given by Dr. Horowitz on March 16, 1956.

Leeuwenhoek is probably the most original figure in the history of biology. He was not an educated man; he was only an *amateur* scientist, and his true calling was the haberdashery business. Fortunately, he was able to spend a great deal of his time at his hobby, which was making microscopes. He made the best microscopes that were known up to that time. Actually, they weren't what we call microscopes; they were magnifying glasses of remarkably high power and resolution. One of them is known to have had a magnification of 270 diameters.

Leeuwenhoek learned how to blow glass and how to grind and polish lenses by going to the fair in Delft and watching professionals. Then he went home and practised by himself, and in this way he learned to make excellent lenses. But he never gave his secrets away; he never told anyone how he made the lenses. He never lent his best instruments, or sold any, or showed visitors his best glasses.

All of Leeuwenhoek's scientific discoveries were communicated in the form of letters to the Royal Society in London. The Royal Society had only recently been formed, and was in search of people doing interesting scientific work. Somebody from Holland told them that Leeuwenhoek was doing interesting things with microscopes, and he was invited to communicate his discoveries. So he wrote a long series of letters which were sent to London, translated and published. He was elected to membership in the Royal Society in 1680. In a way, this was the climax of his career. Elected to the company of people like Newton, Hooke, Robert Boyle, Halley and other great names of his day, Leeuwenhoek was deeply touched. On his death he left 26 of his best microscopes to the Royal Society. Unfortunately, these have all been lost.

Bacteria and decay

The next important date in the history of our subject was 1745, when a Scottish minister named Needham, also a microscopist, published observations and arguments which led him to believe that bacteria were generated spontaneously from decaying organic matter. People no longer believed that worms and mice were generated in this way, but bacteria were so small and primitive, so simple, that it seemed they were really on the threshold of non-living and living matter. It seemed quite reasonable to believe that bacteria were generated spontaneously, especially since it could be demonstrated that they were found wherever decay or putrefaction was going on.

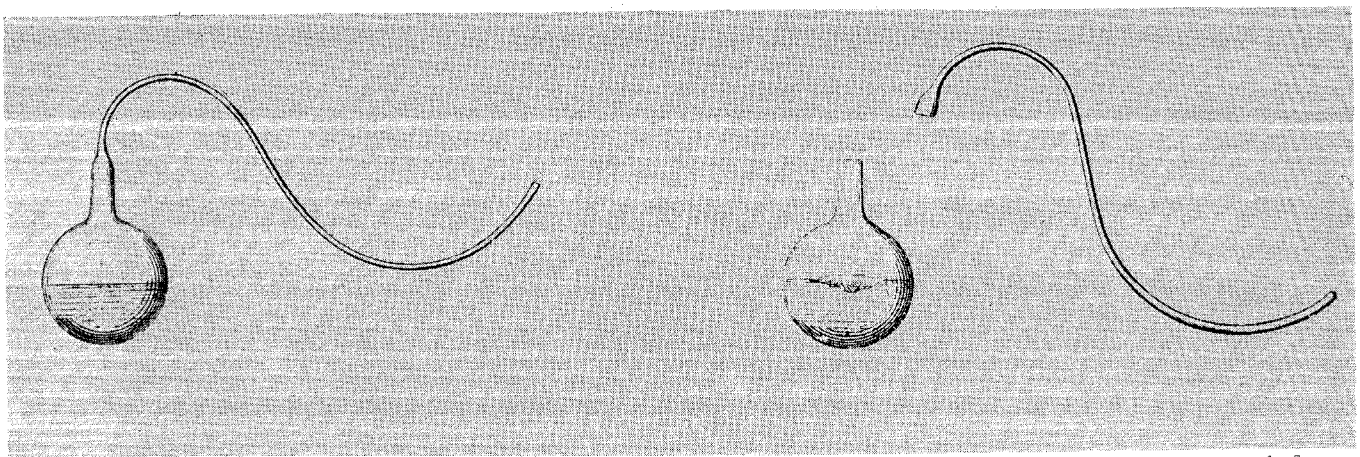
Needham's paper started a controversy. In 1765 an Italian by the name of Spallanzani published a report of an investigation which he thought disproved the claims of Needham. Spallanzani said that if he took mutton gravy, or any other medium suitable for the growth of bacteria, and heated it for a long enough time in a sealed vessel at the boiling point of water, it would no longer give rise to bacterial growth. Needham argued that what Spallanzani had done was to destroy some vital element of the air—some substance which was necessary for spontaneous generation—and that this experiment therefore did not disprove Needham's view.

Origin of canned food

This actually was to some extent true; years later, after oxygen had been discovered, it was shown by the French chemist, Gay-Lussac, that in Spallanzani's experiment the oxygen had, in fact, been used up. What Spallanzani had done was to fill the jar nearly to the top and heat it for 45 minutes; the oxygen was consumed by reacting with the organic material in the jar. So the controversy was not settled at that time. (One important thing did come out of it though. In one of Spallanzani's experiments he used garden peas as his growth medium, and he found that the peas kept indefinitely without spoiling. This was the first time that anything was canned, and it was directly from this observation that the canning industry started).

The argument was finally settled a hundred years later in a famous series of experiments by Pasteur. Pasteur proved, once and for all, that bacteria are not generated spontaneously—any more than Van Helmont's mice were. He showed that bacteria are not the product of decay, but the *cause* of decay. He communicated his results to the general public in a famous lecture which he gave at the University of Paris 92 years ago, and in which he demonstrated three important experiments.

Leeuwenhoek, a 17th century Dutch microscopist, first discovered the world of bacteria and protozoa.



Original illustration from Pasteur's paper on his famous experiments proving that bacteria are the cause of decay. Gooseneck flask at left kept broth sterile for months; open flask at right allowed entry of bacteria-laden dust.

In the first experiment, Pasteur showed that if you destroy the bacteria in a suitable medium by boiling, and allow only sterile air to enter the flask, you get no subsequent growth of bacteria.

In his next experiment he proved that growth of bacteria would start in the medium if it was inoculated with dust collected from the air.

Sterile solution

The third experiment (above)—and the one of which he was obviously most proud—was one in which he prepared his broth in an ordinary flask which he then pulled out in a flame so that it had a gooseneck. He boiled the medium in the flask for three or four minutes, allowing the steam to go up the gooseneck. Then he simply turned off the burner and let the flask sit there until it cooled. Then, without sealing it, and without any other precaution, he put the flask in an incubator and left it there. Nothing grew in it. The flask was completely open to the air, and there was no question of the oxygen being depleted because oxygen had free access to the flask. Yet the broth remained sterile. If you visit the Institut Pasteur in Paris today you will still see such flasks which, it is said, were put there by Pasteur. There is still nothing growing in them.

The explanation which Pasteur gave for this experiment is this: When the broth is boiled, the steam comes out the gooseneck and, of course, drives out the air. When the flame is turned off, air re-enters the flask, but it comes in contact with the liquid which is almost at its boiling point—hot enough to kill bacteria. As the broth cools down, the stream of air entering the flask slows down very much, to the point where dust particles in the air can no longer make the trip; they get caught in the moist gooseneck, so that they never reach the surface of the broth after it is cool.

This experiment, and the others that preceded it, settled once and for all the question of spontaneous generation of bacteria. Of course, many people repeated these experiments after Pasteur, and many failed; but it was a question of technique. Nowadays, it is commonplace to prepare a sterile solution that will remain bacteria-free indefinitely.

Researches that have been carried out since Pasteur's day have shown that bacteria are not nearly so simple as had been assumed up to that time. Although they are very small, they have a very delicate organization and very complicated chemical processes go on in them. They are just as complicated chemically as the individual cells that make up the bodies of higher plants and animals, and the idea of such complicated structures originating by chance in a medium containing nothing but organic chemicals is quite fantastic. As a recent writer has said: "Imagine a factory with smokestacks, machinery, railroad tracks, buildings, and so on springing into existence in a moment—following some natural event like a volcanic eruption. The same sort of event is assumed when one assumes that something as complex as a bacterial cell can originate in a pot of gravy."

Attributes of living matter

It was shown by Pasteur, and by others, that bacteria arise only from other bacteria. This property, which we call self-reproduction, is a very important and fundamental attribute of living matter, true of all cells. There is another attribute of living matter which we must consider: mutability. By mutability we mean the property of undergoing an hereditary change.

For example, if we take a culture of bacteria growing in broth and add some penicillin to the broth, we will destroy most of the bacteria, but there may be a few bacteria—a few mutants—which are resistant to the penicillin. They will continue to grow, and they and all their descendants will be penicillin-resistant. We say that these bacteria are mutated. This represents an elementary step of evolution. These bacteria have evolved to a certain extent; they have changed one of their fundamental properties, and they are a different kind of bacteria than their parents were. These two qualities—the ability to self-reproduce, and the ability to mutate—are probably the most fundamental characteristics of living matter, and the problem of the origin of life as we see it today is that of finding the simplest chemical structure which exhibits these two fundamental attributes.

We think that if we can find a molecule that exhibits these two qualities, we shall have found a simple form of life. Imagine, if you can, a chemical substance that is capable of reproducing itself and that is capable of blindly mutating in various directions. By mutating, our molecule will try out new ways of existence, and after a few generations it will be very different from the thing that it started from. In time, everything else that we associate with living matter will follow by logical necessity. For this reason we feel that these are the two qualities that we must look for when we examine the question of the origin of life.

What are the simplest systems which exhibit these qualities?

About thirty years after Pasteur's experiments — around 1900 — organisms were discovered which are even smaller than bacteria. These are the viruses, and they have played an important part in considerations of the origin of life.

The first virus to be discovered, the tobacco mosaic virus, has been very much studied, especially by Stanley and his collaborators at the University of California. This virus causes a disease of tobacco plants —a disease causing the mottling of the leaves—and in producing the disease the virus multiplies in the plant. If you inoculate the plant with a few particles of the virus, you will find, after a week or two, that the plant contains a great deal of the virus, and you can isolate from the plant juices many times as many virus particles as you put in. The interesting thing about these virus particles is that they are not only much smaller than bacteria—but they really seem to be simpler than bacteria. Viruses do not have a very complicated chemical structure; they don't carry on all the chemical activities of ordinary cells. It appears that the only activities viruses are capable of are reproduction and mutation. The viruses are very close to being particles which possess just the two elementary attributes of living matter.

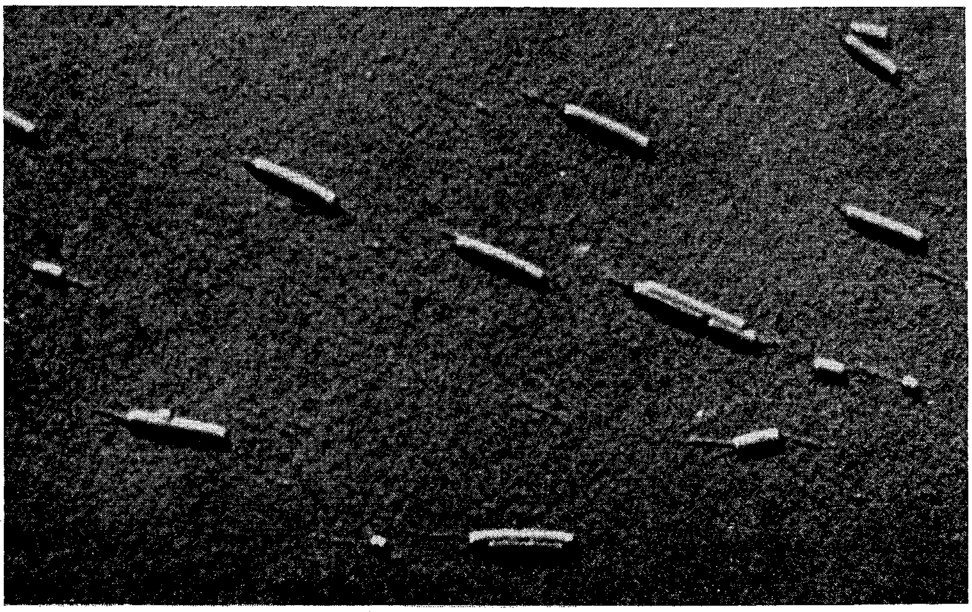
The chemical composition of the tobacco mosaic virus has been studied, and it has been found to consist of only two parts—an outer jacket of protein and an inner core of nucleic acid. Recently two workers — Fraenkel-Conrat and Williams—at the Virus Laboratory of the University of California succeeded in separating the protein part from the nucleic acid part and then putting them back together again, reconstituting the infective virus.

On looking at this experiment more closely a very interesting thing appears. Fraenkel-Conrat and Williams, of course, tested the solution of protein to find out if it had any virus activity by itself, and found that it does not. When they carried out the same test with the nucleic acid, they found a slight infectivity which, at first, they assumed was due to contamination by active virus particles that had not been disintegrated by the chemical treatment. On repeated tests, it appeared that they could not remove the small amount of infectivity from the solution of nucleic acid—and it has now been proven that the nucleic acid by itself has infective properties.

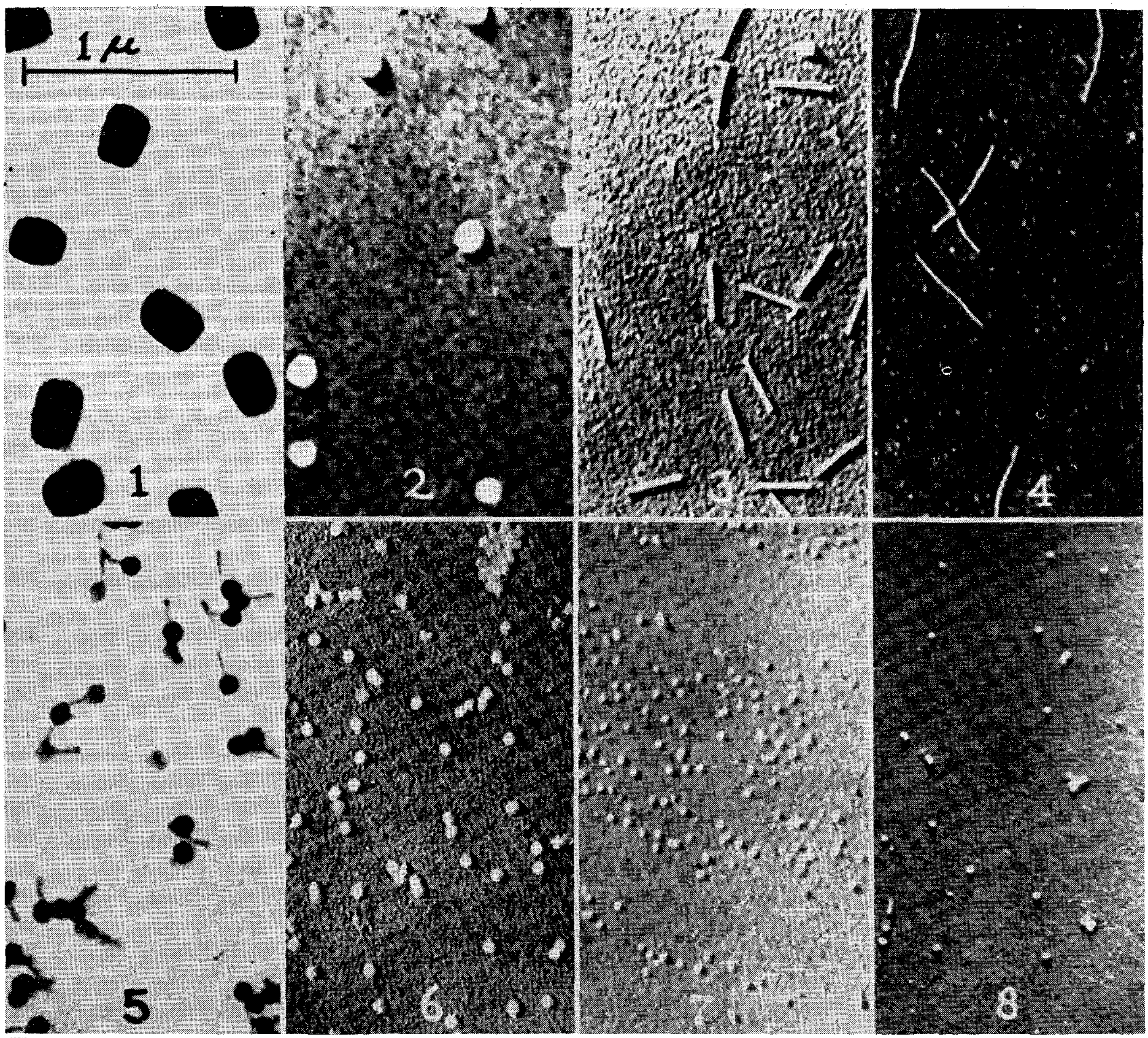
This means that the properties of self-duplication and mutability reside not in the whole virus particle but just in the nucleic acid part.

This finding is in accord with other experiments done on other organisms which indicate that nucleic acids have infective properties and are capable of reproducing themselves and of mutating.

Some biologists now think that the nucleic acids—perhaps combined with proteins — were the original



Electron micrograph of tobacco mosaic virus that has been treated with a hot detergent in such a way as to cause the decomposition of part of the virus—revealing that the virus consists of two parts, an outer jacket of protein and an inner core of nucleic acid.



Electron micrographs of some highly purified viruses: (1) Vaccinia (2) Influenza (3) Tobacco mosaic (4) Potato-X (5) Bacteriophage (6) Shope papilloma (7) Southern bean (8) Tomato bushy stunt.

forms in which living matter first appeared on the earth. These molecules are very much simpler than bacteria, and Van Helmont's mice. There is a chance that, given enough time and given the right conditions, a nucleic acid molecule could be spontaneously generated in the proper chemical medium.

Geophysicists think there were about two billion years between the origin of the earth and the first signs of living matter. That is a long time, of course, and many improbable things can be accomplished in that long a stretch. The conditions of the earth at that time, we are told, were quite different from now. The atmosphere consisted not of oxygen, nitrogen and carbon dioxide, but of hydrogen, ammonia, methane and water. Quite recently Dr. Stanley Miller of the University of Chicago (a post-doctoral fellow at Caltech last year) tried the experiment of passing an electric discharge through an atmosphere consisting of these four gases to see what would happen. He was simulating on a small scale the conditions that the geophysicists say existed on the earth some two billion years ago. He found a large number of organic compounds at the end of the experiment, including a number of amino acids. He found no nucleic acid, or nucleic acid building blocks, but he did find amino acids, which are the building blocks of protein.

There are about 20 different amino acids in an ordinary protein. In Miller's experiment, he found glycine, alanine, aspartic acid, glutamic acid and about 20 others which do not occur in protein. These four amino acids are very far, of course, from a protein, and they are even much farther from a living cell, but that is where this problem stands today—and I guess that is where I had better leave it.