

## Beginning of life

### The beginning of life

#### Mice that are born from a shirt

Philosophers from ancient Greece believed that life was contained in matter itself and when conditions were favourable it would appear spontaneously.

Aristotle synthesized in one of his theories all the ideas on spontaneous generation by the philosophers that came before him.

According to the great philosopher, living beings are born from similar organisms, but sometimes they can also be generated from inert matter. All things, in fact, have a “passive element” which is their matter and an “active element” which is their shape, meaning a sort of inner force which gives the matter its shape.

For example, clay is a nonliving matter that has an active element which enables it to shape inert matter into a living being, such as for example a worm or a frog.

The spontaneous generation theory was supported by famous scientists such as Newton, Descartes and Bacon and in 1500 there were people who still believed that geese were born from certain trees that lived in contact with the ocean and that lambs were generated inside melons.

The first experiments to prove the spontaneous generation theory were done in the XVII century and a doctor called Jean Baptiste Van Helmont declared he had performed a unique experiment: he placed a dirty shirt together with some wheat and according to him, mice were born 21 days later. According to the Doctor the sweat in the shirt was the active element which gave life to the inert matter.

#### First experiments

Following the first incorrect experiments such as Van Helmont’s one, many others were performed.

In 1668 Doctor Francesco Redi led a series of experiments that were supposed to prove that spontaneous generation does not exist. Redi placed in containers some samples of veal and fish, some he sealed while others he left in the open air. As time went by, he noticed that in the open containers on the decomposed meat there were worms (that were in fact larvae!), flies and other insects, while there were no signs of life in the sealed containers.

In that same period, the Dutch naturalist Anton Van Leeuwenhoek (1632 – 1723) built a rudimentary microscope which enabled him to watch microorganisms. This made it possible to see that all substances contained a large number of living organisms, and this obviously brought back the spontaneous generation idea which had apparently been abandoned following Redi’s experiments.

Following numerous examinations with Leeuwenhoek’s instrument new arguments arose among those who sustained the abiogenesis theory (life is born from lifeless substances) and the biogenesis one (life is generated only from living beings).

In 1745 the English naturalist John Needham invented new experiments to prove the abiogenesis theory. He filled some test tubes with chicken broth and herb infusions and then he closed them with some gauze. The test tubes had been sterilized by the heat, nevertheless after a few days hundreds of living beings could be seen inside them. This result reinforced the spontaneous generation theory.

A few years later the abbot Lazzaro Spallanzani, who was not convinced by Needham’s experiment, tried to repeat it heating the nutritious liquid for much longer and at much higher temperatures, actually making it boil for a few minutes. He sealed the test tubes and the result was that even several days later there was no sign of any living organisms in them. As a result, the naturalist Needham, criticized Spallanzani saying that the nutritious liquid had been heated too much, and this had killed the active elements and that sealing the test tubes had not allowed the presence of air which is indispensable for life.

Discussions went on for a long time until half way through the nineteenth century, when a French biologist Louis Pasteur,

ran a new experiment that settled the matter. Pasteur created some glass containers with a long curved neck (called "swan neck balloons"). Inside these the nutritious liquid was boiled for over an hour, letting the vapour out through the container's curved neck. After boiling, the broth inside was left to cool slowly, while the contaminated air carrying microorganisms entered from the outside as a result of the post heating depression. Thus the microscopic organisms that came in contact with the boiling vapour of the liquid inside, would die and even after some months there was no trace of life to be found, instead on the outer part of the container's neck, one could see dust and microorganisms that were coming in from the outside.

This experiment put a definite end to the abiogenesis theory, those that claimed that the long boiling of the nutritious liquid killed the active element. Pasteur instead, proved that once the curved neck of the container was broken, air in contact with the substance would bring germs and microorganisms inside, shortly after. Furthermore, the unsealed container allowed air to enter, even if through a tortuous neck, disproving the objections of those who supported that the active element needed air to generate life.

## Life on Earth

There are two different theories on the origin of life on Earth: the autotrophy theory and the heterotrophy one. The first theory assumes that the first living being was autotrophic, meaning that it is able to produce organic substances from inorganic ones like green plants do, through a complex reaction called "chlorophyll photosynthesis". In the second case instead, the first living organism would have been heterotrophic, meaning that it was not able to produce its own food, but had to feed on other living beings. In fact, animals (heterotrophic) need to eat plants (autotrophic) to survive, while the latter have no such need.

In the 1920s the English biologist John Burdon Sanderson Haldane (1892 – 1964), considering that at the time the environment on Earth was very different from what it is today, began to draw up his own conclusions. Initially on the primitive Earth there was no life as there is today.

According to Haldane if organic matter formed nowadays, it would be immediately eliminated by some living organism, while at the time, in the total absence of any microorganisms that could decompose it, would have had plenty of time to develop and become more complex.

In 1924 the soviet researcher Aleksandr Ivanovic Oparin came forth with theories which were similar to Haldane's one, but with the difference that according to the first, the primitive atmosphere had to be rich in hydrogen, while according to the English scientist, it was rich in carbon dioxide. To have experimental proof of this new theory we shall start from this last supposition.

Oparin and Haldane approached the argument from a scientific point of view omitting any religious interference and this obviously wasn't accepted by the faithful that instead were trying to prove that life couldn't have sprung forth from the random encounters between atoms, but was an act of God. Actually, proteins are extremely complex molecules, that cannot have formed from random encounters between hydrogen, carbon, oxygen and nitrogen atoms, but it has been scientifically proved that the possible combinations of simpler molecules are ruled by physical and chemical laws therefore they are limited and not random.

## Primordial atmosphere

The sun and its planets formed about 5 billion years ago following the explosion of a supernova, which is a big star, that before bursting had generated heavy elements starting from Hydrogen and Helium. At the beginning Earth was an enormous incandescent ball composed primarily of Hydrogen and Helium, but also by heavy elements such as carbon, nitrogen, oxygen, iron and silicon which had been flung into space by the explosion. After that, the planet cooled down and part of the lighter gasses, such as hydrogen and helium, bonded with heavier elements and part got lost in space. Actually Helium was practically totally lost because it is light and not very reactive with other compounds, while a part of hydrogen (the lightest element of all) reacted with other elements forming hydrogenated compounds such as methane (CH<sub>4</sub>), ammonia (NH<sub>3</sub>), hydrogen sulphide (H<sub>2</sub>S) and water (H<sub>2</sub>O). Thanks to gravity the heavier elements began to form a central nucleus, composed mainly of iron and nickel, surrounded by a mantle, formed by heavy elements and an outer

crust, made of the lightest elements such as aluminium, potassium and sodium. While the crust was forming, many volatile gases coming from the center of the Earth were released through the cracks and created what we consider to be the primordial atmosphere. Indirect proof of the primordial atmosphere can be obtained from the mixture of gasses which are still being released nowadays by volcanoes and solfataras, whose composition is very similar to that of the primordial atmosphere.

Another proof of the primordial atmosphere composition theory is given by the atmosphere analysis of the farthest planets of the solar system thanks to a rover, and they have been found to be rich in hydrogen compounds.

The latest proof is obtained by analyzing meteorites which contain all of the assumed substances although in a very low concentration.

In any case scientists are sure that the primordial atmosphere contained no free oxygen ( $O_2$ ) and therefore ozone could not exist either (formed by three oxygen atoms  $O_3$ , instead of two) and so the sun's ultraviolet rays that generally are blocked by a thick ozone layer, could reach the planet's surface in much larger quantities compared to today and help with their energy in the formation of primitive chemical compounds.

## Miller's experiment

In 1952 the American professor Harold Clayton Urey, Nobel prize for chemistry in 1934, asked a young researcher, Stanley Lloyd Miller, to perform an experiment. Inside a glass bottle, Miller put some extremely hot water and in another one he put a Hydrogen mix ( $H_2$ ), ammonia ( $NH_3$ ) and methane ( $CH_4$ ), which are all those gasses that combined with water vapour ( $H_2O$ ) were thought to have created the primordial atmosphere. Hot water, which according to scientists was in the primitive ocean, created vapour that passed through a tube and went to the container that held the gas mix. Inside this container they generated 60.000 volt electric discharges so as to reproduce the probably very frequent and powerful storm phenomena which occurred at the time when the planet was forming. The experiment was carried out for a whole week and in the end they were amazed to see that in the water container there was a red – orange liquid which had many compounds, particularly some amino acids, that are the precursors of proteins that are the main components of every living being. Miller's experiment proved that from simple compounds, that were thought to be in the primordial atmosphere, there could be the formation of complex molecules, those that are found in organic compounds of all living organisms. So the assumption was that the biologic precursors of living beings could have formed with a simple chemical synthesis process in a primitive atmosphere with frequent storm phenomena, heat and ultraviolet radiation. After that rains would have carried these simple organic compounds to the sea, where they could subsequently change and grow. Anyway, creating amino acids in a laboratory doesn't mean that one is creating a living organism, but obviously this was a step forward in the abiotic (that is chemical) formation of living beings.

From then on scientists performed many variations of Miller's experiment. It is possible to modify the gas mixture, change the temperature, use a different energy form from electric discharges, but the final result will always be the same: organic substance.

## The primordial soup

Similar experiments to those done by Miller have definitely proved that in high temperature conditions, with frequent storms and intense ultraviolet rays, which are similar to the kind of conditions present on Venus nowadays, simple inorganic molecules can transform into more complex substances which we call organic because they are part of living organisms. These organic substances spread in the sea and reacted between themselves and with inorganic salts. Even small water basins such as lakes and lagoons offered the right conditions for these reactions to take place leading to the creation of certain compounds and increasing their concentration. Presumably the accumulation of organic substances was remarkable because neither decomposing microorganisms nor oxygen that could modify them existed. This is how a thick substance that scientists call " primordial soup" or "prebiotic soup" was created.

Today this kind of substance would ferment and produce poisonous gasses with an acrid smell. In the sea some molecules will have found shelter from the ultraviolet rays that could destroy them, while others will have found great conditions to gather and bind to become more complex structures, forming the so called "polymers". Therefore the sea is where the chemical evolution of organic substances will have continued.

## Proteins' ancestors

In 1957 Sidney Walter Fox, an American biochemist, invented an experiment that proved how proteins could be formed outside of living beings starting from amino acids.

Fox simply warmed an amino acid mix on a metal plaque. Right after it cooled off it was possible to notice some complex molecules, very similar to proteins, which he called proteinoids so as not to get them mixed up. At that point it was believed that these new molecules had formed from the union of amino acids freed of the water through its evaporation. The same reaction could have happened on the burning hot rocks of the Earth's crust as it had just solidified. The tides might have brought the primordial soup enriched with organic substances on land where water would have evaporated allowing the amino acid molecules to bind together. These protein precursors would then be carried once again to the sea by the rains and the tidal ebb and flow.

## The coacervates

Actually we are still a long way away from what could be defined as a living organism also because nowadays it is surrounded by a wrap called "cellular membrane", that separates it from the outer world.

Starting from these suppositions, Oparin assumed that in the hot primitive seas organic molecules would gather in small drops, similar to the actual cells. These small drops wrapped up in water molecules are called "coacervates" (from cum acervo = gather together) and were already known before Oparin's research. It has been proved that by placing certain proteins which bind well with it in the water, with certain temperature and acidity conditions, numerous small drops will form holding within them most of the larger molecules bound together.

This can be explained by the existence of opposite sign electric charges on the proteins, which are then attracted to one another and gather polar molecules of water on the outer surface to form a membrane around the aggregation transforming it into a small drop.

In 1958 the biochemist Sidney Walter Fox, who discovered protein precursors (proteinoids), made some proteinoids melt in hot salty water. When the solution cooled down, he noticed that there were thousands of small corpuscles similar to bacteria, that he called "microspheres". Through the microscope he saw that the small organic substance corpuscles had a double protection membrane. This membrane is not like a cellular membrane, but in certain conditions it acts as one. In fact, when placing them in solutions at higher or lower concentrations compared to their inner liquid, they shrink or swell exactly like living cells will do in the same situation. Furthermore, the microspheres are able to keep some molecules inside and let others out. These features make the microspheres look very much like living cells..

## The living cell

In 1665, while observing a sliver of cork, Robert Hook discovered the cell, that extremely complex building block in multicellular beings. In fact, each one contains an incredible number of structures which can be observed through an electron microscope and each one of them performs specific biological and biochemical activities that make the cell into a perfectly organized living "factory". One can see the cell's evolution by watching unicellular organisms through the microscope. Within these cells one can see complex structures and organs which are similar to other multicellular organisms. As early as in 1940, following the invention of the electronic microscope, we knew of the existence of a much simpler form of life: the virus. In spite of its tiny size (only a few hundred millionths of a millimeter) and its simplicity compared to other unicellular organisms, it still has a nucleic acid. This nucleic acid known as DNA (deoxyribonucleic acid) is the basic building block of life and is responsible for the transmission of a being's hereditary features. Many viruses contain this nucleic acid that is the same DNA that transmits hereditary features in extremely complex beings such as man. Viruses obviously do not represent life as it was created on Earth because they are parasites that need other plant or animal cells to live on. Nevertheless they show a connecting bridge between chemical and living substances. In 1967 two American scientists, Arthur Kronberg and Mehran Goulian, were able to synthesize, that is to produce artificially in a lab the DNA and joined it with protein molecules taken from a virus. As a result they created a new virus that could reproduce itself. Thus they had reached the moment when life begins.

## Energy for life

All living beings need an energy source to activate chemical reactions. For example, to light a match requires some kind of energy source to trigger the reaction. In this case one simply has to rub the top on a rough surface to produce heat and make it light. This is a case of “activation energy”.

Almost all the experiments we spoke about in the other chapters, used electric discharges, ultraviolet light and heat as energy sources. However these energy sources can be harmful to living molecules, because too much heat can disintegrate the molecules and the coacervate in them, causing irreparable damage.

The primordial earth didn't have a sufficiently thick and dense atmosphere, so ultraviolet radiations could have destroyed everything on the planet's surface. This blocked the evolution of organisms in the areas struck by this energy. Both electric discharges and ultraviolet rays were generally active in the atmosphere, while life began almost certainly in the water or in protected humid places. Therefore, other forms of energy must have helped the beginning of life on Earth.

As time passed, the diluted hot soup that was found in the depressions of the Planet's surface, started to cool down and so the reactions become slower. At this point it is believed that new substances must have appeared which were able to help chemical reactions. These substances actually exist in every living organism: enzymes.

Enzymes activate chemical reactions in living beings even at temperatures so low to be unable to supply the necessary energy to trigger them.

Enzymes generally are formed by two parts: a protein part and a non protein part. The protein part includes the so called “active site”, meaning an area that adheres to the molecules on which it acts. The other non protein part, is often a vitamin and helps the protein part in its function.

Enzymes can function also outside the living cell and this has been useful in multiple lab experiments.

Today living beings tend to use sugars as a source of energy. Sugars or carbohydrates are molecules formed by carbon, oxygen and hydrogen and are synthesized by green plants. Did these substances exist in the primordial ocean?

Melvin Calvin was able to answer this question with a new experiment. He struck different chemical compounds from those used in Miller's experiments but which could have existed nonetheless in the primitive atmosphere, with high energy radiation. Thus, Calvin was able to obtain new molecules like simple sugars such as glucose.

Thanks to specific enzymes, glucose and other similar sugars can create more complex structures such as starch and cellulose.

Primordial oceans might have contained glucose molecules that could be usable as an energy source, but a lot of activation energy is needed to undo the ties among atoms of glucose and produce other energy. So presumably there was a similar mechanism to the one that happens in living beings nowadays, which means that they must bind some atoms to the molecule that must be divided so that they will attract electrons that will form a bond to weaken the molecular structure and break it down. In the case of glucose, the phosphorous groups (a group of phosphor, oxygen and hydrogen atoms) bind to a sugar molecule and transform it into glucose – phosphate, a weaker molecule than the initial one which consequently requires less activation energy to break down. ATP (acid adenosinotriphosphate) is a chemical compound that supplies not only energy to add phosphoric groups to glucose, but also the necessary phosphoric group to weaken the molecule.

## Fermentation and respiration

ATP or adenosinotriphosphate is a complex molecule formed by a nitro compound called adenine, by one sugar with five carbon atoms called ribose and three phosphoric groups. The phosphoric groups were present in the Earth's crust as phosphates, that is, salts found in the rocks that the hot water of the primordial earth could have melted and carried to the sea. Adenine and ribose instead formed spontaneously and we have experimental proof of this.

In 1960 the American biochemist Juan Oro made hydrocyanic acid (one of the products of Miller's experiment) react with ammonia thus obtaining adenine. In a further experiment, the biochemist added formaldehyde, a compound used as a disinfectant also known as formalin, obtaining ribose.

As we said before, ATP has three phosphoric groups of which, when two are detached, will release a huge amount of energy. For this reason the terminal binds of phosphoric groups are called “high energy” binds. When one of the

phosphoric groups detaches from the ATP, what remains is called ADP (adenosyndiphosphate) because it has only two phosphoric groups.

Thanks to a particular enzyme a phosphoric group can pass from an ATP molecule to a glucose one creating glucose – phosphate and ADP. ADP must transform into ATP again to become once again an active molecule. The transformation of ADP into ATP actually happens through chemical reactions that release energy. If these reactions happen without oxygen they are called fermentation, with oxygen instead they are called respiration. In the primitive atmosphere, however, there was no oxygen and so we can assume that in primitive heterotrophy something similar to fermentation took place. Today fermentation happens in many unicellular organisms but also in many complex organisms, including man, which allow cells to survive, even for short time , in the absence of oxygen.

The best known fermentation reaction, from a chemical point of view, is the one that transforms grape juice into wine. A sweet liquid such as grape juice thanks to the presence of glucose turns into a watery solution of ethyl alcohol: wine. Most of the energy produced in this transformation is stored in the phosphoric binds of the ATP. The transformation of glucose into ethyl alcohol releases carbon dioxide, of which there was very little in the atmosphere in those times, but then it will become vital for the subsequent evolution of metabolism. The anaerobe primitive organisms needed glucose and other simple organic substances for vital processes that were found easily in the water at the time, storing the energy produced in ATP.

## Protein or DNA?

Today, proteins are formed following instructions given by DNA (deoxyribonucleic acid) which in turn is synthesized by specific enzymes that are proteins. So which came first, protein or DNA?

Nucleic acids (DNA and RNA) are made up by nucleotides which are molecules formed by one sugar with 5 carbon atoms, one phosphoric acid molecule and a nitro base.

Sugars with 5 atoms of carbon are ribose, which is found in the RNA (ribonucleic acid) and the deoxyribose in the DNA. Nitro bases are compounds with basic proprieties (which means that they can receive protons) that have nitro atoms and are: adenine, cytosine, guanine, thiamine, and uracil. We find the first four bases in the DNA, whereas in the RNA there are the first three and the ... DNA contains the genetic information of all living organisms.

Proteins are large molecules made up by 20 small molecules called amino acids. All living organisms have the same 20 amino acids, but they are arranged in different ways and this determines the different function for each protein. Proteins perform all of an organisms' vital functions, but the unique disposition of the amino acids within them is determined by a specific sequence of the nitro bases in the DNA. The RNA carries the message contained in the DNA to the cell area where protein is synthesized and will have to perform the synthesis as well.

So in a living being nucleic acids contain the information that is passed to the proteins that are in charge of many functions, including rebuilding these nucleic acids.

It seems rather unlikely that two molecules which are so important for life appeared at the same time, but on the other hand it seems absurd to have one without the other.

Some biologists among which Francis Crick and Leslie Orgel, each one of them on his own, assumed that there was a compound which could both duplicate without the help of proteins as well as catalyze each phase of the protein synthesis. This compound is supposed to be the RNA, because it is a simpler molecule compared to DNA and it is easier to synthesize.

Subsequently, many studies confirmed this supposition, including the discovery of enzymes made of RNA so they understood that not all chemical reactions are performed by proteins. They even succeeded to modify some RNA molecules with enzyme functions to make them able to bind nucleotides of RNA itself.

It still is not possible to prove for sure that the cells' ancestor had an RNA which could synthesize proteins, as well as duplicate and modify itself; but it is even more important to be able to understand how this RNA was born.

We have already seen how adenine synthesis, one of the four nitrogen bases of DNA, was obtained in a lab experiment. Subsequently, other reactions among compounds that existed in this ancient atmosphere created also the other three nitrogen bases of nucleic acids.

## The origin of photosynthesis

The first cells fed on organic substances in the primordial soup as its concentration gradually diminished. It is likely that the scarcity of energy resources imposed a selection. Some cells acquired the ability to feed on others, while other cells developed the ability to synthesize new organic substances by using energy from oxidation. Even today there still are prokaryotes (cells that don't have an actual nucleus but a nuclear "equivalent") that draw energy in this way to live, they are the so called chemosynthetic bacteria. Other cells instead could exploit energy from light to transform water and obtain the hydrogen necessary for photosynthesis (a reaction that can transform simple inorganic substances into organic ones such as carbohydrates thanks to the energy given by light). Living organisms that are able to perform photosynthesis however released simple oxygen which, thanks to its high affinity with organic substances, must have killed most primitive cell forms. Only those cells that could handle the increasing oxygen concentration survived. Subsequently some prokaryotes learned to use free oxygen as an oxidant for energy production. This is how respiration appeared bringing with it the great advantage of being a more efficient energy stocking method than fermentation and guaranteeing survival in the oxygen rich atmosphere.